Strathclyde Institute of Pharmacy and Biomedical Sciences

Genetic and Environmental Drivers of Fruit Composition in Relation to Sensory Quality in Blueberry (*Vaccinium corymbosum*)

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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.

Signed:

Date:

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Abbreviations

13-HPL	13-hydroxyperoxide lyase
13-HPO	13-hydroxyperoxide
13-LOX	13-lipoxygenase
2,4-Hexal	(E,E)-2,4-hexadienal
2-Hexal	(E)-2-hexenal
2-OH C16:0	2-hydroxy-hexadecanoic acid
2-OH C24:0	2-hydroxy tetracosanoic acid
3-HexeOH	(Z)-3-hexenol
4-CCoA	4-coumaroyl CoA
4CL	4-courmarate CoA ligase
6-MHO	6-methyl-5-hepten-2-one
A	absorbance
a	appearance descriptors
a.m.u	atomic mass unit
ACT	anthocyanin acyltransferase
ANOVA	analysis of variance
ANR	anthocyanidin reductase
ANS-LDOX	anthocyanidin synthase
AU	Aurora
BE	Berkeley
BG	Bluegold
BL	Bluecrop
BR	Brigitta
C14:0	tetradecanoic acid
C15:0	pentadecanoic acid
C16:0	hexadecanoic acid
C18:0	octadecanoic acid
C18:1	octadenoic acid
C18:3	linolenic acid
C20:0	eicosanoic acid
C21:0	heneicosanoic acid
C21-OH	heneicosanol
C22:0	docosanoic acid
C22-OH	docosanol
C23:0	tricosanoic acid
C23-OH	tricosanol
C24:0	tetracosanoic acid
C24:OH	tetracosanol
C25:0	pentacosanoic acid

C26:0	hexacosanoic acid
C26-OH	hexacosanol
C3H	coumarate CoA ligase
C4H	cinnamate 4-hydroxylate
CA	citric acid
caf-hex	caffeoyl hexose
CAR	carboxen
cat	catechin
CGA	chlorogenic acid
CH	Chandler
ChA	chorismic acid
CHI	chalcone isomerase
CHS	chalcone synthase
CHT	Chanticleer
CO_2	carbon dioxide
CSh	4-coumaroyl shikimate
cya-ara	cyanidin-3-O-arabinoside
cya-gal	cyanidin-3-O-galactoside
cya-glc	cyanidin-3-O-glucoside
Da	dalton
DA	Darrow
Daid	daidzein
del-ara	delphindin-3-O-arabinoside
DFR	dihydroflavonol 4-reductase
dimer B2	(-)-epicatechin-(4β→8)-(-)-epicatechin
DNA	deoxyribonucleic acid
DP	Descriptive profiling
EGC	(epi)gallocatechin
EI	electron impact
EL	Elliott
ESI	Electro spray ionisation
EtOAc	ethyl acetate
EtOH	ethanol
Euc	eucalyptol
eV	electron volt
F3’5’H	flavonoid 3’,5’ hydroxylase
F3’H	flavanoid 3’ hydroxylase
F3βH	flavanone 3-β-hydroxylase
F6P	fructose-6-phosphate
FAE	fatty acid elongase
FCP	Free-choice profiling
FLS	flavonol synthase

Fru	fructose
G1P	glucose-1-phophate
G6P	glucose-6-phophate
GC	gas chromatography
GC-MS	gas chromatography - mass spectrometry
Gen	genistein
Glc	Glucose
GPA	General Procrustes Analysis
GPP	geranyl pyrophosphate
H_2O_2	hydrogen peroxide
HAc	acetaldehyde
HCl	hydrochloric acid
HDL	high density lipoprotein
Hex acid	hexanoic acid
Hexal	hexanal
Hexen acid	(E)-2-hexenoic acid
HPLC	high pressure liquid chromatography
HQT	hydroxycinnamoyl-CoA Quinate hydroxycinnamoyl transferate
i.d.	internal diameter
IFR	isofavone reductase
IFS	isoflavone synthase
Il	isoliquiritigenin
int	intensity
IOMT	isoflavone O-methyltransferase
IS	internal standard
kae-deox	kaempferol hexose deoxyhexoside
kae-mal	kaempferol hexoside-malonate
KCl	kalium chloride
LAR	leucoanthocanidin reductase
lar-glc	laricitrin-3-O-glucoside
LC-MS^2	Liquid chromatography-tandem mass spectrometry
LDL	low density lipoprotein
LDOX	leucoanthocyanidin dioxygenase)
LDP	linalyl pyrophosphate
LI	Liberty
Lim	D-limonene
Lin	linalool
Liq	liquiritigenin
L-Phe	L-phenylalanine
m/z	mass-to-charge-ratio
MA	malic acid
mal-ac	malvidin-3-(6-acetyl)-hexoside

mal-pen	malvidin-3-O-pentoside
MCoA	malonyl CoA
Med	medicarpin
MEK	2-butanone
MS	mass spectrometry
MS/MS	tandem MS
MSTFA	n-methyl-n-(trimethylsilyl)-trifluoroacetamide
MW	molecular weight
myrc	ß-myrcene
myr-hex	myricetin hexoside
NA	not applicable
NaCl	Sodium chloride
NaOH	sodium hydroxide
Nar	naringenin
Nc	naringenin chalcone
ND	not detected
NF	not found
NIST	National Institute of Standards and Technology
No.	number
O_2	oxygen
OA	organic acid
OCH3-CA	3- or 4-methoxy, 3- or 4-hydroxy cinnamic acid
OMT	o-methyltransferase
OZ	Ozarkblue
PAL	L-phenylalanine ammonia-lyase
PC	principal component
PCA	principal component analysis
PDA	photodiode array detector
PDMS	polydimethylsiloxane
Penal	pentanal
peo-pen	peonidin-pentose
PEP	phosphoenolpyruvate
PEPC	phosphoenolpyruvate carboxylase
pet-ara	petunidin-3-O-arabinoside
pet-hex	petunidin-3-O-hexoside
Phen	phenol
PLS	partial least squares
PPO	polyphenol oxidase
PTFE	polytetrafluoroethylene
PTV	programmable temperature vaporising
PU	Puru
que-ara	quercetin-3-O-arabinoside

que-glc	quercetin-3-O-glucoside
que-hex	quercetin hexoside
que-mal	quercetin hexoside-malonate
que-rut	quercetin3-O-rutinoside
r	Pearson's correlation coefficient
R^2	regression coefficient factor
RE	Reka
RI	retention index
ROS	reactive oxygen species
s	aroma descriptors
ShA	shikimic acid
SO_2	sulphur oxide
SP	Spartan
SPME	solid-phase microextraction
Suc-CoA	succincyl-CoA
sug	total sugar content
sug/OA	sugar to organic acid ratio
SUSY	sucrose synthase
t	taste descriptors
T-Anth	total anthocyanins
T-AO	total antioxidants
TCA cycle	tricarboxylic acid cycle
t-CA	trans-cinnamic acid
TO	Toro
TOF	time of flight
T-Phen	total polyphenols
t_R	retention time
U	unit
UDP glc	uridine diphosphate glucose
UDP	uridine diphosphate
UFGT	flavonoid glucosyltransferase
UV	ultraviolet
VR	vestitone
α-ketoglu	α-ketoglutarate
α-terpe	α-terpineol
α-terpo	α-terpinolene
λ	wavelength

Abstract

The consumption of blueberries in the UK is increasing, but currently most of the available blueberries are imported. There is therefore great potential for UK growers to supply high quality blueberries to meet the consumer preferences. At present little is known regarding specific UK consumer sensory preferences, the relationship between fruit metabolite and sensory profiles or the impact of UK climatic conditions on fruit metabolite profile in relation to sensory and health beneficial characteristics. The aim of this study was to understand the sensory expectations of UK consumers and the relationship between fruit phytochemistry and sensory profiles in order to provide underlying tools to assist in breeding of an elite UK adapted germplasm. Sensory tests were conducted on a range of currently grown cultivars. Free-choice profiling identified the range of descriptors used by consumers and subsequent descriptive profiling using the consensus vocabulary resulted in the generation of a multivariate product space describing relationships between the cultivars. The most important attributes were size, sourness and sweetness. The assessors had difficulties in differentiating between aroma, because of the limited flavour volatiles profile. Sugars, organic acids, polyphenols, anthocyanins, flavour volatiles, lipids and brix were quantified to establish links between phytochemicals and sensory character. Sugar and organic acids were the major determinants of sensory scoring for flavour characters. Limited genetic influences were observed in phytochemical content of individual cultivars grown in the same location, based on the similar pedigrees of the cultivars. Environmental influences were manifested as differences in the same cultivar across seasons or growing locations. This study highlights the need for the development of a more diverse product offering. In the short term, further research on the environmental impact on blueberry chemistry should be conducted leading to the development of growing practices that enhance metabolites associate with favourable sensory properties. In the longer term breeding of flavour intensive cultivars is required and the work presented here linking fruit chemistry to sensory properties provides a valuable resource for the indirect breeding of varieties with an appropriate sensory profile.

Chapter 1

Introduction

1.1 Market review

Blueberries (*Vaccinium* spp.) belong to the large group of soft fruits with blueberry production becoming globally the second most economically important soft fruit after strawberry (Giongo et al., 2013).

The blueberry plant is native to North America where it is widely cultivated with Canada and the USA accounting for approximately 85 % of global production (Carew et al., 2005). In addition to the North American centres of cultivation, blueberries are commercially cultivated in South America (Bañados, 2006), parts of Asia (Yu et al., 2012, Tamada, 2009), New Zealand (Buck et al., 2012) and across a number of European countries including Poland, Germany, the Netherlands and France (Prodorutti et al., 2007). The all-year global market depends on northern (USA and Europe) and southern (mainly Chile and Argentina) centres of production, consequently the storage ability is an essential factor for the global retail market (Giongo et al., 2013).

The commercial sale of blueberry was launched in the early 1800's however the market for blueberries has yet to mature in many parts of the world (Wood, 2004). According to statistics obtained from the UN Food and Agriculture Organisation (http://faostat.fao.org) blueberry production showed a growing trend in the USA and Canada over the first decade of this century rising from a combined harvest of approximately 195,000 tons in 2000 to 309,000 tons by 2011. In other key production centres the last decade saw mixed fortunes with some areas such as

Poland seeing steep production declines from a peak of approximately 30,000 tons in 2001 to a minimum of less than 5,000 tons by 2006, although production had recovered to 10000 tons by 2010 (FAO Stat).

In recent years global highbush blueberry production raised by nearly 70 % between 2008 and 2012 primarily due to the fast increase of acreage in North America, Europe and Asia (Table 1.1) (Brazelton, 2013). Much of the success of the blueberry industry in North America can be ascribed to the extensive research and marketing effort surrounding the fruit. North America produces over 70 % of the global highbush blueberry crop (Table 1.1). Since 2008 the production of fruit for the fresh and process increased by an average of 20 % every two years. Between 2005 and 2012 the acreage under cultivation increased from 71075 acres to 123635 acres (Brazelton, 2013). This expansion was supported by governmental funding amounting to in excess of £1.8 million between the years 2000 and 2003, an amount almost matched via non-federal channels with the majority of funds directed towards breeding, and pest and disease management (Carew et al., 2005). In addition to this funding, considerable research has been undertaken into the potential health benefits of blueberry consumption. It has long been established that blueberries exhibit a high antioxidant capacity and anthocyanin content in relation to similar fruits (Moyer et al., 2002). More recently, studies in animal models and human intervention trials have demonstrated positive benefits of blueberry consumption in relation to activity against certain tumors (Adams et al., 2011), protection against neurodegeneration (Krikorian et al., 2010), protection against heart disease (Ahmet et al., 2009) and in the prevention of type II diabetes (Stull et al., 2010). These findings have been skillfully used by marketing groups to grow the appeal of blueberries within Northern America correlating with an increase in average consumption of fresh fruit from approximately 120 g per person per annum in 1995 up to 250 g in 2005 with 45 % of US consumers purchasing the fruit in 2008 (Hummel et al., 2012). Growth in consumption continues with more recent figures published by the North American Blueberry Council putting US fresh per capita consumption at approximately 500 g in 2010 (Anonymous, 2011).

Table 1.1. Global fresh, processed and total highbush blueberry production in 10^3 tons within the last three years (2008 - 2012).

	2008			2010			2012		
	Fresh	Process	Total	Fresh	Process	Total	Fresh	Process	Total
North America	45.72	61.76	185.6	135.27	84.06	219.33	152.77	114.91	267.68
South America	18.35	10.28	51.4	61.19	7.29	68.48	85.06	36.54	121.6
Europe	10.85	0.88	25.17	30.64	5.58	36.22	40.43	3.42	43.85
North Africa	0.16	0	0.36	0.97	0.02	1.00	2.42	0.04	2.46
South Africa	0.22	0.20	0.69	0.94	0.09	1.03	1.37	0.22	1.59
Asia & Pacific	2.45	2.11	7.59	7.28	2.77	10.04	16.95	4.54	21.48
World Production	174.15	96.65	270.8	236.29	99.82	336.12	299.02	159.69	458.66

The European highbush blueberry market is still small, but production accelerated in recent years; between 2008 and 2012 production grew by almost 75 % from 25178 to 43840 tons. This was underpinned by an increase in the acreage under production which grew by nearly 150 % from 9736 to 24101 acres between 2005 and 2012. The main European producers for highbush blueberries are Poland, Spain, Germany, The Netherlands, Italy, France and the UK (Figure 1.1). Spain, Germany and Poland are the largest producers (30980 tons) of highbush blueberries in Europe accounting for 70 % of total production (43840 tons), mainly for the fresh market (Brazelton, 2013).

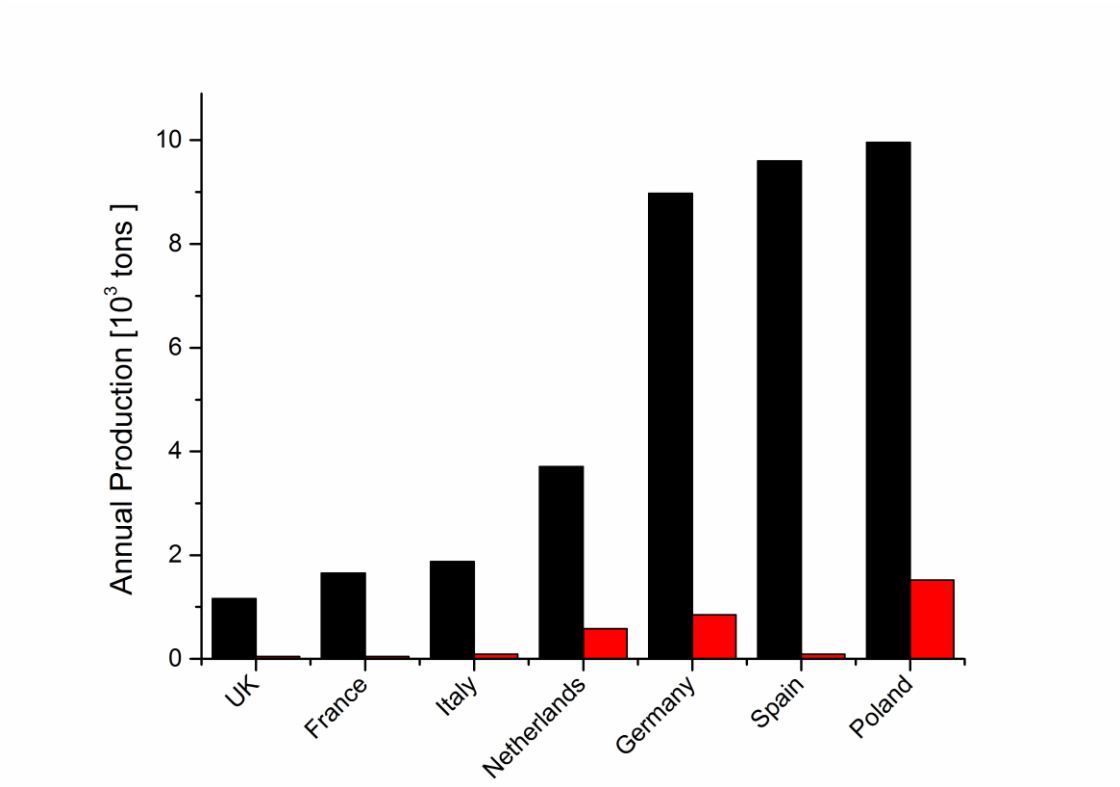


Figure 1.1. Leading European countries for fresh and processed annual highbush blueberry production in 2012. Fresh production refers to ■ and processed production refers to ■.

In the UK, statistics specific to blueberries are difficult to obtain however, according the UK Department of the Environment, Food and Rural Affairs (DEFRA) there was a huge increase in imports of soft fruit excluding strawberries, raspberries and blackcurrants from 4200 tones with a value of approximately £8 million in 2000 to 25400 tons with a value of £128 million by 2009. This was accompanied by a modest increase in UK production of blueberries from 6200 to 9400 tons with the value doubling from approximately £13 million to £26 million over the same period. The

UK is the leader for blueberry consumption in Europe (Brazelton, 2013). In fact, UK consumer demand for blueberries is at record levels and as the production data suggest UK growers are currently far from being able to meet current demand (McCallum et al., 2012) representing a potential opportunity for UK soft fruit growers. UK retailers are looking for quality, traceability and transparency to the consumer. In the last ten years the UK has moved from no commercial highbush blueberry production to production of nearly 800 acres. 30 – 40 % of the blueberry production is in containers because of the difficulties growing in soil. It is important to adapt the available varieties to the growing conditions. Growers reported that the soil and the short growing season make it difficult to reach high yields (Brazelton, 2013).

1.2 Emerging market

As outlined above, global blueberry production is rising dramatically however, growth over the next five years is unlikely to be as rapid as over the previous five years due to global weather changes, old fields, labour challenges and disease pressure. Diseases in blueberry are increasing; however the possibilities to fight against these diseases are not rising similarly. It is often difficult to recruit sufficient labour to harvest all available fertile crops. The changes in global weather may contribute to the improbability of desired crops in all growing regions. Extreme weather conditions in certain growing regions leads to beneficial crops in other growing regions. Regions with rapid growth are contributing to underperformance because of various reasons. For instance, fields at weaker sites are used due to limited field space. Furthermore poor varieties are planted and growing experience is often limited. Therefore the likehood of underperformance is higher in newly developing growing regions. New processed products with a longer self-life that do not require maintenance of the cool chain are expected to be a major growth market however, global demand will still depend on the quality of the product (Brazelton, 2013). Such products could present an opportunity to provide blueberry products outside the harvesting season in UK. Unlike Brazelton (2013), Garner (2013)

estimated that the growth trends observed between 2008 and 2012 are likely to continue 2017 due to increases in emerging and developing markets in China, Europe and South America. China is increasing the acreage to meet the increasing demand for the blueberry (Garner, 2013) and it is predicted that the leading market for blueberries will be China in ten years with the largest consumption of blueberries in the world. This growing demand is likely to lead to problems in China producing sufficient volume of fruit for a competitive price and quality in the next five to ten years. This represents a potential opportunity for other countries such as the United States and South America which could gain the blueberry production for China. The North American Blueberry Council World Blueberry Acreage and Production Report published the global blueberry production forecast (Figure 1.2) (Brazelton, 2013).

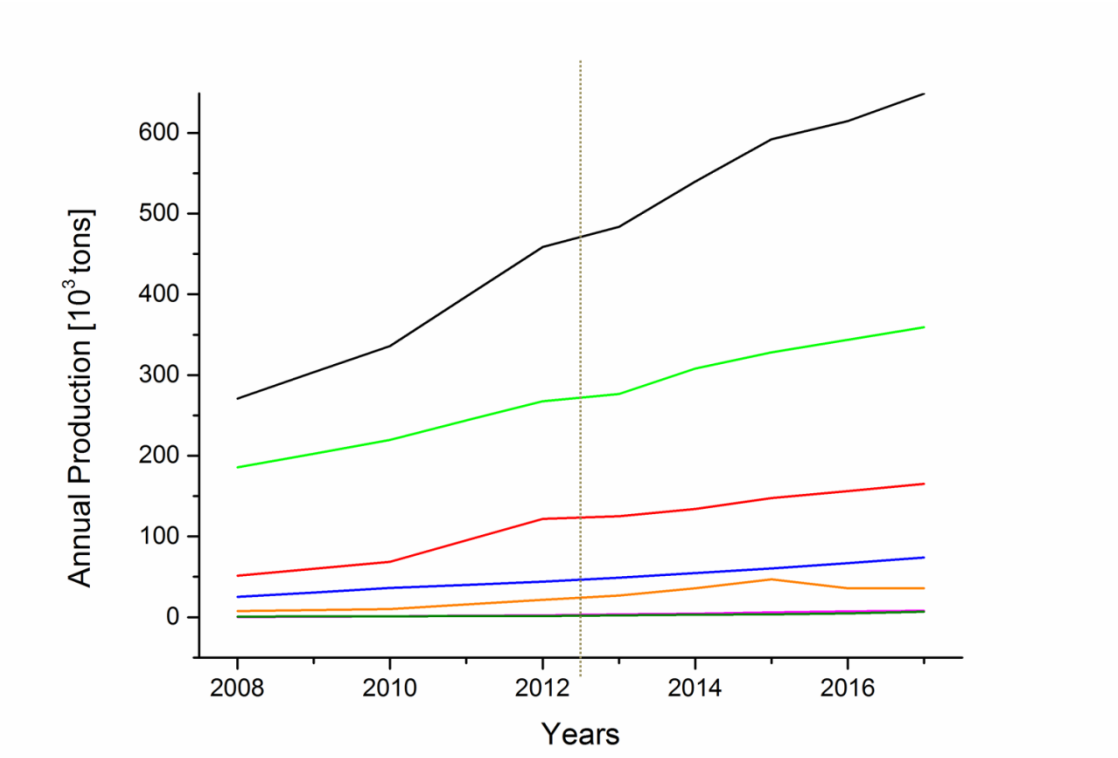


Figure 1.2. Prediction of global highbush production within the years 2008 – 2017 adapted from Brazelton (2013). World production refers to ■, North America refers to ■, South America refers to ■, Europe refers to ■, Asia & Pacific refers to ■, North Africa refers to ■, South Africa refers to ■.

The global blueberry production is suggested to increase by over 165180 tons through to 2017 (Table 1.2). The largest increase will be achieved in North America which is expected to account for 50 % of the growth globally. The annual production

will grow by 7.7 % with the largest increase in southern Africa and northern Africa by 300 % and 225 %, respectively. The European market is predicted to grow in the next years by 50 % (Brazelton, 2013).

Table 1.2. Prediction of highbush blueberry production in 10^3 tons within the years 2013 - 2017.

	2013	2014	2015	2016	2017
North America	276.79	308.04	328.13	343.75	359.38
South America	125	133.93	147.32	156.25	165.18
Europe	49.11	54.46	60.27	66.96	73.66
North Africa	3.57	4.46	5.8	7.14	8.04
South Africa	2.23	2.9	3.57	4.91	6.7
Asia & Pacific	26.79	35.71	46.88	35.71	35.71
World Production	483.48	539.51	591.96	614.73	648.66

An overview regarding the forecast of the highbush blueberry market is shown in Figure 1.3. In detail, South America especially Brazil, Mexico, Colombia and Chile will be an important market for blueberries. New blueberry growing regions like China, Romania, Republic of Georgia, Mexico and Peru will contribute to the growing blueberry production. The existing growing regions are likely to attempt to improve production by using new technologies and improved cultivars. Mainland Europe is entering blueberry production and it is likely that the European market will see a steady increase in consumption. The Pacific Northwest coast of America is expected to lead the highbush processed market. China, Korea, Taiwan, India, Middle East, Russia, Brazil, Mexico, Republic of South Africa, Saudi Arabia, United Arab Emirates are suggested to represent new sales markets. Growing regions like South America could cover the demand of blueberries in the UK outside the growing season (Brazelton, 2013).

Figure 1.3. Summary about the global highbush blueberry market, blue stars indicate the existing market, yellow the growth market and white the developing market (Brazelton, 2013).

1.3 Breeding program

Blueberry is a low-growing shrub, which belongs to the *Vaccinium* genus (Family Ericaceae) with 150 to 450 species, native to North America and widespread perennial flowering plants (Luby et al. 1991). The *Vaccinium* genus can be divided further in sections. The predominant section in terms of blueberry production is cyanococcus, which includes the three most commonly cultivated species, the wild lowbush (*Vaccinium angustifolium* Ait.), highbush (*Vaccinium corymbosum* L.) and rabbiteye (*Vaccinium ashei*). According to winter hardiness and chilling requirements the highbush cultivars are further divided into southern and northern types; the northern cultivars are grown in a colder climate than southern cultivars and also more highly represented in retail (Hancock, 2008).

1.3.1 Fruit quality

The key parameters relating to premium character are flavour, large size, light blue color, small scar of the pedicel detaches and long storage life (Hancock et al., 2008). In addition to these attributes uniform shape, high aroma and retained texture during storage are key quality traits. Beside the flavour and appearance, extended storage time, better disease resistance, and the capacity for mechanical harvesting are

important requirements for northern highbush blueberry cultivars. Different native species can be used to introduce the relevant traits into breeding programs (Hancock et al., 2008). V. *corymbosum* exist as diploid and tetraploid, (Draper and Scott 1971, Krebs and Hancock, 1989). Crosses between different sections could achieved the best fruit quality, but intersectional crosses occurred difficulties in blueberry breeding (Hancock et al., 2008). Nevertheless the following intersectional crosses could improve the fruit quality. The best fruit quality of native species in US terms will be achieved by the highest soluble solids (V. *angustifolium*, V. *pallidum* and V. *stamineum*), lowest titratable acidity (V. *ashei*, V. darrowii, V. *myrsinites* and V. *pallidum*), largest size (V. *ashei* and V. corymbosum), smallest scar (V. *elliottii*), easiest detached fruit (2x V. *corymbosum*, V. *darrowii*, V. *elliottii*, V. *myrtilloides* and V. *tenellum*) and most firm (V. *darrowii*, V. *tenellum* and V. *ashei*) (Ballington et al., 1984). V. *darrowii* is the most suitable species for the following characteristics blue color, intense flavour and good fruit condition in hot weather (Draper and Hancock, 2003, Ballington, 2001, Ehlenfeldt and Prior, 2001). Surprisingly, crosses of light-blue genotypes tend to produce black progeny. In general hybrids from V. *angustifolium* are softer and V. *darrowii* firmer (Martin, 2002). The fruit of most progeny in hexaploid families of V. *ashei* and V. *ashei* x V. *constablei* are appreciated for color, fruit scar, firmness and flavour (Ballington et al., 1986). The hybrid of V. *constablaei* produces small fruits, but gives the best result for machine harvesting (Hancock et al. 2008).

1.3.2 Disease and pest resistance

Blueberries contract a wide range of diseases. Diseases are dependent on the species and external influences like weather conditions.

In northern highbush cultivars virual diseases are the most significant problem (Hancock et al., 2008). In addition to disease, significant damage to highbush blueberries can be caused by insects and arthropods such as blueberry maggot (*Rhagoletis pomonella* Walsh), blueberry gall midge (*Dasineura oxycoccana* Johnson), blueberry bud mite (*Acalitus vaccinii* Keifer), flower thrips (*Franklinellia* ssp.), Japanese beetle (*Popillia japonica* Newman), sharp-nosed leafhopper which is a vector for virally transmitted stunt (*Staphytopius magdalensis* Prov.), blueberry

aphid (shoestring and blueberry scorch virus vector) (*Illinoia pepperi* Mac. G.), cranberry fruit worm (*Acrobasis vaccinii* Riley), cherry fruit worm (*Grapholita packardi* Zell), and the plum curculio (*Conotrachelus nenuphar* Herbst). A large group of southern highbush blueberries has resistance to blueberry gall midge and many cultivars are resistant to blueberry bud mite (Lyrene, 2008). Lowbush and rabbiteye blueberries are more sensitive to pests (Hancock et al., 2008), therefore intersectional crosses to V.*ashei* or V. *elliotti* may contribute to a resistance to leafhoppers due their non-feeding preference to these species (Meyer and Ballington, 1990).

1.3.3 Environmental influences

An important factor in blueberry breeding programs is increasing the harvesting period. Early cultivars have been developed by selecting earlier bloom dates and shorter ripening periods, but the earliness of bloom date is limited by the potential for frost damage (Hancock et al., 2008). Ripening interval is correlated to crop load to obtain high yield and uniformly ripe genotypes (Luby and Finn, 1986). Soil pH and tolerance to mineral soil are the main abiotic drivers for blueberry breeding (Chandler et al., 1985), while progenies of highbush blueberries demonstrated a significant high variation in pH tolerance (Hancock et al., 2008). Rabbiteye blueberries can tolerate higher temperature and drought better than northern highbush blueberries (Hancock et al., 2008).

1.4 Fruit ripening

Fruit ripening starts by pollination and fruit set. Ripening is characterized by physical and chemical changes that lead to attractive and palatable fruits, such changes affecting flavour, texture and aroma (Gortner et al., 1967). Ripening can be divided into three ripening stages, pre-maturation, maturation and senescence (Ismail and Kender, 1974).

The first berry growth stage appears in the pre-mature berry which starts at fertilization and can take thirty to sixty days (Ismail and Kender, 1974, Bell, 1957). Cell tissue undergoes cell division immediately after fertilization, resulting in endosperm and zygote formation; these changes increasing the berry diameter. The second growth stage also occurring in pre-mature berry, is characterized by the embryo growing to penetrate the endosperm plug and initiate seed development (Bell, 1957). During this stage colour does not change and berry diameter rises more slowly than in the initial stage of endosperm formation. The length of this stage depends on the berry size with large berries completing the second stage after eight days but smaller berries taking up to 25 days. The third growth stage occurs in the mature berry. In this stage, berries obtain their full size due to a rapid increase in the volume of pericarp tissue and water uptake (Ismail and Kender, 1974). Size changes are associated with colour changes, from green to blue through red and biochemical changes for instance pH, sugar composition and soluble solid content. Similar to the second stage, the duration of the third stage is dependent on berry size (Ismail and Kender, 1974).

The last developmental stage is ripening and ultimately senescence (Ismail and Kender, 1974, Bell, 1957) with senescence associated with a drastic reduction in the firmness of berries (Ismail and Kender, 1974). These developmental stages are also associated with significant changes of metabolites in berries that are described in the following sections.

Although berry development follows a similar programme independently of environmental conditions, factors such as latitude and prevailing climate influence the rate of fruit development. Growth in northern areas are slightly behind of growth in southern regions (Hall et al., 1979). But regional microclimates can mask this tendency between growing areas, which are less than one hundred kilometers apart (Bell, 1957). Light, temperature and moisture influence on blueberry development and ripening are further described under section 1.5.2.

1.5 Flavour quality of blueberries

1.5.1 Metabolites in fruits

Metabolites in fruitsDuring the ripening process of fruit there are profound biochemical and physiological changes associated with the shift in function from an organ providing a protective environment for the development and ripening of seeds to one which acts in seed dispersal. The main climatic factors that have an impact on ripening are temperature, light and soil moisture with warmer conditions stimulating faster ripening (Hall and Aalders, 1968). Pigmentation is dependent on anthocyanin accumulation which in turn is dependent on the exposure to light during growing (Zhou and Singh, 2002). The first growth stage is especially important for the anthocyanin production; the following growing stages increase the anthocyanin accumulation and change the accumulated anthocyanin compounds (Vvedenskaya and Vorsa, 2004). Moisture has an impact on berry acidity and firmness; harvesting in wet conditions produces softer berries (Seymour et al., 2004).

Key changes associated with this shift in function include fruit softening, color change, changes in carbohydrates, organic acids, lipids and flavour volatiles (Eccher Zerbini, 2008, Lizada, 1993). The processes that are associated with ripening are important for fruit quality and consumer acceptance. Beside firmness, sugar and organic acid accumulation, flavour volatiles, pigment production and size all contribute to quality. All of these quality attributes are dependent on genetic, environmental and agronomic factors (Steyn et al., 2002).

1.5.1.1 Cell wall degradation

Modifications of cell wall polymers contribute to softening in blueberries (Cantu et al., 2008). Most of the firmness changes are observed between green and red stages in highbush blueberries (Figure 1.4) (Proctor and Peng, 1989). During blueberry ripening the firmness of fruit decreases in parallel with the cell wall yield (Vicente et al., 2007). Figure 1.4 illustrates the cell wall changes that contribute to changes in fruit firmness during ripening.

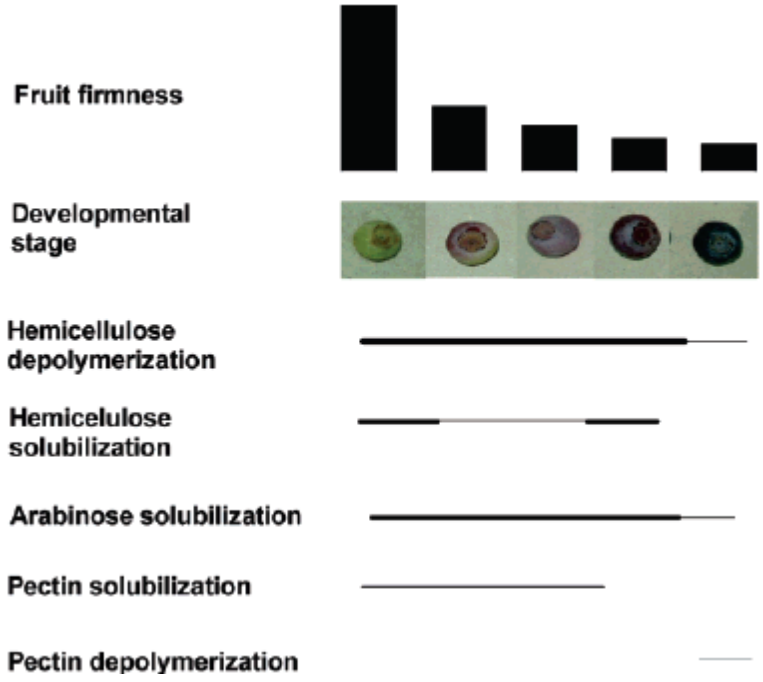


Figure 1.4. Fruit firmness and associated changes in cell wall composition during ripening in blueberry fruit, the degree of the line thickness correspond with the proposed reaction during ripening in blueberries (Vicente et al., 2007).

During blueberry development the main modifications in the cell walls are changes in hemicelluloses, which decrease in polymer size and increase in solubility (Vicente et al., 2007). The dimension of pectin polymer degradation varies between fruits (Brummell, 2006). Large changes in the polymer size of pectin were shown in avocado and peach (Huber and O'Donoghue, 1993, Brummell et al., 2004), while smaller changes of pectin sizes occur in tomato (Huber and O'Donoghue, 1993), and even less in blueberries (Vicente et al., 2007). Similar to blueberries other fruits like peppers, banana, apples and some strawberry cultivars demonstrated minor changes in pectin size (Brummell, 2006, Vicente et al., 2007, Huber, 1983). Softening during ripening may occur rather by modifications of the hemicelluloses than by a depolarization of pectin (Vicente et al., 2007). Treatment with calcium chloride enhanced the firmness of blueberries (Hilz et al., 2005) that was associated with decreased pectin solubilisation (Vicente et al., 2007). It is likely that the formation of ionic bridges between calcium and the free carboxylic groups of the galacturonic acid in pectin results in less accessibility of the polymers to depolymerizing enzymes (Figure 1.5). In addition to its impact on pectin solubilisation, calcium treatment

reduces indirectly hemicellulose degradation by reduction of the access of hemicellulolytic enzymes to substrate in fruit that have lower levels of pectin solubilisation (Vicente et al., 2007). Conversely, Angeletti (2010) reported that the hemicellulose content wasn't influenced by calcium treatment, despite the fact that these treatments resulted in a decrease in the solubilisation of pectin.

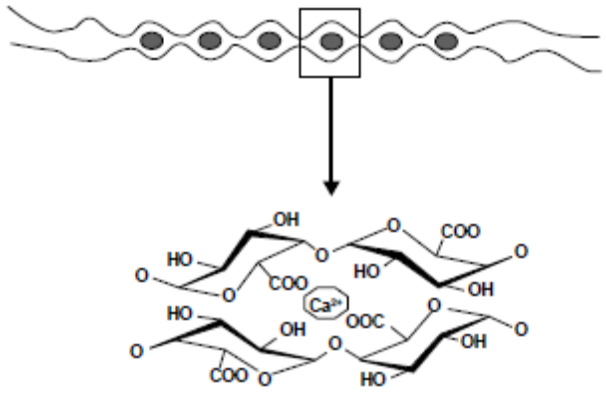


Figure 1.5. Schematic structure of calcium crosslinking to galacturonate and the polygalaturonate sequences (Raj et al., 2012).

The pectin depolymerisation may have an minor effect on fruit softening (Vicente et al., 2007). Studies in tomato exhibited that pectin depolymerisation might cause the softening during the overripe stage (Brummell and Harpster, 2001). In summary, the depolymerisation of the main polysaccharides pectin, hemicellulose and cellulose lead to fruit softening during maturation. The level of depolymerisations varies between cultivars. At early ripening stages the pectin solubilisation contributes to the main cell wall modifications; especially the cell wall matrix polysaccharides such as pectin go through discruptions. These modifications are proposed to be responsible for the reduction in tissue firmness (Tucker and Grierson, 1987). Pectin is the main uronate containing polymer that are dominant in galacturonate, rhamnose, galactose and arabinose. During ripening the loss of arabinoside and galactoside side chains from pectin occurs resulting in increased solubility and decreased molecular weight (Seymour et al., 1990, Redgwell and Fischer, 2002). Only the hemicellulosic polymers are depolymerized in blueberries (Vicente et al., 2007).

1.5.1.2 Sugar and organic acid accumulation

Sugars and organic acids contribute extensively to flavour quality via their substantial impact on sweetness and sourness. Sweetness and sourness are important for the organoleptic character with consumer preference being highly dependent on the sugar/acid (sug/OA) ratio. A low level of citric acid (CA) produces less tart fruit, but the absence of acids produces bland fruits while low levels of both sugars and organic acids produce tasteless fruits (Kader, 1991). An appropriate level of CA is therefore required to produce desirable fruits, because the acids influence the sweetness perception. Studies on sweetened tomato puree have shown, that CA masks the sweetness (Schifferstein and Frijters, 1990, Baldwin et al., 2008). Beside size and colour, sweetness and sourness have a large impact in sensory fruit quality. Sugar and organic acids accumulation varies between species, developmental stages and tissue types. In general malic acid (MA) and CA are the predominant organic acids in blueberries (Ehlenfeldt et al., 1995), and contribute to several cellular functions (Sweetman et al., 2009).

Sucrose is produced by photoassimilation and is transported from photosynthetic leaves to the developing fruit (Walker and Thornley, 1977). In tomato it has been demonstrated that the sucrose concentration gradient between leaves and fruit regulates the portion of sucrose import (Wang et al., 1993). In fruit sucrose synthase (SUSY) and acid invertase cleave imported sucrose into its component hexoses thus maintaining fruit sink strength (Wang et al., 1993) (Figure 1.6). In detail, acid invertase metabolises sucrose into fructose (Fru) and glucose (Glc) and SUSY metabolises sucrose into Fru and uridine diphosphate glucose (UDP glc). It seems that SUSY plays the dominant role for the metabolism of imported sucrose in tomato fruits (Wang et al., 1993).

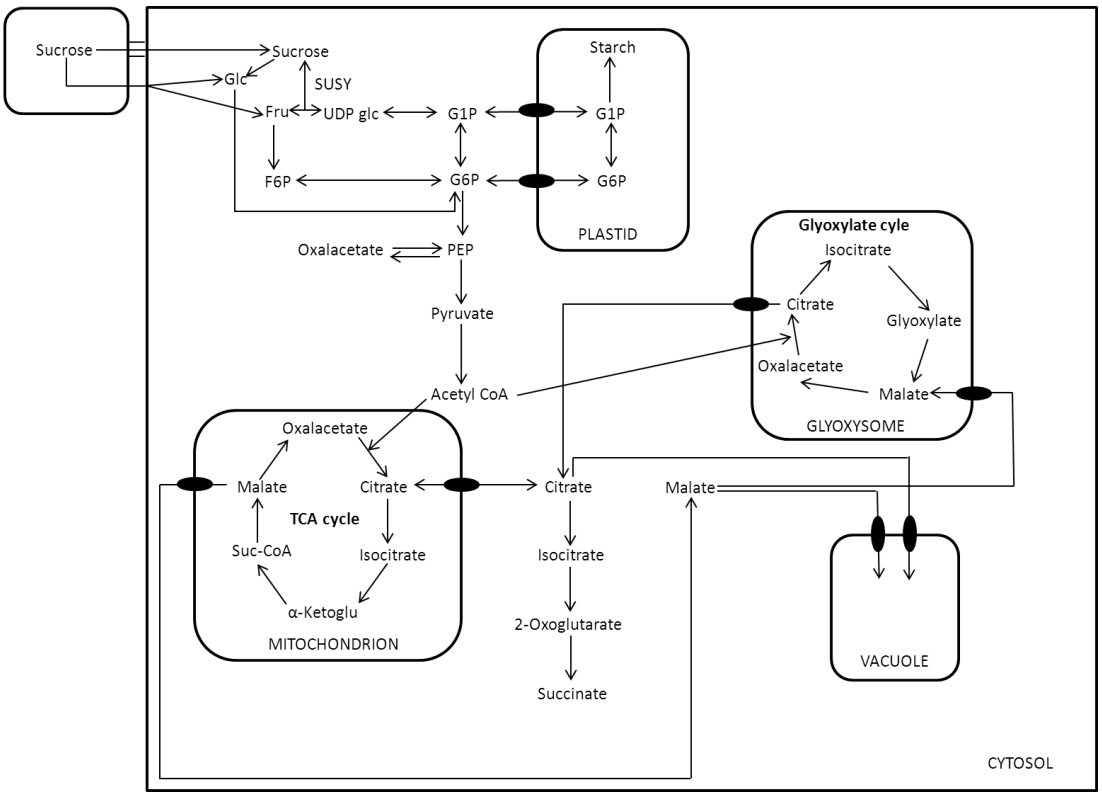


Figure 1.6. Biosynthesis pathway of involving malate and citrate in the fruit cells. Glc, glucose; Fru, fructose; UDP glc, uridine diphosphate glucose; SUSY, sucrose synthase; F6P, fructose-6-phosphate; G1P, glucose-1-phosphate; G6P, glucose-6-phophate; PEP, phosphoenolpyruvate; α-ketoglu, α-ketoglutarate; Suc-CoA, succincyl-CoA; TCA cycle, tricarboxylic acid cycle.

CA and MA are formed in the tricarboxylic acid (TCA) and glyoxylate cycles and stored in the vacuole (Moskowitz and Hrazdina, 1981), while during ripening these organic acids were transported out of the vacuole to be available for a range of pathways like TCA cycle, gluconeogenesis, respiration, amino acid interconversion, ethanol fermentation and production of complex secondary compounds like polyphenols (Famiani et al., 2000, Farineau and Laval-Martin, 1977). A high level of phosphoenolpyruvate carboxylase (PEPC) activity increases malate accumulation during early fruit development and decreases the malate accumulation during ripening (Diakou et al., 2000, Guillet et al., 2002, Moing et al., 2000). During the green stage of fruit development large amounts of sugar from photoassimilation are available for the formation of MA by glycolysis and then the production of CA via the TCA cycle. A high sugar supply during growing contributes to the production of larger fruit with a resulting higher respiration rate due to a high source to sink ratio. An increase of fruit respiration rate may facilitate glycolysis and the transformation

of malate into citrate due to a high supply of sugars (Etienne et al., 2013). Conversely during ripening sugars may no longer be available for respiration, as they are diverted to the vacuole for storage (Coombe, 1976). Therefore at the later stages the main respiratory substrates are organic acids dominated by citrate (Lobit et al., 2003, Wu et al., 2007).

CA and MA decrease when the temperature is raised during fruit growth in grapes (Gautier et al., 2005, Wang and Camp, 2000) and banana (Bugaud et al., 2009). The modified enzyme activities might influence the reaction rate of glycolysis and TCA cycle (Araújo et al., 2012). Temperature influences the sugar and organic acid accumulation in plants, due to a reduced malate accumulation. The impact of temperature on the fruit acidity depends on the fruit cultivar and species (Wu et al., 2007).

1.5.1.3 Fruit phenolic compounds

Fruit antioxidant capacity is influenced by the total anthocyanin (T-Anth) content and the total polyphenol (T-Phen) content which in turn is influenced by genotype, environmental and climatic conditions, soil type, geographic location, maturity and the storage conditions (Connor et. al 2002a, Bravo 1998).

The major source of the high content of antioxidant capacity in blueberry is from the polyphenols rather than from ascorbic acid, which is generally low in blueberries (Kalt and Dufour, 1997). T-Phen and T-Anth contents are two to three fold higher in lowbush cultivars (Giovanelli and Buratti, 2009) than in highbush blueberries. Fruit skin contains higher levels of anthocyanins and polyphenols than the flesh with the result that larger genotypes have a lower antioxidant capacity due to smaller relative surface area of skin in relation to pulp (Cho et al., 2004). The antioxidant capacity of flavonoids depends on the degree of glycosylation and the substituted groups of anthocyanins (Wang et al., 1997). Cyanidin-3-O-glucoside is an example of phenolic glycosylation shown in Figure 1.7.

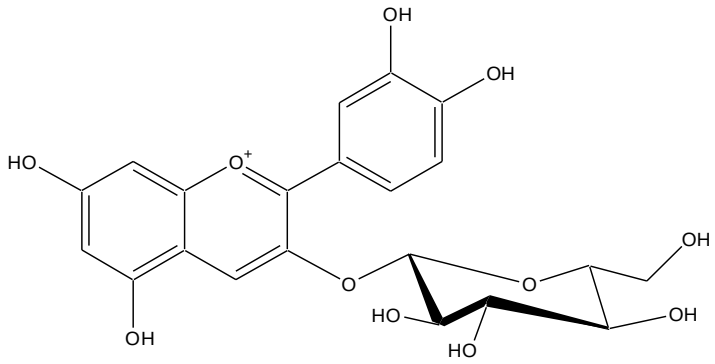


Figure 1.7. Structure of cyanidin-3-O-glucoside.

Colour is an important quality index for consumer preferences (Sapers et al., 1984). Beside the pigmentation, anthocyanins attract pollinators, block UV light, protect against fungal diseases, decrease stress damage and contribute to flavour. Anthocyanins contribute to astringent, bitter and unenjoyable taste (Koes et al., 1994). The colour depends on the type and amount of anthocyanins in plants (Koes et al., 1994) and the changes during maturity occur by the degradation of chlorophyll and the production of anthocyanins and carotenoids (Kalt et al., 2003).

The amount of anthocyanins depends on the blueberry species with lowbush and rabbiteye blueberries having a significantly higher content than highbush blueberries, because both the flesh and skin accumulate anthocyanins in lowbush and rabbiteye blueberries (Lohachoompol et al., 2008, Kalt et al., 2001). The predominant anthocyanidins are delphinidin and malvidin followed by petundin, cyanidin and peonidin, mainly in 3-monoglycosylated forms (Rodriguez-Mateos et al., 2012, Cho et al., 2004, Gao and Mazza, 1994). The more polar anthocyanins (cyanidin, delphinidin) are more active and less stable antioxidants (Fukumoto and Mazza, 2000). The level of anthocyanins depends on genetic and environmental influences and on ripeness (Connor et al., 2002a, Prior et al., 1998). Different structures of anthocyanins have an impact on the colour and antioxidant capacity (Wang et al., 1997) with the antioxidant capacity dependent on the degree of glycosylation and the number of hydroxyl groups (Velioglu et al., 1998, Boelens, 1991).

Beside anthocyanins, phenolics, including quercetin, kaempferol, myricetin, chlorogenic acid and procyanidins are responsible for the high antioxidant capacity of blueberries (Prior et al., 2001, Sellappan et al., 2002) and they are strongly

correlated to the antioxidant capacity (Prior et al., 1998). Chlorogenic acid is the predominant phenolic acid (Rodriguez-Mateos et al., 2012). In crushed blueberries polyphenol oxidase (PPO) expedited the degradation of anthocyanins and chlorogenic acid can stimulate this reaction (Kader et al., 1997) suggesting that low chlorogenic acid varieties may be preferred for the processing market.

The anthocyanin and phenolic content and the antioxidant capacity of blueberries varies by genotype, environment (Prior et al., 1998, Connor et al., 2002a, Moyer et al., 2002, Kalt et al., 2001), maturity (Prior et al., 1998, Moyer et al., 2002) and postharvest storage conditions (Kalt et al., 1999, Connor et al., 2002b). The content of phytochemicals within genotypes differentiates between various factors like maturity, harvesting days. Some genotypes differ in phenolic content and antioxidant capacity among environmental differences (Howard et al., 2003). Therefore biotic (e. g. herbivory, disease) and abiotic parameters (e. g. temperature, moisture irradiation) influence the phenolic content in plant. One of the key drivers for the environmental influence on T-Phen content appears to be the expression of genes encoding phenylalanine ammonia lyase, a key enzyme controlling entry to the phenylpropanoid biosynthetic pathway (Figure 1.8) (Jones and Hartley, 1999).

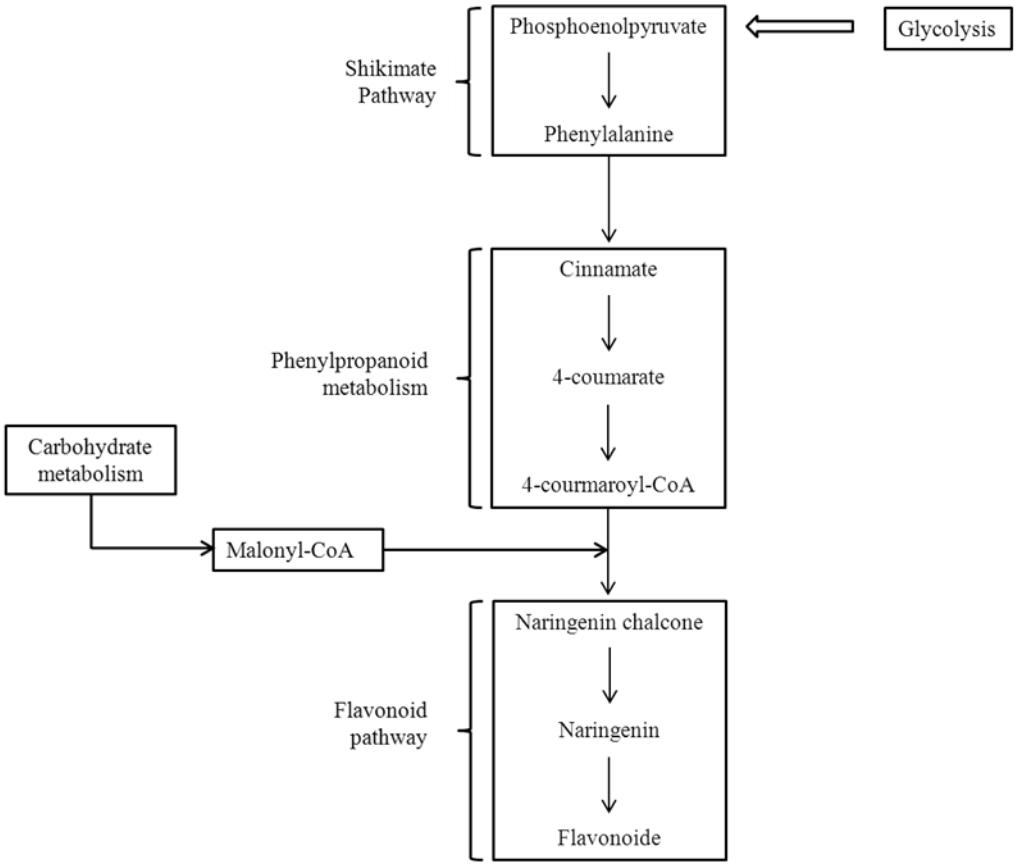


Figure 1.8. Biosynthesis pathway of flavonoids (Dixon et al., 2002).

The total antioxidant (T-AO) capacity was more highly correlated to T-Phen than to T-Anth contents (Moyer et al., 2002, Sellappan et al., 2002). Similar to anthocyanins, flavonols occur mainly in the 3-O-monoglycosylated form (Cho et al., 2004) and the levels of flavonols in smaller fruits are higher than in larger fruits (Prior et al., 1998). Flavonoids are secondary metabolites that are present in stems, leaves, flowers and fruits (Koes et al., 1994) and can be classified into flavones, isoflavones and anthocyanins. Phenolics have an aromatic ring with different numbers of hydroxyl groups (Taiz and Zeiger, 2006). All flavonoids are produced from phenylalanine and acetyl-CoA, products of primary plant metabolism. These compounds are important substrates in primary and secondary metabolism.

Figure 1.8 illustrates the biosynthetic pathway of phenolic compounds in plants. The pathway starts with the production of phenylalanine in the shikimate pathway, the following phenylpropanoid metabolism forms 4-courmaroyl-CoA. In the malonic acid pathway acetyl CoA converts to malonyl-CoA. 4-courmaroyl-CoA and malonyl-

CoA forms naringenin chalcone in the flavonoid pathway. The resulting phenolics are dependent on the substrate availability and external signals (Dixon et al., 2002, Ryan et al., 1999).

1.5.1.3 Flavour volatiles

Flavour volatiles contribute to flavour quality (taste, smell). The flavour volatiles profile varies dependening on the blueberry type; highbush blueberries (*Vaccinium corymbosum*) produce mainly hydrocarbons, esters, terpenes and long chain alcohols while esters and alcohols predominate in lowbush bluerries (*Vaccinium angustifolium*) (Forney, 2001) and terpenes, C_6 aldehydes and alcohols in rabbiteye blueberries (*Vaccinium ashei*) (Horvat and Senter, 2006). The aroma components (E)-2-hexenal, (E)-2-hexenol, (Z)-3-hexenol, linalool and geraniol were found in different amounts between species and are described as the typical aroma compounds in blueberries (Horvat and Senter, 2006, Hirvi and Honkanen, 1983). The C_6 flavour volatiles are mainly described in fruit as green and grassy; linalool and geraniol are described as floral; terpinolene as herb-spicy, octanol and nonanal, α-terpinene, p-cymene, D-limonene and geranial as citrus– and lemon-like (Rohloff, 1999, Du et al., 2012). The type and quantity of flavour volatiles differs by genotype, maturity, pre- and postharvest environmental conditions and storage conditions (Forney, 2001). At the moment there is limited knowledge regarding the impact of these factors on the flavour volatiles. Although data specific to blueberry is limited it is well known that in soft fruit generally different genotypes exhibit different types and quantities of flavour volatiles. For example in strawberry up to 35-fold difference the total flavour volatiles between cultivars has been reported (Forney et al., 2000). Further it is well established that the flavour volatile profile and their quantity is strongly increased during ripening. Similar to strawberries, rabbiteye blueberries reduce the quantity of low molecular weight volatiles like (E)-2-hexenol, (Z)-3-hexenol, α-terpineol and β-caryophyllene and increase the higher molecular weight volatiles like linalool and geraniol during ripening (Horvat et al., 1996, Horvat and Senter, 2006).

Postharvest conditions have an impact the flavour volatile profile and their concentration. After harvesting the flavour volatile profile changes with new flavour

volatiles produced and others lost. Such changes have positive and negative impacts on the fruit quality. Harvesting is an important factor in obtaining a high quality fruit, because the harvesting of unripe fruits will never allow the full flavour profile to develop. The postharvest temperature has an impact on some flavour volatiles (Forney, 2001). Studies in strawberries have shown that the postharvest temperature has an impact on the composition of the flavour volatiles profiles. Additionally correct storage conditions increase shelf-life and prevent the production of off-flavours. Packaging strawberries under high concentrations of CO_2 and O_2 can start fermentation leading to the generation of volatiles like ethanol, acetaldehyde and ethyl acetate resulting in high concentrations off-flavours (Ke et al., 1994, Larsen, 1994), while studies in apples have shown that low levels of O_2 could influence some volatiles (Lidster et al., 1983). More than 10 % of CO_2 reduced the decay of fresh blueberries, but high levels of CO_2 may influence the flavour volatile profile (Forney, 2001). Bluecrop has shown a production of off-flavours and reduction of the flavour volatile profile at a CO_2 concentration above 20 %, compared to packages with less than 20 % CO_2 (Rosenfeld et al., 1999). Previous studies recommended for highbush blueberry cultivars a CO_2 concentration of 15 % in packages (Forney, 2001).

1.5.2 Impact of genetics and environment

Considerable research has previously been conducted regarding genetic and environmental influences on quality in fruits and vegetables. Many papers have described the influence of genetic and environmental diversity however, although a genetic linkage map has recently been published for highbush blueberry (Brevis et al., 2007) there are at present no reports of its exploitation with respect to breeding in the scientific literature. Previous studies demonstrated a genetic and environmental interaction of phytochemicals in blueberries (Connor et al., 2002a, Finn et al., 2003).

Seasonal conditions such as light, temperature and moisture have an impact on berry development and berry ripening. Sun exposure is an important factor for photosynthesis and anthocyanin accumulation (Gibson, 2011), but UV radiation may damage the deoxyribonucleic acid (DNA). Moisture is required for respiration and

nutrient transport, but a too high water supply may decay the root (Taiz and Zeiger, 2006). Temperature influences the fruit development, but frost may damage the floral buds during development (Hicklenton et al., 2002, Olson and Eaton, 2001).

Light

Light influences photosynthesis and facilitates the accumulation in plants (Krause, 2006), but there is not a linear relationship between light and photosynthesis. When the plant is light saturated, photosynthesis may affected due to photoinhibition (Taiz and Zeiger, 2006). The yield of highbush blueberries grown in Chile increased after supplying shading with coloured nets, because the photoinhibition is prevented (Retamales et al., 2008). Parameters like time of day, age of the plant, lifecycle and season have an impact on the photosynthetic rate of plants (Hicklenton et al., 2000, Hall et al., 1972). For example, photosynthesis is increased in the morning possibly as a result of the depletion of leaf carbohydrate reserves via respiration during the night (Hall et al., 1972). Furthermore the level of photosynthesis is higher in newer leaves than in fully developed leaves and higher in newer shoots than in overwintered shoots (Hicklenton et al., 2000, Hall et al., 1972).

Sun exposure is essential for the complete expression of genes required for anthocyanin metabolism. Light exposure increases anthocyanin accumulation and produces darker pigmentation in cranberries (Zhou and Singh, 2002), while a lower light exposure decreases anthocyanin pigmentation in grapes and cranberries (Dokoozlian and Kliewer, 1996, Zhou and Singh, 2002).

Temperature

Beside light, temperature has an impact on both photosynthesis and anthocyanin accumulation in grapes (Mori et al., 2005, Cohen et al., 2008). Previous studies reported that the anthocyanin biosynthesis in grapes is similar to the biosynthesis in bilberry (Boss et al., 1996, Jaakola et al., 2002). Reduction of daytime temperature increase cyanidin and peonidin and decreased anthocyanins in acylated form in grapes (Cohen et al., 2008). Additionally, temperature influences indirectly the activity of pollinators, which relate to fruit yield and berry size and disease outbreaks (Hildebrand et al., 2001). The optimal field temperature for net photosynthesis is

about 25°C in lowbush blueberries. In terms of berry ripening, temperature is suggested to be a more significant factor than sun exposure (Glass et al., 2005b).

Moisture

Water is an important factor for photosynthesis (Taiz and Zeiger, 1998). Insufficient water supply reduces the efficacy of photosynthesis, with the result of smaller fruits and increased soluble solid content. The amount of water varies between clones and plant life stages (Hicklenton et al., 2002, Glass et al., 2005a). Further the pollination activity is decrease in cold and wet conditions. The lower pollination activated contribute to fewer seeds and smaller berries at maturity (Barker et al., 1963). A large water supply leads to softer berries that may be damaged more easily during harvesting (Seymour et al., 2004).

1.5.3 Health promoting properties

Blueberries are known for contributing to healthy nutrition being rich in anthocyanins, flavonols, hydroxycinnamic acid derivatives and flavan-3-ols, which are associated with physiological benefits. Numerous studies *in vitro* and *in vivo* have demonstrated that high intake of fruit and vegetables can reduce the risk of cardiovascular disease, cancer, diabetes, Parkinson's and Alzheimer's disease (Szajdek and Borowska, 2008). The food industry uses these simple statements to promote the health benefits of fruits, but in many cases these scientific studies are often contradictory.

Antioxidant potential

Plant and animal tissues have a variety of natural compounds with antioxidant properties (Antolovich et al., 2002). Berries contain a four times higher antioxidant capacity than non-berry fruits (Hancock et al., 2007). The bioavailability distinguishes largely between polyphenols; the highly available polyphenols have not necessarily the greatest beneficial impact (Manach et al., 2005). Many epidemiological studies demonstrated an inverse relationship between the above described diseases and oxygen stress. During normal metabolism reactive oxygen species (ROS), which are chemical reactive compounds, are produced and react with

lipids, proteins and DNA. Normally a range of compounds contribute to defend cells themselves against ROS damage in order to retain a low cellular ROS level (Hancock et al., 2007). Environmental stress such as UV or heat exposure can increase the level of ROS intensively with the potential to contribute to tissue destruction which can result in the above mentioned diseases (Devasagayam et al., 2004). Polyphenols and anthocyanins containing in fruit and vegetables may contribute in preventing of ROS damage by scavenging hydrogen atoms from their phenolic hydroxyl groups. The potential to donate hydrogen atoms varies between polyphenols (Hancock et al., 2007).

Anticancer properties

A healthy lifestyle comprised of a diet consisting largely of fruits and vegetables may reduce the risk of cancer mortality (Donaldson, 2004). A large range of studies examined the impact of fruit and vegetable consumption on cancer. Animal studies have demonstrated that the consumption of an extract from several berry fruits decreased the development of cancer in rats (Boateng et al., 2007). Animals who consume a high anthocyanin diet had decreased skin, lung and throat cancer (Wang et al., 2008). Rats were fed with either high antioxidant berry fruits (blueberry, blackberry or cranberry juice) or other high antioxidant fruits (pomegranate, watermelon, mangos and plum) and injected the carcinogen azoxymethane three weeks into the trial. Rats that were fed on a berry supplemented diet had lower levels of cancerous colon cells compared to the control. Additionally rats fed with blueberries exhibited a significant decrease in the development of colon cancer cells compared to rats consuming blackberries, plum and mango (Boateng et al., 2007). In other animal studies rats were fed with black raspberries, blueberries, red raspberries, strawberries, noni, goji berry and acai, however, no significant differences were found between fruits in their ability to limit development of cancer cells (Stoner et al., 2010). Results from animal studies cannot directly transfer to humans, because of the variation in biological processes. 128 out of 156 studies reported a protective effect of fruits and vegetables against lung, colon, breast, cervix, oesophagous, oral cavity, stomach, bladder, pancreas and ovary cancer (Block et al., 1992). The risk of cancer was twice as high for consumers with the least consumption of fruits and

vegetables (lower quartile) compared to consumers with the most consumption of fruit and vegetables (upper quartile). The intakes corresponded to the average fruits and vegetable consumption in North America. This study was limited because the impact of a very high consumption of fruits and vegetables on cancer risk were not determined (Block et al., 1992). Combinations of several substances in fruits and vegetables have protective properties (Steinmetz and Potter, 1996). Currently the extent of the contribution of a high fruit and vegetable intake to the antioxidant effects is not clear. Further studies are required to determine the antioxidant effect (Halliwell, 2007). According to these studies the consumption of high flavonoid foods may have protective effects against certain cancers. In general is difficult to conduct human intervention studies with long incubation times. It is important to determine if specific high flavonoid fruits have a significant protective effect against cancer.

Cardioprotection

Cardiovascular diseases describe diseases of heart and blood vessels. Phytochemicals contribute to cardioprotection by sustaining vascular permeability, decreasing inflammatory responses and platelet aggregation and providing vascular protection (Youdim et al., 2000). Further biological factors like low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL), regulation of blood flow, blood pressure and gene expression relates to cardiovascular diseases. The diet has an impact of the balance of these factors. Animal studies demonstrated a slower development of high blood pressure compared with the control by feeding hypotensive stroke-prone rats a 3 % blueberry supplemented diet for eight weeks (Shaughnessya et al., 2009). Kalt et al. (2008) found that pigs fed a 1, 2 and 4 % blueberry supplemented diet showed a decrease in total, LDL and HDL cholesterol with the largest observed reduction at 2 %. Furthermore hamsters were fed with a commercial product that contained 36 % bilberry anthocyanins (10 mg per 10 g body weight) for two or four weeks. An improved capillary perfusion and less adhesive leukocytes in capillaries were found in comparison to the controls (Kadar et al., 1979). Evidence of a relationship between consumption of berries and reduction of cardiovascular diseases in epidemiological studies is limited (Beattie et al., 2005).

Hooper et al. (2008) reported that dark chocolate, soy protein isolates and green tea have significantly benefits on the described risk factors, while other food including berries couldn't confirm this evidence. The impact of high blueberry consumption on protection against cardiovascular diseases is still not clear.

Neuroprotection

Advancing age can affect brain functions such as balance, coordination and short-term memory and reduce the cognitive performance whilst enhancing susceptibility to neurodegenerative diseases including Alzheimer's disease and dementia. Several studies demonstrated that blueberry consumption can reverse the decrease in brain function (Juranic and Zizak, 2005, Joseph et al., 1999, Casadesus et al., 2004). A human observation study demonstrated that high flavonoid consumption contributes to reduce the risk of Alzheimer's disease. A study that examined 1640 French males aged 65 and older associated a high flavonoid intake with a significantly better cognitive performance over a ten year period (Letenneur et al., 2007). In animals studies Joseph et al. (1999) found an inverse relationship between supplementation of fruit and vegetable extracts and age-related reduction in neuronal and cognitive function. 344 19 month old rats were fed with supplements of strawberry, spinach or raspberry dried aqueous extracts over eight weeks. These rats demonstrated an improved memory and motor performance and anti-aging effects. These studies revealed a possible relationship between age related declines of neurodegenerative disorders and high flavonoid intake. It is still unclear what specific compounds are responsible for this effect.

Summary of health benefits

In summary a further study is required to analyse which polyphenols contribute to health benefits of blueberries. The main challenge is to analyse complex matrices (fruit) in combination to complex biological systems (human body) without confounding effects. There are a large number of scientific studies, but there is limited evidence that certain compounds in blueberries provide any significant health benefits. It is a debatable point whether anthocyanins have any significant health benefits due to the low bioavailablity in the human body (Mazza et al., 2002).

1.5.4 Sensory characteristics

In the past breeders were more focussed on size, colour and shape than on sensory attributes like flavour and aroma, however, nowadays an increasing amount of customers are willing to pay a higher price for premium crops, hence sensory character has moved up the breeding agenda (Klee, 2010). Appearance, especially colour as well as mouthfeel and texture, influence the subjective expectation of flavour (Christensen, 1983, Causse et al., 2004, Stommel et al., 2005). Sweet, sour, salty, bitter and umami contribute to flavour, where sugars and acids are the dominant phytochemicals. Generally the appropriate balance between sugars and acids provides a desirable flavour (Stevens et al., 1977, Petro-Turza, 1986).

Flavour

Flavour is an important character for sensory evaluation that combines taste, aroma, texture, chemical irritation and thermal sensation and is influenced by chemical (taste and olfaction) and physical (mechanical, sound, temperature) parameters (Rawson and Li, 2004). Flavour active compounds are perceived through taste buds on epithelial cells on the tongue and palate (Figure 1.9) (Chandrashekar et al., 2006).

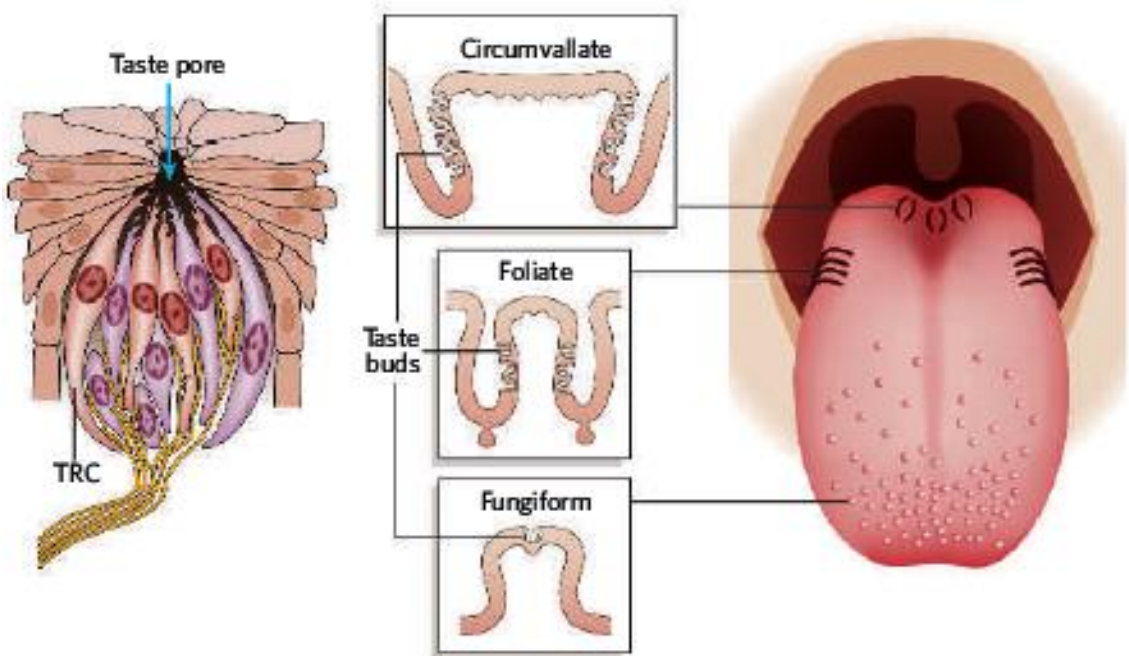


Figure 1.9. Anatomy of the human tongue (Chandrashekar et al., 2006).

The perception of taste can be divided between sweet, bitter, sour, salty and umami based on the interaction with different molecules or ions. Other factors like smell, texture and temperature have an impact on taste perception. Many taste buds located on the surface of the tongue are found within taste papillae, fungiform papillae in the front part of the tongue, circumvallate in the back and foliate in the posterolateral tongue (Figure 1.9). Newer studies suggest that sweet, sour, bitter, salty and umami are detected across the tongue and not detected on specific regions (Figure 1.10) (Chandrashekar et al., 2006). Saliva dissolves taste substances and transports them to the taste receptors. Some saliva components interact with some taste substances. For example bicarbonate molecules in the saliva reduce the concentration of free hydrogen ions with a resulting less sour perception. Furthermore the perception of bitterness is reduced due to the binding of some salivary proteins with bitter taste substances (Hershkovich and Nagler, 2004). Indeed it is now recognised that saliva has a regulatory effect on sour, salt and umami taste and contains immunoglobulin, proteins, enzymes, mucins, Glc and nitrogenous products such as urea and ammonia in order to ease the transport of molecules to receptors and improve taste perception. Age has an impact the sensitivity and ability to discriminate flavour intensities (Humphrey and Williamson, 2001, Spielman, 1990).

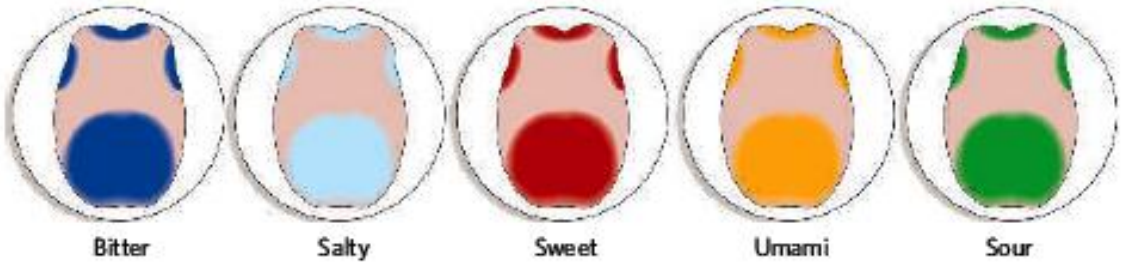


Figure 1.10. Tongue map of the bitter, salty, sweet, umami and sour (Chandrashekar et al., 2006)

The diversity of flavour in fruits is modulated by ca. 350 olfactory receptor genes that interact with different compounds. Soft fruits contain several hundred flavour volatiles, but only 15 – 20 are present in sufficient quantity to have an impact on flavour (Buttery, 1993, Baldwin et al., 2000). Volatiles are involved in taste and retonasal stimulation and contribute to taste character (Klee, 2010), however the

consumer finds it easier to identify sweetness, sourness, salty and bitterness than odour (Darnell and Williamson, 1997).

Visual influences on flavour

A range of factors like taste, odour, price, nutritional information, attitude and previous experience of the product influence the perception of a product (Raats et al., 1995, Krondl and Au, 1978). Consumers mainly associate quality with appearance, flavour and texture (Schutz and Wahl, 1981). The visual sensation; especially colour, plays an important role for acceptance or rejection of products and influences flavour perception. A range of studies have shown a significant link between colour intensity and higher scoring of taste and flavour intensity in fruit drinks (Hyman, 1983). The colour intensity has an impact on the perception of overall flavour intensity. Studies on orange-flavoured beverages have shown that flavour perception increases with higher colour intensities (DuBose et al., 1980), while yellow colour reduced the rating of perception in cherry-, raspberry- and strawberry-flavoured beverages (Spence et al., 2010). Similar findings were observed during the study of pear nectars where green nectars were perceived as less sweet than other coloured samples (Clydesdale, 1993). Additional work has shown that dark red solutions are rated sweeter than light red solutions, while light green solution rated sweeter than dark green solution (Spence et al., 2010). In fact as early as the mid-1970's it was suggested that consumers associate red with sweetness and green with sourness perception (Maga, 1974).

1.5.5 Relationship between flavour and fruit phytochemistry

The relationship of flavour to fruit composition is important to understand the underlying phytochemical drivers of the blueberry sensory experience. Instrumental analysis is often preferred because sensory analysis needs large quantities of fresh fruits, trained assessors and considerable time. Sensory analysis is impossible to conduct with a large range of fruits during the short harvesting times thereby making breeding directly for sensory quality a highly demanding task. Modeling of the relationships between compositional and sensory data is required to determine the prediction of instrumental analysis to sensory assessment. Appearance, flavour and

texture all contribute to sensory quality. Color and size are the main sensory attributes in appearance. Measured firmness values correlated to juiciness and crispness. In general maturity has a larger impact on firmness than genotype. The anthocyanin content contributes to the characteristic blue colour in blueberries. Relationships between colour and consumer preferences identified that consumers prefer brighter blueberries. Relationships between the measured size and preference scores have shown that smaller blueberries were less preferred than larger ones (Saftner et al., 2008). The appropriate level of sugars and acids are required to achieve an appropriate blueberry flavour. A 1 % increase in sweetness perception is achieved with a reduction of 0.1 % in acid concentration (Saftner et al., 2008). In general phenols and flavonoids contribute to astringency and bitterness (Bravo, 1998). The bitterness depending on the molecular weight of polyphenols with large molecules more bitter than small ones (Peleg et al., 1999).

1.5.6 The influence of processing on fruit phytochemistry

Nowadays consumers are interested in healthy consumption of fresh fruits and vegetables. However, due to seasonal availability and costs processed products are often preferred. In most fruit and vegetable juice manufacture the process is divided into three basic steps, pre-treatments, juicing and pasteurisation.

Juice manufacturing

Pre-treatments are applied to obtain maximum juice recovery and enhance the quality of the juice. Temperature treatments are most frequently applied parameter in this step. Freezing of fruits is often done because they can be stored for an indefinite period and the juice can be produced when it is necessary. Additionally freezing destroys the fruit tissues, resulting in a high yield during juice extraction (Stewart, 2005). Heating of fruits is often applied, because heating breaks down cell tissues. Blanching is a common pre-treatment operation resulting in a reduction in microbiological growth and the inactivation of plant enzymes that contribute to browning of fruits (Kader et al., 2002). Typically fruits are heated to 95 ºC for only few minutes and then cooled quickly. Other pre-treatments are the addition of cell wall degrading enzymes to break down pectin, cellulose and starch. High levels of

pectin in particular can complicate the extraction during juice production and significantly reduce the yield of juice. It is common to heat blackcurrant or cranberry pulp with one hour enzyme treatment at 55 ºC. The benefit is that anthocyanins which are mainly located in the skin can be release to the pulp, due to the removal of their sugar moieties to the pulp in presence of cellulase enzymes (Hohn et al., 2005).

Juicing separates the solid material from the aqueous phase, mainly mechanical presses like a screw press with adjustable pressure is applied for fruit juicing (Ashurst, 2005). The clarification removes small solid particulates from the liquid in order to obtain clear juice. This step can be done before or after pasteurisation. The types of clarification are decantation, centrifugation and filtration. In decantation juice is stored until solid particulates settle from the liquid, fining agents accelerate this process. Centrifugation separates the solids from the liquids by using a spinning conical bowl. Filtration use pores with a defined size to pass only small particles (Ashurst, 2005).

For the majority of fruit juices extensive thermal processing is not required, because of their low pH (Kader et al., 1997). Only yeast and moulds can grow under this environment, nevertheless the pasteurisation step is often required to deactivate fruit enzymes. In general pasteurisation is conducted between 85 and 90 ºC for 15 – 60 sec. There are two possibilities to package the berry juice, cool filled or hot filled, depending what packaging is used. For hot filling the package must withstand high temperature, while cold filled packaging requires sterile bottles. An alternative is to use in-pack pasteurisation. The produced juice is filled in adequate packaging; the filled product is immersing into different tanks with heated water. The in-pack pasteurisation is conducted at 70 – 75 ºC for maximum 20 minutes (Ashurst, 2005).

Changes of phytochemicals during juice manufacturing

The content of phytochemicals varies according to the steps the applied during juice processing like thawing, blanching, clarification, different extract and press methods. It is difficult to evaluate the optimal conditions because different studies used different processing methods, variation and cultivars. The degradation during food processing and storage influences the colour quality and nutritional properties (Kader

et al., 2002, Patras et al., 2010). A discussion about the loss of the key phytochemicals will follow.

Anthocyanins

Anthocyanins are bioactive compounds and contribute to the characteristic colour of fruits (Patras et al., 2010). The largest loss occurs at the pressing step, because at this step the aqueous phase separates from the solids and the majority of anthocyanins are located in the skin. Only 22 % of anthocyanin content in frozen blueberries can be measured in blueberry juice (Lee et al., 2002). Total monomeric anthocyanins were decreased during puree processing by 43 % compared to fresh fruits (Brownmiller et al., 2008). Nevertheless the blanching steps improve the anthocyanin recovery in pressed juice (Lee et al., 2002). Several studies reported that blanching inhibited the activity of enzymes like PPO. Chlorogenic acid is the predominant phenolic acid and accelerates the browning pigmentation due the degradation of anthocyanins. PPO catalyse in presence of chlorogenic acid the oxidation to produce quinone, quinone is responsible for the degradation of anthocyanins to brown pigments (Figure 1.11) (Kader et al., 1997).

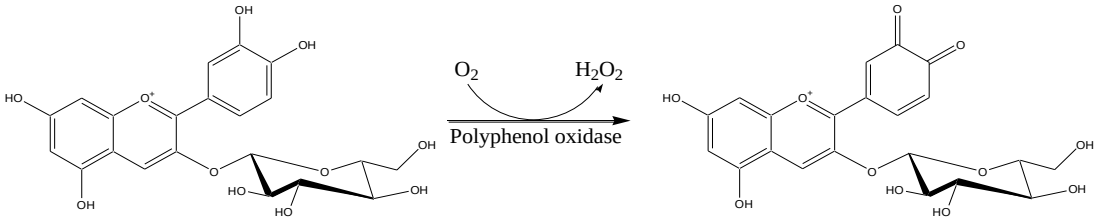


Figure 1.11. Polyphenoloxidase catalyse the oxidation of cyanidin-3-O-glucoside to produce quinone, adapted from He et al. (2010).

Temperature has a strong impact on anthocyanin degradation (Patras et al., 2010), because heat treatment inactivates PPO (Kader et al., 1997). Previous studies demonstrated that an extraction at 60 ºC compared to 25 ºC reduced the loss of anthocyanin, phenolic and antioxidant content (Kalt and McDonald, 2000). Lee and Wrolstad (2004) reported similar results with a heating temperature of 80 ºC compared to 50 ºC. A combination of faster thawing and blanching have a large impact on the anthocyanin content (Brownmiller et al., 2008). During processing and storage the loss of anthocyanins is decreased by lower temperature and shorter

heating time (Patras et al., 2010). The clarification step is done to obtain a clear juice but this step leads to a reduction of anthocyanins (Lee and Wrolstad, 2004). Heat treatment decreases T-Anth content and individual anthocyanins. The anthocyanins differ in their stability. During juice processing the level of malvidin derivatives reduce proportionally less than delphinidin derivates (Skrede et al., 2000, Lee et al., 2002). Pre-treatment with sulphur dioxide (SO_2) could reduce the loss of the anthocyanin content because PPO activity is inhibited (Lee et al., 2002). Further Lee and Wrolstand (2004) analysed the impact of different SO_2 concentrations with the conclusion that the highest anthocyanin content was obtained with 80 ^oC and 50 ppm SO_2. After clarification, heat- or SO_2 treated juices contained four times higher anthocyanin content than the control clarified juice ($p < 0.05$) and this was suggested to be due to the inactivation of PPO activity (Lee et al., 2002). Additionally oxygen may contribute to anthocyanin degradation during juice manufacturing. The impact of oxygen on the stability of anthocyanins, polyphenols and antioxidant capacity were analysed. Tubes was filled or half filled with juice; the anthocyanins, polyphenols and antioxidant capacity were analysed after six days of exposure. The full tubes demonstrated no losses, while the half-filled tubes demonstrated 76 % losses of anthocyanins, 30 % of phenolic and 46 % of T-AO capacity (Kalt and McDonald, 2000).

Other phenolic compounds

Levels of other flavonoids change during juice processing. Different flavonoid classes vary in the loss during juice manufacturing. The smaller loss of chlorogenic acid compared to anthocyanins results from their higher water solubility, additionally chlorogenic acid is mainly found in cell vacuole. Studies compared three treatments (control, blanching and SO_2) without any differences in the levels of cinnamic acid derivatives or flavonols. The highest loss was obtained in the pressing step, because compounds may degrade (Lee et al., 2002). Blanching reduces the loss of cinnamic acid derivates, higher levels of cinnamic acid derivatives were obtained by blanching compared to the control, because blanching deactivates PPO (Rossi et al., 2003). Especially chlorogenic acid is degraded in presence of PPO. The levels of chlorogenic acid, flavonols and procyanidins were measured without using any pre-

treatments; 40 % of chlorogenic acid obtained in the pressed juice and only 1 % in the press cake. The remaining 59 % of chlorogenic acid was suggested to have degraded (Skrede et al., 2000).

The above described thermal treatment reduced the nutritional quality of juice products, while non-thermal treatments like pulsed electric fields, high hydrostatic pressures, dense phase CO_2, ultrasound, ultraviolet light and ionizing radiation preserve the high nutritional quality (Hancock and Stewart, 2010) and could alternatively use.

Retention of phytochemicals during storage

Several studies analysed the optimal storage conditions in order to retain phytochemicals. 168 ml blueberry juice was stored in glass bottles at 25 ºC for six months. The monomeric anthocyanin level was measured after 1, 3 and 6 months with a linear decrease. The clarified juice contained lower levels of anthocyanins compared to non-clarified juice. The clarified juice contained 15 % of the original content of anthocyanins, while the non-clarified juice contained 23 %. Additional Brownmiller et al. (2008) demonstrated that the losses of flavonols and chlorogenic acid are smaller compared to anthocyanins during storage at 25 ºC. Further studies analysed the impact of the different storage temperature to phytochemicals. Blueberry juice samples of two different cultivars in 30 ml glass bottles were stored for sixty days at -20, 6, 23 and 35 ºC. Samples stored at -20 or 6 ºC had higher levels of anthocyanins and polyphenols and higher antioxidant capacities compared to samples stored at ambient temperature (23 and 35 ºC). Additionally Srivastava (2007) demonstrated that the stability of individual anthocyanins during storage depends on their chemical structure. Malvidin and petunidin derivatives are more stable because the single hydroxyl group and two methoxy group on the phenolic ring results in low reactivity and high stability. The levels of flavonoids and phenolic acids had reduced by 85 – 80 %, independently of the storage temperature.

1.6 Metabolite analysis methods

Metabolomics is described as the analysis of small molecules and products occurring in the metabolism. Initially Oliver et al. (1998) used the term metabolome to describe the set of synthetized low-molecular-mass compounds by an organism (Oliver et al., 1998). Later Fiehn et al. (2002) introduced metabolomics for identification and quantification of each single metabolite in a biological system (Fiehn, 2002).

The metabolome comprises a large number of small metabolites with a molecular weight of less than 1500 Da. A variety of different compound classes with very different properties such as amino acids, peptides, organic acids and lipids belong to the metabolome. Metabolic profiling provides a snapshot of the metabolome at a given point in time. The main task of systems biology and functional genomics is to combine proteomic, transcriptomic and metabolomic data in order to develop models describing how individual components combine to result in the emergent properties observed in living organisms.

Studies of transcriptome and proteome were applied in functional genetics methods with the aim to identify the function of genes. These studies have a number of limitations in particular the observations that transcript levels do not reliably predict protein levels and that simple protein quantification via proteomic methods does not necessarily predict the level of protein activity (Trethewey et al., 1999). Thus the emergence of metabolomic studies may support functional genomics studies due to its capacity to supply additional information regarding gene function, particularly their contribution to metabolism.

Metabolomics analyses can be divided into two major groups, targeted and non-targeted analysis, and dependent on the aim of the research. The different approaches used in targeted and non-targeted analyses are described below.

1.6.1 Non-targeted analysis

Non-targeted metabolite analysis supplies an overview of abundant metabolites. Compounds are initially identified during a non-targeted approach. The characteristic of all potential compounds are considered for additional analyses. This approach is

frequently referred to as metabolic fingerprinting. Metabolite fingerprinting does not necessarily identify or quantify the metabolites and instead compares large scale patterns within the total metabolite profile (Dettmer et al., 2007). The non-targeted approach may not be completely accurate, because the selected analytical method and experimental influences may change the metabolite outcome. After the choice of metabolites from the non-targeted analysis, a targeted analysis is recommended for biological interpretation. Identification and quantification of selected metabolites is needed in order to understand the underlying mechanism.

1.6.2 Targeted analysis

Targeted analysis is the most established approach in metabolic studies. The aim is to identify and quantify a certain number of specific metabolites. Therefore the structure of target metabolites needs to be known and analytical methods have to be developed that are appropriate to quantify their concentration (Shulaev, 2006). For instance targeted analysis have been applied to examine terpenoids (Opitz et al., 2008), amino acids (Thornton et al., 2007) and water soluble carbohydrates (Pavis et al., 2001). But the targeted analysis does not supply any information about pathway-related metabolites that can be important for a particular response (Shulaev, 2006).

1.7 Sample preparation

A key aim of metabolomic analysis is to achieve a characteristic snapshot of the metabolic state in the cell at a specific time. Therefore one of the key requirements for sample preparation is to stop all cellular processes at the time of sampling and to stabilize the cellular metabolite pool. Similar to transcriptomic and proteomics studies samples are often flash–frozen in liquid nitrogen and afterwards stored at -80 ºC to preserve the state of the metabolite pool prior to further extraction (Dunn and Ellis, 2005). Additionally inactivation of enzymes may be required to prevent degradation or inter-conversion of metabolites (Shulaev, 2006). The key is to develop universal, robust, reproducible sampling and sample reparation methods that have no bias towards chemical classes (Dunn and Ellis, 2005). The purification of

one compound may destroy or exclude other unrelated compounds, therefore it is important that researchers adjust their methodology to the already used analytical platform (Shulaev, 2006).

One of the main crucial steps is the sample workup, which depends on the type of sample, the analytical method and whether targeted or non-targeted approaches are of interest.

1.8 Chemical analysis methods

Metabolites in plants are highly dynamic and changing from second to second. At the moment it is impossible to analyse the entire range of compounds by one analytical method. Liquid chromatography coupled with mass spectrometry (LC-MS) and gas chromatography coupled with mass spectrometry (GC-MS) are the most used analytical techniques for metabolomics studies (Oresic, 2009) and the principles are described in this section.

1.8.1 Mass spectrometry

Mass spectrometry became one of the dominant methods for metabolomics analysis based on its high sensitivity and selectivity. Mass spectrometry has a wide range of applications. This analytical method generates charged molecules or molecular fragments by ionization of chemical compounds and measures their mass to charge ratios. The molecules in the analyte can be quantified through characteristic fragmentation pattern.

Mass spectrometry is limited in its capacity to distinguish between isomers and enantiomers due to their identical molecular masses (Kopka et al., 2004). Therefore mass spectrometry is frequently coupled with chromatographic separation techniques like gas chromatography and liquid chromatography (Shulaev, 2006).

1.8.2 Gas chromatography – mass spectrometry

Gas chromatography is a frequently used chromatographic technique that can be used to separate compounds and the coupled with mass spectrometry to generate characteristic fragment patterns for specific compounds and chemical classes allowing differentiation and quantification of co-eluting chemical compounds (Kopka et al., 2004). This analytical technique can detect simultaneously several hundred different chemical compounds such as organic acids, sugars, sugar alcohols, amino acids, fatty acids, aromatic amines and flavour volatiles (Shulaev, 2006). These non-volatile compounds require derivatisation to vaporise without decomposition (Dunn and Ellis, 2005). Mostly derivatisation is achieved using silyating agents in a two-stage reaction (Kopka et al., 2004) in which first the carbonyl groups are oximated (C=O → C=N-OH) followed by the replacement of protons with trimethylsilyl (TMS) groups (Dunn and Ellis, 2005).

After chromatographic separation with GC, the compounds are ionized mostly using electron impact (EI) which is a reproducible ion source that prevents ion suppression effects. Single, triple or quadruple ion trap detectors or time of flight detectors (TOF) are usually used for detection (Kopka et al., 2004). Often GC-MS displays a large number of unknown metabolites (Kopka et al., 2004) that cannot be deconvoluted by reference to readily available MS databases. A characteristic GC-MS chromatogram is shown in Figure 1.12.

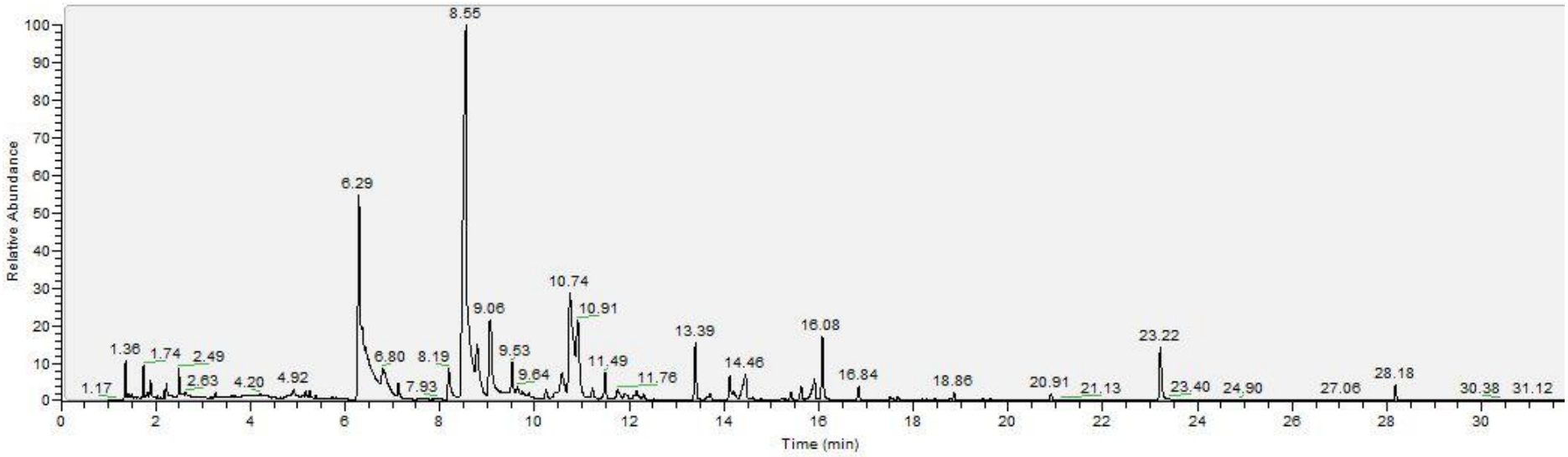


Figure 1.12. GC-MS chromatogram of a headspace-SPME profile of *Vaccinium corymbosum* L. variety Elliot grown in 2012.

1.8.3 Liquid chromatography – mass spectrometry

After separation of compounds chromatographically the obtained compounds are transferred to the ion source and mass spectrometer similar to GC-MS. Compared to GC-MS, LC-MS uses a liquid mobile phase instead of gas. The eluted samples from the chromatographic system are transferred to the ion source in order to convert the molecules to charged or ionised forms. Electro spray ionisation (ESI) is the most frequently used ion source in metabolomics (Dettmer et al., 2007). ESI produces ions by protonation or deprotonation. In general specific analytes will provide stronger spectra either by protonation or by deprotonation. Dunn and Ellis (2005) recommended an operation in both modes in order obtain the widest possible coverage. The identification of unknown analytes is difficult due to the absence transferable mass spectral libraries (Shulaev, 2006). Tandem MS (MS/MS) induces collision-induced dissociation in order to achieve structural information. The fragmentation of known compounds can be compared with the resulting fragmentation pattern (Kopka et al., 2004). The fragmentation pattern provide structural information that may support the characterisation and identification of unknown metabolites (Shulaev, 2006). A characteristic LC-MS chromatogram is shown in Figure 1.13.

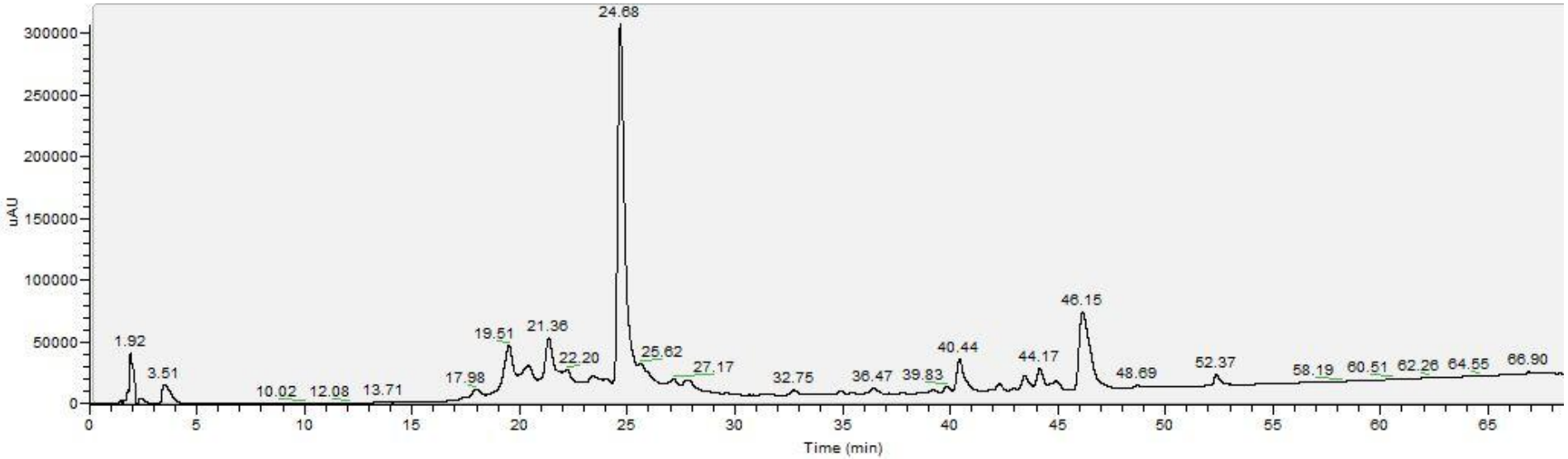


Figure 1.13. LC-MS^2 chromatogram of *Vaccinium corymbosum* L. variety Chandler grown in 2010 measured in the positive mode.

1.9 Data analysis

Metabolomic studies provide a large data set with thousands of variables in each sample. Large datasets are multidimensional and it is often difficult to visualise and extract the relevant information. Chemomteric methods deconvolute the multivariate data into easier interpretable structures (Wold 1987). In the following section the most common chemometric methods are briefly described.

1.9.1 Principal component analysis

The multivariate data analysis technique principal component analysis (PCA) visualizes the relationships between samples and variables. The goal of PCA is to filter the crucial information, to find patterns of similarity of the samples and variables by reducing the number of variables without losing important information. PCA has a widespread area of application in nearly all scientific disciplines.

The data structure is defined by N rows of samples (observations) and K columns of sensory or instrumental response. The extensive set of data is structured, simplified and illustrated in order to reduce the large number of interrelated variables to a small amount of significant variables (Wold, 1987). A new set of uncorrelated data is obtained with new variables, called components. These new components are uncorrelated with others and variance of each one carries greater variance than any subsequent ones. Each component is described by scores and loading. Scores characterise the properties of the samples, while the loadings describe the relationship between the variables. Samples are considered similar if they located together in the two dimensional PCA plot, while samples that are more distantly located are considered different (Naes and Risvik, 1996). Analysis of loading plots allows identification of the variables that act as the drivers of sample variability. Variables with large negative or positive values on the loading axes have a strong influence in describing differences between samples.

1.9.2 Partial least square regression

Partial least square (PLS) regression is the most frequently applied chemometric method for quantification. PLS analyses the relationship between two independent data sets. It is a linear regression model that projects the predicted and measured variables to a new space. PLS reduces the predictor to less uncorrelated components and performs a linear regression model that projects the predicted and measured variables to new spaces (Westerhuis et al., 2008). This technique is used to model the relations between matrices to find the latent variable in X with the best prediction in latent variables in Y. Cross validation estimates the strength of the model. The quality of the model depends on R^2 and the differences between the predicted and calculated variables.

1.10 Aims of the project

The overall objective of the project was to support the developing UK blueberry industry by defining consumer expectations and sensory preferences for blueberries and to provide tools to allow academia and industry to produce blueberry fruit that meets those consumer expectations.

The hypothesis is that there is a correlation between sensory profile and the compositional data of varietal blueberries.

The aim of the project was to examine the impact of cultivars and growing regimens on fruit metabolites and to conduct sensory trials using a variety of fruit germplasm and compositional data to model metabolite profiles onto sensory data in order to gain an understanding of the phytochemical drivers of fruit flavour characteristics.

The first step of the study was to understand the sensory differentiation of blueberries from UK grown highbush cultivars and profile development of current and potential blueberry consumer's expectations. A questionnaire was used to reveal consumer perception towards blueberries. A range of sensory tests were conducted to determine a sensory profile for premium blueberries, which fulfill the expectations of

UK consumers. Initial free-choice profiling was used to understand the range of descriptors used by consumers to differentiate the fruit; subsequent conventional profiling by trained assessors using a consensus vocabulary to generate a multivariate product space describing relationships between the cultivars. Preference mapping was established for liking or disliking of blueberry cultivars by consumer preferences. Further the preferences of UK consumers towards a range of blueberry cultivars were examined and linked to the sensory perceptions in order to understand the influence of attributes to consumer liking. The second step of the project was to analyse a range of components with potential sensory (sugar, organic acid, flavour volatiles, lipids and anthocyanins) and health promoting (polyphenols, anthocyanins) properties in a range of germplasm grown in three growing seasons. Furthermore, metabolite diversity across a range of cultivars grown under different environments was studied. The third step of the project was to link phytochemical to sensory properties to develop a model of how fruit chemistry drives sensory character.

A range of sensory characteristics driven by underlying knowledge of phytochemical-sensory relationships will allow accelerated breeding of desirable UK adapted germplasm to meet consumers' expectations in order to replace the import market for blueberries. Further growers and agronomists could manipulate growing conditions of blueberries in order to improve the desired fruit quality.

Chapter 2

Materials and Methods

2.1 Materials

Chemicals were purchased from the following suppliers (Table 2.1).

Table 2.1. Chemical list including supplier.

Chemicals	Supplier
4-aminophenazone	Sigma-Aldrich, Poole, UK
4-chlorophenol	Sigma-Aldrich, Poole, UK
acetaldehyde	Sigma-Aldrich, Poole, UK
acetic acid (ACS reagent grade, ≥ 99.7 %)	Sigma-Aldrich, Poole, UK
catechin hydrate (≥ 98 %)	Sigma-Aldrich, Poole, UK
chloroform	Fisher Scientific, Loughborough, UK
citric acid (anhydrous)	Sigma-Aldrich, Poole, UK
eicosane	Sigma-Aldrich, Poole, UK
ethanol	Fisher Scientific, Loughborough, UK
Folin-Ciocalteu reagent	Sigma-Aldrich, Poole, UK
formic acid, 90 %	BDH, Poole, UK
fructose (> 99 %)	Sigma-Aldrich, Poole, UK
glucose (anhydrous, 96 %)	Sigma-Aldrich, Poole, UK
horse radish peroxidase (type II, lyophilized powder)	Sigma-Aldrich, Poole, UK
hydrogen peroxide (30 % solution, medical grade, stabilised)	Merck, Hertforshire, UK
kalium chloride	Sigma-Aldrich, Poole, UK
malic acid (99 %)	Sigma-Aldrich, Poole, UK
methanol (HPLC grade)	Fisher Scientific, Leicestershire, UK
morin	Sigma-Aldrich, Poole, UK
n-methyl, n-trimethylsilyl trifluroroacetamide	Sigma-Aldrich, Poole, UK

Chemicals	Supplier
n-nonadecanoic acid methyl ester	Sigma-Aldrich, Poole, UK
octatriacontane	Sigma-Aldrich, Poole, UK
oxalic acid (≥ 99 %)	Hopkin & Williams Ltd., Poole, UK
potassium chloride (≥ 99 %)	Sigma-Aldrich, Poole, UK
potassium carbonate	Sigma-Aldrich, Poole, UK
potassium phosphate monobasis (≥ 99 %)	Sigma-Aldrich, Poole, UK
potassium phosphate dibasic (ACS regent grade, 37 %)	Sigma-Aldrich, Poole, UK
pyridine	Sigma-Aldrich, Poole, UK
quinic acid	Sigma-Aldrich, Poole, UK
reserpine	Sigma-Aldrich, Poole, UK
sodium acetate (electrophoresis grade)	Sigma-Aldrich, Poole, UK
sodium carbonate (anhydrous)	BDH, Poole, UK
sodium chloride	Sigma-Aldrich, Poole, UK
sodium hydroxide, 50 % w/v	VWR, Leicestershire, UK
sodium sulphate (anhydrous)	Sigma-Aldrich, Poole, UK
sucrose (> 99.5 %)	Sigma-Aldrich, Poole, UK
sulphuric acid	Sigma-Aldrich, Poole, UK
tetracosane	Sigma-Aldrich, Poole, UK
triacontane	Sigma-Aldrich, Poole, UK
tridecane	Sigma-Aldrich, Poole, UK
trolox (97 %)	Fluka, Poole, UK
undecane	Sigma-Aldrich, Poole, UK

2.2 Plant growth and fruit sampling

Plants were grown under standard commercial conditions either on site at the James Hutton Institute, Dundee, UK (56°27'N, 3°03'W; open field) or at two separate commercial farms located close to Dundee in Muirhead (56°30'N, 3°05'W; open field) or Blairgowrie (56°35'N, 3°18'W; polytunnel or open field). All fruit were harvested by hand from individual plants at commercial maturity in the years 2010, 2011 and 2012. Following harvest, plants were returned to the laboratory and stored less than four days at 4 °C prior to sensory testing. A separate sample of each harvest was snap frozen in liquid N_2 and stored at -80 °C until extraction for phytochemical analysis (Table 2.2). Additionally juice samples were prepared by puréeing fruit in a

Waring blender followed by filtration through two layers of cheesecloth. Juice samples were stored at -80 ^{o}C for up to two months until sensory or phytochemical analysis.

A total of fifteen cultivars grown at several locations were evaluated (Table 2.2) for their sensory properties by free-choice profiling (FCP) and descriptive profiling (DP) and phytochemical profile over three different harvest years.

Table 2.2. Sensory and phytochemical analysis of blueberry cultivars grown at three different locations in three different years (FCP, free-choice profiling; DP, descriptive profiling).

Name of the cultivars	Growing place	Sensory analysis	Phytochemical analysis
Aurora (AU)	Blairgowrie	FCP 2010; DP 2010, 2011, 2012	2010, 2011, 2012
Berkeley (BE)	Dundee	DP 2010, 2011, 2012	2010, 2011, 2012
Bluecrop (BL)	Dundee Muirhead	DP 2010, 2011, 2012	2010, 2011, 2012 2012
Bluegold (BG)	Muirhead	DP 2011	2011, 2012
Brigitta (BR)	Blairgowrie Muirhead	DP 2011, 2012	2011, 2012 2012
Chandler (CH)	Blairgowrie Muirhead	FCP 2010; DP 2010, 2011, 2012	2010, 2011, 2012 2012
Chanticleer (CHT)	Blairgowrie	DP 2012	2012
Darrow (DA)	Blairgowrie	FCP 2010; DP 2010, 2011, 2012	2010, 2011, 2012
Elliott (EL)	Blairgowrie	DP 2011, 2012	2011, 2012
Liberty (LI)	Blairgowrie	FCP 2010; DP 2010, 2011, 2012	2010, 2011, 2012
Ozarkblue (OZ)	Blairgowrie	FCP 2010; DP 2010, 2011, 2012	2010, 2011, 2012
Reka (RE)	Blairgowrie	DP 2011, 2012	2011, 2012
Puru (PU)	Blairgowrie	DP 2012	2012
Spartan (SP)	Blairgowrie Muirhead	FCP 2010; DP 2010, 2011, 2012	2010, 2011, 2012 2012
Toro (TO)	Blairgowrie	DP 2012	2012

2.3 Sensory analysis

All samples were labeled with a random three digit code and assessment was undertaken in purpose-built sensory booths at ambient temperature with artificial daylight for appearance and red light for aroma and taste (Figure 2.1). Blueberry juices were provided for aroma in whisky glasses and for taste in 15 ml cups, entire berries for appearance in 15 ml cups for cultivars 2010; in 2011 and 2012 blueberry juice were provided for aroma in whisky glasses and entire blueberries for taste and appearance in 15 ml cups. Approximately 100 g fruit were pureed in a Waring blender, filtered through two layers cheesecloth and stored in a fridge prior testing. The assessors were asked to drink cold tap water before profiling of each sample. For experimental design and data collection FIZZ software were used.

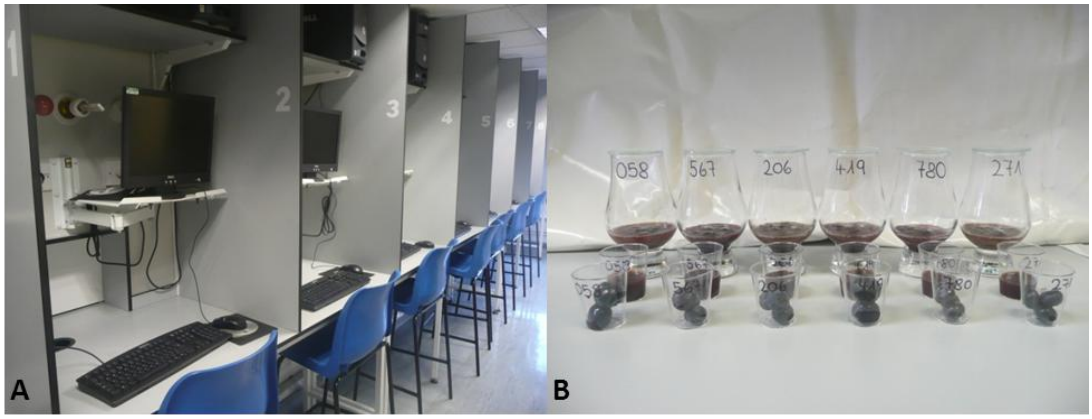


Figure 2.1. Images depicts the sensory laboratory (A) and sample preparation (B).

2.3.1 Questionnaire

A questionnaire was designed to determine the profile of current and potential blueberry consumer's expectations and to recruit assessors for sensory analysis.

The online survey for consumer fruit perceptions is attached and divided into 2 parts; the first part referred to the attributes of the perfect blueberry and the second part to demographic background (Appendix A). The survey for consumer about fruit perceptions was placed online at the University of Strathclyde (322 responses), the James Hutton Institute (79 responses) and the John Innes Institute (20 responses). The distribution of the assessors is shown in Table 2.3. The questionnaire required

about 10 minutes to complete and was accessible for a period of 4 weeks. As an incentive to complete, each person who finished the questionnaire was entered into a draw to win a £50 book token. The data were collected into a matrix that was analysed by using SPSS version 20.1 (SPSS Inc., Chicago, IL, USA).

Table 2.3. Summary statistics of assessors in the questionnaire (n =421).

		% of assessors
Age	under 18	0
	18 - 24	28.1
	25 - 44	34.3
	over 45	37.6
Gender	Female	71
	Male	29
Ethnicity	UK	59.9
	Asia	13.5
	Rest of Europe	17.1
	Rest	1.4

2.3.2 Free-choice profiling

FCP supplied information regarding how people judge blueberries without intensive training in advance. This sensory evaluation technique supplies important information on quality, range of perception and the product space, additionally it generates a vocabulary.

Assessors

25 non-smokers, of different ages and ethnicity were selected from the questionnaire, 15 females and 10 males tested the products (Table 2.4). They were students or staff of the University of Strathclyde without any experience in sensory evaluation.

Table 2.4. Summary statistics of assessors from the free-choice profiling (n = 25).

	% of assessors
Age	
under 25	60.6
25 - 44	36.4
over 45	3.0
Gender	
Female	60.6
Male	39.4
Ethinicity	
UK	39.3
Asia	30.3
Rest of Europe	21.2
Rest	9.1

Procedure

In 2010, participants assessed juices prepared from individual blueberry cultivars for aroma and taste; additionally entire blueberries were assessed for appearance. In the first session each participant described three blueberry cultivars with as many attributes as they needed (Figure 2.2). In a second session three additional blueberry cultivars were supplied to extend the own vocabulary. The following two sessions scaled the blueberries on a 10 cm anchored-scale from absence to intensive according to the described attribute using FIZZ software (v. 2.46, Biosystemes, Couternon, France). The last two sessions repeated the scoring.

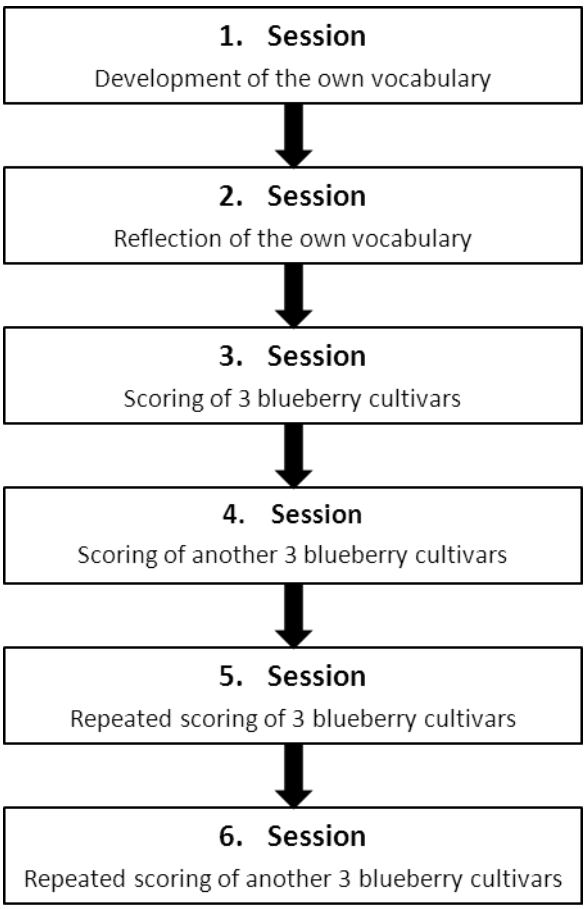


Figure 2.2. Flow diagram of free-choice profiling procedure.

2.3.3 Descriptive profiling

DP determined the intensity of the attributes of blueberry cultivars by a trained panel in order to establish quality. The panel performed scaling on the generated lexicon from the FCP of 17 attributes; 6 appearance, 4 aroma and 7 taste (Table 2.5). Reference points were introduced by training the panel using standard solutions and are required to reduce variability hence DP is a time-consuming technique, based on a training of a panel (Meilgaard et al., 1999).

Table 2.5. Attributes and reference standards for training.

Attribute class	Attributes	Reference material	Range
Appearance	size	Photograph	see Figure 2.3
	round vs oval	Photograph	
	matt vs shiny	Photograph	
	blue	Photograph	
	red	Photograph	
	Firmness	Fresh blueberries	subjective measurement by touch
Aroma	sweetish	Honey	7 – 25 g/l
	fruity	Forest fruits[a]	600 g – 1200 g/l
	acid	Vinegar	1 – 10 g/l
	intensity	Blueberry juice	500g/l – undiluted
Taste	sweet	Granulated sugar	10 – 80 g/l
	sour	Forest fruits[a]	600 g – 1200 g/l
	bitter	Tonic water	0.5 – 50 g/l
	fruity	Forest fruits[a]	600 g – 1200 g/l
	acid	CA	0.06 – 1 g/l
	intensity	Blueberry juice	500 g/l – undiluted

[a] purchased from Tesco Ltd.

Assessors

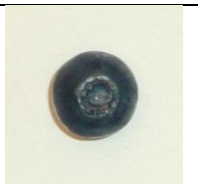
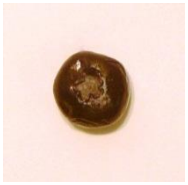
Eight assessors (six females, two males) were recruited for the DP 2010 from the FCP sessions and eight assessors (four and four in 2011, two and six in 2012) in 2011 and 2012 by sending emails to participants of previous sensory tests and blanket email to students and staff of Strathclyde Institute of Pharmacy and Biomedical Sciences. All assessors were non-smokers.

Procedure

Assessors were required to perform ranking tests, in which they were required to sort a diluted solution by ascending order to demonstrate a minimum performance in their capacity to distinguish flavour intensities in standardised solutions. The solutions used were sucrose (0 g/l – 10 g/l), CA (0 g/l – 10 g/l), tonic water (0 g/l – 20 g/l) and blueberry juice (100 g/l – undiluted) dissolved in water to determine the assessors capacity to taste sweetness, acidity, bitterness and intensity, respectively. In order to

minimise variation, reference standards (Table 2.5) were used for the definition and determination of attributes (Meilgaard et al. 1999). In the first training session three reference standards per attribute were supplied, in the second session four reference standards. For appearance photographs (Figure 2.3) were used, except for firmness (subjective measurement by touch).

Figure 2.3. Photographs of reference standards.

Attributes	No. of Reference standards 2	5	7	9
blue				
matt vs shiny				
red				
round vs oval				
size				

For aroma and taste different solutions were used according to the attribute. In order to control the training an examination (Appendix B) was performed in session three, the criterion for passing was 60 % correct answer. The two following sessions were dedicated to scoring on a nine point scale using FIZZ software (v. 2.46, Biosystemes, Couternon, France) of fruit attributes in eight thawed blueberry cultivars in 2010 (juices for aroma and taste, entire blueberries for appearance), twelve fresh blueberry cultivars in 2011 and fourteen in 2012 (juice for aroma, entire blueberries for taste

and appearance). Repetitions followed in the sessions six and seven in order to construct the profile by means and determine the reproducibility of achieved results.

2.3.4 Consumer preferences

Preference maps on fresh blueberries were performed at University of Strathclyde to get information about consumer preferences.

Assessors

56 assessors were recruited in the first consumer test and 70 assessors in the second consumer test from staff and students of the University of Strathclyde. The distribution of the assessors is shown in Table 2.6.

Table 2.6. Summary statistics of assessors in the consumer preferences (n = 56 in the first test; n = 70 in the second test).

	% of consumers	
	first test	second test
Age (years)		
under 25	10.7	49.3
26 - 34	37.5	21.7
35 - 44	23.2	14.5
over 45	28.6	14.4
Gender		
female	55.4	49.3
male	44.6	50.7
Occupation status		
professional	25.9	23.5
office	17.9	8.4
student	56.2	68.1
Sport		
every day	7.1	8.7
more than once a week	37.5	42.0
once a week	21.4	20.3
fortnightly	7.1	1.4
occasionally	26.8	27.5

Procedure

The participants assessed the entire blueberry cultivars 2011 according to appearance, taste and overall. After the experimental work was explained to each assessor, they completed a questionnaire about gender, age, occupation and sports exercise (Appendix C). During the tasting sessions assessors were asked not to discuss anything with others. The hedonic testing was done twice, first with nine cultivars (BE, BL, BR, CH, DA, LI, OZ, RE and SP) and secondly three (AU, BG and EL) based on the cultivar availability. All samples were labeled with a random three digit code and assessed at the common area at the Strathclyde Institute of Pharmacy and Biomedical Sciences at ambient temperature. Assessors rated liking of blueberries on a seven-point hedonic scale from dislike extremely to like extremely.

2.4 Analytical methods

2.4.1 Metabolite extraction

For the extraction of sugars, organic acids, anthocyanins, polyphenols and T-AO capacity frozen fruit were homogenised in a Waring blender at a ratio of 1:3 (v/w) in methanol/water/formic acid (50/49/1) followed by centrifugation (16000 g, 10 min, 1 ^{o}C). Samples were then frozen at -80 ^{o}C in separate aliquots until metabolite quantification.

The method for the production of blueberry juice was described under 2.2 with the exception that in addition to filtration through cheesecloth juice destined for phytochemical analysis was further clarified by centrifugation (16000 g, 10 min, 1 ^{o}C). The supernatant was used as described below for the phytochemical analysis. Reference standards were used in every metabolite analysis in order to confirm the efficacy of the applied method.

For the metabolite quantification blueberry extracts and juices were analysed.

2.4.2 Metabolite quantification

Sugars and organic acids

Blueberry juices were diluted 10 fold, boiled for 5 min, and then centrifuged (16000 g, 10 min, 1 °C). The supernatant was diluted by a further 500 fold and transferred to HPLC vials. Blueberry extracts were boiled for 5 min, and then centrifuged (16000 g, 10 min, 1 °C). 500 µl aliquots of supernatant were evaporated to 100 µl using speed vac, followed by lyophilisation to remove formic acid. 2 ml ultrapure water was added to the dried material and an aliquot was diluted 250 fold with ultrapure water. Sugars were separated by anion exchange chromatography on a Dionex Carbopac PA-100 250 x 4 mm column (Dionex (UK) Ltd., Camberley, UK) using a mobile phase of 200 mM NaOH at 1.0 ml/min. The sugars were detected by pulsed amperometric detection according to Souleyre et al. (2004) and quantified with references to external calibration curves of Glc, Fru and sucrose in the range of 2 – 30 µg/ml.

Similarly, for organic acid quantification blueberry juices were 10 fold diluted, boiled for 5 min, and then centrifuged (16000 g, 5 min, 1 °C). The supernatant was directly transferred to HPLC vials. Blueberry extracts were boiled for 5 min, and then centrifuged (16000 g, 5 min, 1 °C). 500 µl aliquots of supernatant were evaporated to 100 µl using a speed vac, followed by lyophilisation to remove formic acid. 2 ml purified water was added to the dried material and directly transferred to HPLC vials. The organic acids were quantified by HPLC according to Masson (2000) with conductivity detection on a Dionex IonPac AS11-HC 4 x 250 mm (Dionex (UK) Ltd. Camberley, UK) with ion suppression via a 4 mm ASRS 300 set at 240 mA in external mode with ultrapure water at 2 ml/min. The mobile phase consisted of 10 % methanol in ultrapure water (A) and 100 mM NaOH in 10 % methanol (B). A gradient was applied with 0 min, 10 % B; 1 min, 10 % B; 5 min, 25 % B; 8 min, 30 % B; 18 min, 60 % B; 23 min, 80 % B; 24 min, 10% B; 30 min, 10 % B with a flow rate of 1.5 ml/min. Organic acids were quantified by external calibration curves, CA in a range of 0.1 – 1.0 mg/ml and MA, oxalic and quinic acid in a range of 0.003 to 0.025 mg/ml.

Brix

Brix measurements were undertaken on juice samples using a portable Jencons PR-100 digital refractometer against distilled water.

Antioxidant properties

T-Anth content was estimated by pH differential method (Lee et al., 2005). Juice or extract was diluted 40 fold into either 50 mM KCl/ HCl buffer pH 1.0 or 50 mM acetate buffer pH 4.5. Absorbance was measured at 510 nm and 700 nm against buffer blanks at each pH. Anthocyanin concentration was calculated using the following equation

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$$

with anthocyanin concentration = A/12100 M. Results were presented as delphinidin 3-rutinoside equivalents with a molecular mass of 647 Da.

T-Phen content was estimated by the method of Stevenato et al., 2004. A mixture of 100 µl 20 fold diluted juice or extract, 690 µl 50 mM potassium phosphate pH 8.0, 100 µl 30 mM 4-aminophenazone, 100 µl 20 mM H_2O_2 and 10 µl 100 U/ml horse radish peroxidase were incubated at room temperature for 5 min. Absorbance was measured at 500 nm. T-Phen content was quantified by external calibration curve against catechin in a range of 10 – 200 µg/ml.

T-AO capacity was estimated by the Folin-Ciocalteu method (Everette et al., 2010). 250 µl 100 fold diluted juice or extract and 250 µl of 10 fold diluted Folin-Ciocalteu reagent were incubated 3 min at room temperature. After addition of 500 µl 13 % (w/v) Na_2CO_3 samples were incubated for 60 min in the dark. The absorbance was measured at 750 nm. T-AO capacity was quantified by external calibration against trolox in a range of 10 – 200 µg/ml.

Individual polyphenols and anthocyanins

In addition, individual polyphenols were quantified by LC-MS^2 in the negative ion mode and individual anthocyanins in the positive ion mode according to a modification of Gavrilova et al., 2011. Chromatographic separations were carried out on a 150 x 2.0 mm i. d., 5µm Phenomenx Gemini-NX C18 column (Phenomenex, Macclesfield, UK) on a LCQ-FLEET system, Accela autosampler, Accela photodiode array (PDA) detector, ThermoFinnigan mass spectrometer iontrap. Eluent A was 0.1 % formic acid in ultrapure water and eluent B was 0.1 % formic acid in methanol. Eluent flow rate was 200 µl/min and the following gradient was applied: 0 min, 5 % B; 5 min, 5 % B; 45 min, 50 % B; 55 min, 75% B; 65 min, 100 % B; 70 min, 5 % B. Relative polyphenol content was estimated by comparison with 5 µl 10 mM morin (3.022 mg morin/ml in 0.1 % formic acid, 50 % methanol) as internal standard while relative anthocyanin content was estimated by comparison with 1 µl 200 µM reserpine (0.024 mg reserpine/ml in 0.1 % formic acid, 50 % methanol). Compounds were identified according to their relative retention, UV/ visible and mass spectra of parent and daughter ions according to previous publications (Table 2.7) (Gavrilova et al., 2011, Määttä et al., 2003).

Table 2.7. Identification of phenolic compounds in extracted blueberries.

t_R (min)	λ_{max}	MW	MS (m/z)	MS^2 (m/z)	compounds
			Anthocyanins		
21.00	280, 518	449	449[a]	287 (100)	cyanidin-3-O- glucoside (cya-glc)
23.41	278,518	419	419[a]	287 (100)	cyanidin-3-O-arabioside (cya-ara)
33.75	280,518	449	449[a]	287 (100)	cyanidin-3-O-galactoside (cya-gal)
41.59	278,526	435	435[a]	303 (100)	delphinidin-3-O-arabioside (del-ara)
35.71	278,532	535	535[a]	331 (100)	malvidin-3-(6″-acetyl)-hexoside (mal-ac)
28.16	278,530	463	463	331 (100)	malvidin-3-O-pentoside (mal-pen)
26.84	280,518	433	433[a]	301 (100)	peonidin pentose (peo-pen)
25.05	278,530	449	449[a]	317 (100)	petunidin-3-O-arabioside (pet-ara)
23.05	276,528	479	479[a]	317 (100)	petunidin-3-O-hexoside (pet-hex)
			Flavonols		
44.46	264, 348	594	595[a]	287 (100)	kaempferol hexose-deoxyhexoside (kae-deox)
47.90	264,290	535	489[b]	285 (100)	kaempferol hexoside-malonate (kae-mal)
51.28	262,360	494	493[b]	331 (100)	laricitrin-3-O-glucoside (lar-glc)
36.06	254,300	480	479[b]	316 (100)	myricetin hexoside (myr-hex)
19.15	254,262	464	465[a]	303 (100)	quercetin hexoside (que-hex)
43.54	254,300	550	551[a]	303 (100)	quercetin hexoside-malonate (que-mal)
43.08	256,356	434	433[b]	301 (100)	quercetin-3-O-arabionside (que-ara)
40.63	256,356	464	463[b]	301 (100)	quercetin-3-O-glucoside (que-glc)
40.12	254,262	610	611[a]	303 (100)	quercetin-3-O-rutinoside (que-rut)
			Flavan-3-ols		
19.12	242,280	578	577[b]	425 (100), 407 (100)	(-)-epicatechin-(4β→8)-(-)-epicatechin (dimer B2)
21.06	242,278	290	289[b]	245 (100), 205 (31)	(+)-catechin (cat)
13.33	ND	306	305[b]	179 (100)	(epi)gallocatechin (EGC)
			Hydroxycinnamic Acid Derivatives		
23.06	246,330	342	341[b]	179 (100), 161 (39)	caffeoylhexose (caf-hex)
24.24	244,324	354	353[b]	191 (100)	chlorogenic acid (CGA)
27.38	244,324	354	353[b]	191 (100)	chlorogenic acid (CGA)

[a] positive ions; [b] negative ions; t_R, retention time; ND, not detected

Flavour volatiles

Flavour volatiles were estimated according to the method of Rohloff et al. (2009). 3 g of crushed blueberries were transferred to 20-ml headspace vials with 5 ml of 20 % (w/v) NaCl solution and 100 µl of 0.2 mg/ml 4-chlorophenol as internal standard prior to immediate closure with a PTFE/silicone septum (Supelco, Bellefonte, PA). Volatile compounds were analysed using a Thermo Finnigan Trace gas chromatograph coupled to a Finnigan Tempus Plus TOF mass spectrometer (Thermo Scientific, UK). CAR/PDMS-coated 85 µm SPME fibre (Supelco, Bellafonte, USA) with a 23-gauge protective sheath (Supelco, UK) was exposed at 280 °C within a programmable temperature vaporising (PTV) injector, fitted with a silcosteel coated stainless steel liner (1.3 mm × 120 mm), operating in constant temperature splitless mode. Fruit samples were extracted 30 min at 35 °C using a COMBIPAL® autosampler equipped with the fiber to adsorb the volatiles onto the coated fiber (extraction step). This low extraction temperature were selected to identify flavour volatiles that consumer perceive during blueberry consumption in order to correlate the flavour volatiles to sensory attributes. Blueberry headspace volatiles were desorbed in the injection port (280 °C) for 3 min and resolved on a DB1701 GC column (30 m × 0.25 mm × 0.25 µm; J&W Scientific, Folsom, CA, USA) using helium as a carrier gas at 1.0 ml min^{-1} in constant flow mode. The GC temperature was held isothermally at 45 °C for 2 min, ramped from 45 °C to 180 °C at 5 °C /min, and 180 °C to 220 °C at 20 °C /min. Detection was using a TOF mass spectrometer in electron impact (EI) mode at 70 eV. Data were acquired and subsequently analysed using the Xcalibur™ software package v. 1.4 (Thermo Scientific, UK). Compounds were identified by comparing mass spectra of parent ions with the NIST or Wiley database and by comparing the retention indices to those from database of the James Hutton Institute (Table 2.8). The flavour volatiles were positively identified, if the retention indices (RI) were close to those from the database.

The RI of each compound was determined with alkanes that elute before and after that peak in order to compare compounds measured by different analytical laboratories. The RI was calculated by using Equation 2.1 (Van den Dool and Kratz,

1963). Standards of acetaldehyde and ethanol were injected, because no retention indices were available.

$$RI = \frac{t_R(x) - t_R(n)}{t_R(n+1) - t_R(n)}$$ **Equation 2.1**

$t_R(x)$ retention time of compound x
$t_R(n)$ retention time of closest alkane that elute before the compound x
$t_R(n+1)$ retention time of closest alkane that elute after the compound x
n number of Carbon atom of closest alkane that elute before compound x

Table 2.8. Identification flavour volatiles in highbush blueberry cultivars.

Peak	t_R (min)	MW	MS (m/z)	RI[a] (IST)	RI[b] (SOLL)	Compounds
1	1.51	44	44,			acetaldehyde (HAc)
2	1.78	96	45			ethanol (EtOH)
3	2.54	88	43, 70, 61	689	691	ethyl acetate (EtOAc)
4	2.62	72	43, 72	694	682	2-butanone (MEK)
5	3.95	86	44, 58	775	775	pentanal (Penal)
6	6.35	100	56, 44, 72, 82	880	883	hexanal (hexal)
7	8.54	98	69, 83, 55, 98, 41	958	962	(E)-2-hexenal (2-Hexal)
8	8.83	206	41, 55, 67, 82	969	970	(Z)-3-hexenol (3-HexeOH)
9	10.33	136	93, 69, 41, 91	1025	1021	β-myrcene (myrc)
10	10.81	96	81, 96, 67	1035	1039	(E,E)-2,4-hexadienal (2,4-Hexal)
11	11.31	136	67, 94, 77, 107	1051	1057	D-limonene (Lim)
12	11.89	154	108, 71, 81, 93, 154	1070	1072	eucalyptol (Euc)
13	12.24	126	108, 69, 43, 93	1082	1085	6-methyl-5-hepten-2-one (6-MHO)
14	13.15	136	93, 121, 136, 79, 105	1112	1119	α-terpinolene (α-terpo)
15	14.44	116	60, 73	1157	1160	hexanoic acid (Hex acid)
16	15.49	154	71, 93, 55, 80	1192	1193	linalool (Lin)
17	15.90	114	73, 99, 55	1205	1206	(E)-2-hexenoic acid (Hexen acid)
18	16.16	94	73, 99, 68, 55	1216	1218	phenol (Phen)
19	18.36	154	93, 59, 136, 121, 67	1296	1302	α-terpineol (α-terpe)
20	23.25	129	64, 128			4-chlorophenol (IS)

[a] superscript indicates the obtained retention indices; [b] superscript indicates retention indices from the data base at the James Hutton Institute, Dundee, Scotland; IS, internal standard.

Metabolomic analysis

Extraction

Metabolites were extracted according to the method of Dobson et al. (2004), approximately 100 mg of accurately weighed freeze-dried blueberry samples were extracted with 3 ml methanol and the mixture vigorously shaken at 1500 revs/min at 30 °C for 30 min, followed by addition of 100 µl of internal standard (0.2 mg/ml n-nonadecanoic acid methyl ester). After 0.75 ml water and 6 ml chloroform were added sequentially to the mixture and shaked at 1500 revs/min and 2500 revs/min at 30 °C for 30 min, 1.5 ml water was added and shaken by hand for several min. Upper (polar) and lower (non-polar) fractions were separated by centrifugation (1200 rpm, 10 min), the polar fraction were discarded and the non-polar fractions were stored in a -20 °C freezer overnight.

Derivatisation

The non-polar fractions were evaporated to dryness, then 1 ml chloroform and 2 ml freshly made 1 % (v/v) methanolic sulphuric acid were added and samples transesterified at 50 °C for 16 hours. After the addition of 5 ml 5 % (w/v) aqueous sodium chloride and 3 ml chloroform, the vials were shaken and left to separate into two layers. The upper layer was discarded and 3 ml 2 % (w/v) aqueous potassium hydrogen carbonate and 2 % (w/v) potassium carbonate was added to the lower layer. The tubes were shaken and left them to separate. The lower chloroform layer was dried over anhydrous sodium sulphate and evaporated to dryness, then 50 µl chlororform, 10 µl anhydrous pyridine and 40 µl MSTFA (n-methyl-n-(trimethylsilyl)-trifluoroacetamide) were added and silylation allowed to continue at 37 °C for 30 min.

Vials were prepared by addition of 50 µl of retention standard mixture comprising 0.2 mg/ml undecane, tridecane, hexadecane, eicosane, tetracosane, triacontane and octatriacontane in isohexane that was then allowed to evaporate to dryness at room temperature. 40 µl dry pyridine was added to the prepared vials followed by 40 µl of the silylated samples. Samples were then directly analysed by GC-MS as described below.

Analysis of metabolites with GC-MS

The non-polar compounds were analysed using a Thermo Finnigan Tempus GC-(TOF)-MS system (Thermo Scientific, UK). 1 µl samples were injected with a programmable temperature vaporising (PTV) injector, operating at a constant split ratio of 80:1. The following PTV conditions were selected: 132 ^{o}C for 1 min, ramped to 320 °C at 14.5 ^{o}C/sec, 320 °C for 1 min, ramped to 400 °C at 14.5 ^{o}C/sec and 400 °C for 2 min. Chromatographic separations were carried out on a DB5-MS™ column (15 m x 0.25 mm, 0.25 um; J&W, Folsom, CA). The carrier gas was helium at a constant flow of 1.5 ml/min. Oven temperature conditions were 100 °C for 2.1 min, 25 °C/min ramped until 320 °C, and then held isothermally at 320 °C for 3.5 min. Mass/z detection was operating in the EI mode (ionization energy, 70 eV, source temperature 200 °C). Data acquisition was performed in scanning mode (mass range m/z 35 – 900; a.m.u: four scans per second). Data were acquired and subsequently analysed using the Xcalibur™ software package v. 1.4 (Thermo Scientific, UK). Compounds were identified according to their relative retention, mass spectra of parent to those from the database of the James Hutton Institute (Table 2.9).

Table 2.9. Identification of lipids in highbush blueberry cultivars. TMS, trimethyl silyl; IS, internal standard.

Peak	t_R(min)	MS (m/z)	Compounds
1	5.32	242.4	n-tetradecanoic acid (C14:0) methyl ester
2	5.37	256.1	n-petadecanoic acid (C15:0) methyl ester
3	6.19	270.2	n-hexadecanoic acid (C16:0) methyl ester
4	6.26	250.2	3- or -4 methoxy, 3- or 4-hydroxy cinnamic acid (OCH3-CA) methyl ester (TMS)
5	6.86	292.3	α-linolenic acid (C18:3) methyl ester
6	6.88	264.4	octadecenoic acid (C18:1) methyl ester
7	6.94	343.3	2-hydroxy hexadecanoic acid (2-OH C16:0) methyl ester TMS
8	6.96	298.4	n-octadecanoic acid (C18:0) methyl ester
9	7.33	269.5	n-decanoic acid (IS) methyl ester
10	7.67	326.5	n-eicosanoic acid (C20:0) methyl ester
11	8.01	340.3	n-heneicosanoic acid (C21:0) methyl ester
12	8.08	369.5	n-heneicosanol (C21-OH) TMS
13	8.33	354.5	n-docosanoic acid (C22:0) methyl ester
14	8.39	383.5	n-docosanol (C22-OH) TMS
15	8.64	368.6	n-tricosanoic acid (C23:0) methyl ester
16	8.70	397.5	n-tricosanol (C23-OH) TMS
17	8.95	382.5	n-tetracosanoic acid (C24:0) methyl ester
18	9.00	411.5	n-tetracosanol (C24-OH) TMS
19	9.24	396.5	n-pentacosanoic acid (C25:0) methyl ester
20	9.46	411.6	2-hydroxy tetracosanoic acid (2-OH C24:0) methyl ester TMS
21	9.53	410.5	n-hexacosanoic acid (C26:0) methyl ester
22	9.56	439.5	n-hexacosanol (C26-OH) TMS

2.5 Statistical analysis

Analysis of variance (ANOVA) was used to determine the significant differences ($p < 0.05$) of each group from independent variables in the data set. The significance between treatments was tested by Fisher's protected least significance method. ANOVA was carried out using Genstat, 16th ed. (VSN International Ltd., Hemel Hempstead, UK).

Pearson's correlation coefficient was applied to determine pair-wise correlations among data using Genstat, 16th ed. (VSN International Ltd., Hemel Hempstead, UK).

Hierarchical cluster analysis using Euclidean distances was applied to estimate the similarity of scoring. Hierarchical cluster analysis was carried out using Genstat, 16th ed. (VSN International Ltd., Hemel Hempstead, UK).

General Procrustes Analysis (GPA) was performed to analyse the free-choice profiling data using Senstools (v. 3.3.2, OP&P Product Research BV, Utrecht, The Netherlands). The statistical analysis of the generalized Procrustes analysis (GPA) was used to evaluate data based on the description of participant's own vocabulary to characterize products.

Principal Component Analysis (PCA) visualizes the relationships between samples and variables. A new set of uncorrelated data was obtained with new variables, called components. The PCA evaluate dimensionality and provide an overview of dominant pattern and major trends and outliers, visualised in two dimensional spaces. PCA was carried out using Genstat, 16th ed. (VSN International Ltd., Hempel Hempstead, UK).

Partial least squares (PLS) analyse the relationship between two independent data sets. It is a linear regression model that projects the predicted and measured variables to a new space. Cross-validation estimated the strength of the model. PLS was performed using Unscrambler (X 10.1, Camo AS, Oslo, Norway) (Yeniay and G., 2002).

2.5.1 Sensory analysis

Free-choice profiling

Individual data sets were analysed with one-way ANOVA to assess individual assessor reproducibility. The data sets were analysed with GPA to determine the interrelationship among the sensory characteristics of the six blueberry cultivars evaluated.

Descriptive profiling

Individual data sets were analysed with one-way ANOVA to assess individual assessor reproducibility and to define significant differences between cultivars. PCA was used to produce two dimensional product spaces for the mean panel. Significance of attributes was also assessed by one-way ANOVA. Hierarchical cluster analysis was applied to estimate the scoring similarities between assessors using Euclidean distances.

Consumer preferences

The consumer acceptance scores were analysed for cultivars and clusters using one-way ANOVA. Additionally one-way ANOVA was conducted on socioeconomic variables to analyse the impact of socioeconomic variables to consumer preferences. Hierarchical cluster analysis was applied to understand the acceptance of certain cultivars by particular consumers using Euclidean distances. PLS was used to analyse the relationship between the cluster of consumers and sensory profiling using and PCA was used to detect trends between consumer preferences and sensory profiling.

2.5.2 Analytical analysis

Individual data sets from independent variables were analysed using one-way ANOVA. PCA was applied to detect trends between variables and blueberry cultivars, between variables and harvesting years and between variables and growing locations. Pairwise correlation analysis was applied by Pearson's correlation coefficient for each harvesting year.

2.5.3 Modelling of relation between sensory and phytochemical data

The correlation analysis was applied to determine direct correlations between every variable and every sensory attribute within the cultivars. PLS carried out to determine the relationship between sensory and phytochemical data, and PCA was applied to determine relations between sensory and phytochemical data.

Chapter 3

Genetic and environmental determinants of fruit quality

3.1 Introduction

Sensory analysis is a range of strategies to characterize products or food items with sensory data (sight, smell, taste) obtained from trained and consumer assessors. The evaluation of a product can be done by affective testing, which deals with subjective facts (hedonism), ranking or scaling. The choice of the participants depends on their interest, availability, health and their ability to rank products and identify their threshold. For establishment of a panel of assessors training is required, while consumer assessors only have to understand the present interrogation. Sensory analysis contributes to the quality and the success of the product (Meilgaard et al. 1999).

In the present chapter a range of techniques used to obtain sensory and quality data related to fresh and processed blueberry cultivars is described. In initial work, a questionnaire was designed to obtain basic information on consumer perceptions and for the purpose of recruitment of assessors for additional studies. This was followed by an initial sensory evaluation in which consumers described sensory attributes of blueberries via FCP. This technique provided a lexicon in order to understand the attributes of blueberries by DP using a trained panel. Finally, hedonic scoring was used to define the relationship between sensory attributes and consumer preferences.

3.2 Questionnaire

3.2.1 Introduction

The questionnaire was performed in order to determinate the profile of current and potential blueberry consumer's expectations and additionally consumer expectations on their ideal blueberry; blueberry attributes and recruits assessors for sensory analysis. A questionnaire is very cost effective and easy to analyze. . Due to no verbal or visual clues, there are no influences to the respondent, but well-designed questions are important for high response rates. Short sentences, basic vocabulary and the usage of simple and direct language were used to get as many responses as possible. For delicate questions e.g. ethnic origin, the participants had a choice to refuse to answer (Mellenbergh, 2008).

3.2.2 Results and Discussion

A questionnaire was designed and placed online to obtain information about consumer attitudes to blueberries and to relate consumption to demographics. The survey determined the profile of current and potential blueberry consumer's expectations and additionally consumer expectations regarding their ideal blueberry attributes. Furthermore, the questionnaire was used to recruit potential assessors for sensory analysis. The online survey used for consumer fruit perceptions is attached (Appendix A) and was divided into two parts; the first part referred to the attributes of blueberry and the second part to demographic background.

Analysis of responses to the online survey revealed that consumer's attitude to life was an indicator of consumer perception towards blueberries. 421 consumers at three sites: University of Strathclyde (322 responses); the James Hutton Institute in Invergowrie (79 responses); and the John Innes Institute in Norwich (20 responses) were obtained to the questionnaire with the following results. The respondents were asked about socioeconomic background. The summary statistic of the questionnaire is shown in Table 3.1. More females (71 %) than males (29 %) participated in the online-questionnaire, consumers within different age groups were similarly distributed in this questionnaire. Married/ co-habiting people were more represented

than singles. 58.2 % of the consumers rated their health very good, 39.7 % rated it fair and 2.1 % poor. Non-smokers and optimists were over-represented.

Table 3.1. Number and percentage (%) of participants in the online questionnaire (n = 421).

	Numbers	%
Gender		
female	299	71.0
male	122	29.0
Age in years		
18 – 24	118	28.1
25 – 44	144	34.3
over 45	159	37.6
Domestic status		
married/ co-habiting	248	58.9
single	173	41.1
Health status		
good	245	58.2
fair	167	39.7
poor	9	2.1
Optimist	314	74.5
Pessimist	107	25.5
Smokers		
yes	35	8.4
no	381	90.4
don’t know	5	1.2

Consumers demonstrated a strong liking for blueberries (Table 3.2). Importantly, a considerable proportion of those questioned were prepared to pay a premium for high quality UK grown fruits (Figure 3.1). As these web-based surveys were self-selecting further studies should be conducted in conjunction with multiple retailers to obtain the opinions of a broader cross-section of the UK buying public. More females preferred blueberries and would accept a higher price than males and were also generally more concerned with health than males. This finding was in agreement with previous research that indicated that men’s approach toward food is pleasure orientated, whereas women are more likely to follow nutritional guidelines (Kiefer et al., 2005). Respondents to the web survey regarding attitudes to blueberries were asked about their liking for blueberries and whether they were prepared to pay a

premium for UK grown blueberries. Smokers exhibited a similar optimist/ pessimist distribution contrary to the previous findings of Giltay et al. (2007). Optimists felt in control of their lives and were active in looking for health-promoting strategies, this included consuming blueberries (Lightsey, 1996). Pessimists were less interested and active in controlling their lives and felt less healthy and were less likely to buy blueberries (Kelloniemi et al., 2005). Based on their health orientation, optimists prefer healthy food and implement dietary recommendations, contrary to pessimists who do not believe in a correlation between healthy food and healthiness (Ylöstalo et al., 2003).

Table 3.2. Summary statistic of liking of blueberries and acceptance for higher price for premium UK blueberries with socioeconomic breakdown (n = 421). The percentages of acceptance for higher price for premium UK blueberries is a breakdown of those consumers (75,8 %), who like blueberries.

	Liking of Blueberries [%]	Acceptance of higher prices [%]
Total	75.8	32.3
Gender		
female	53.2	25.0
male	22.6	7.3
Age in years		
18 - 24	18.2	2.7
25 - 44	26.4	13.4
Over 45	21.2	16.1
Optimist	59.1	60.6
Pessimist	16.7	15.2
Smokers		
yes	5.5	5.7
no	69.3	69.5
don’t know	1.0	0.6
Health Status		
good	46.6	54.6
fair	27.5	20.1
poor	1.7	1.1

Respondent answers were broken down according to marital status (Figure 3.1). There was an interaction between marital status and healthy lifestyle (Johansson et al., 1999). In general married/ co-habiting people had a stronger preference for

blueberries than singles, independent of the gender. A higher percentage of married females would accept a higher price for British premium blueberries than unmarried females possibly because they were more focused on healthy food for their family, especially their children. In accordance to Roos et al (1998), married women, especially married women with young children were correlated to health promoting behaviour and followed dietary guidelines more closely than singles. Older age is an additional indicator of healthy lifestyle and awareness (Tinker et al., 2007). Participants between 18 - 24 years had a lower preference for blueberries, possibly because they consume less fruits in general and those that they do consume may tend to be the more common fruits like apples or orange (Anonymous, 2005).

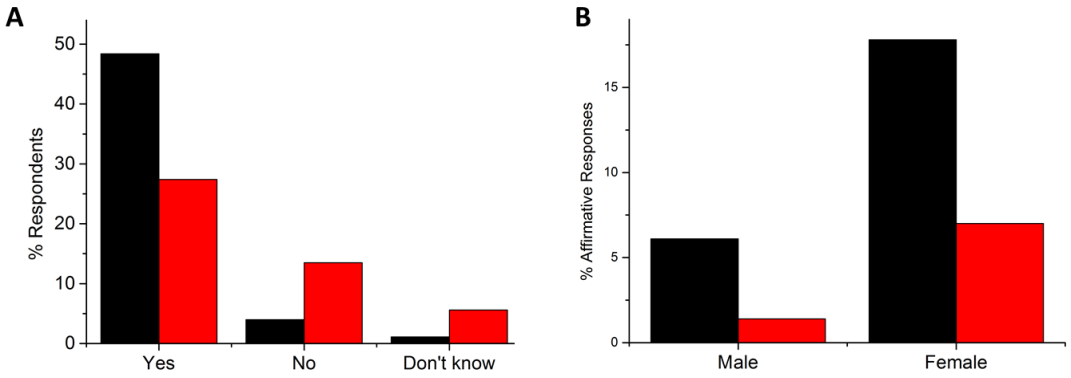


Figure 3.1. Percentage distribution about consumer (n = 421) attitudes to UK blueberries of strong liking of blueberries (A) and willing to pay a higher price for premium high quality UK grown blueberries (B). Married/co-habiting refers to ■ and singles refers to ■.

In Figure 3.2 is shown the frequency of consumption of different types of fruit. Out of ten fruits blueberries were ranked in the lower half by all consumers. Strawberry and raspberry have the highest consumption rate, followed by blueberry and blackberry. In general the soft fruits were consumed monthly or less than once per month. This may relate to cost and availability with seasonal influences. The questionnaire was carried out in February and possibly a repeated questionnaire during the soft fruit season would increase the apparent consumption rate.

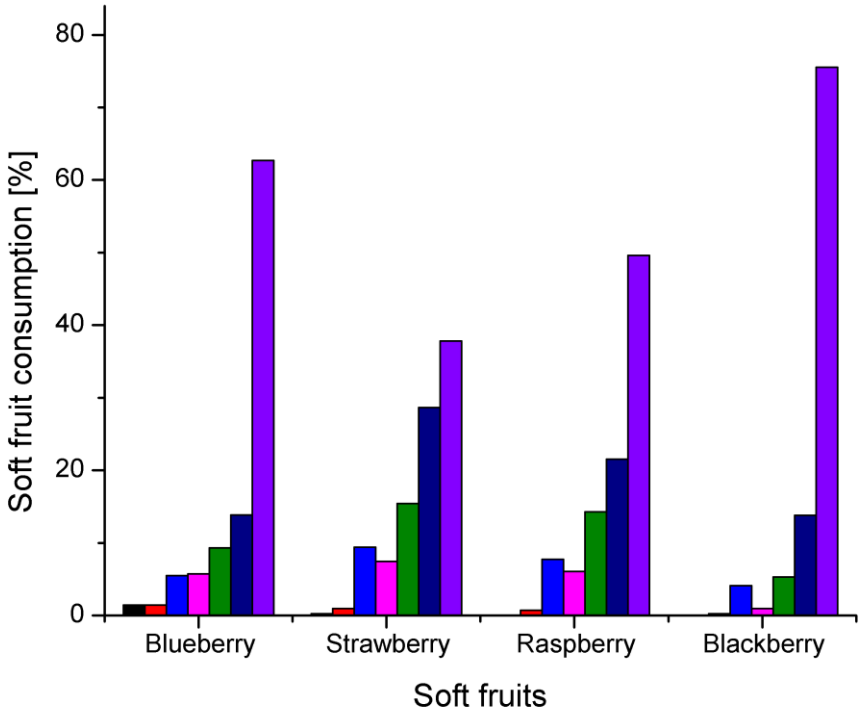


Figure 3.2. Percentage distribution of soft fruit consumption. Every day rated soft fruit consumption refers to ■; 5 to 6 times a week refers to ■; 2 to 4 times refers to ■; once a week refers to ■; fortnightly refers to ■; monthly refers to ■ and less than once a month refers to ■. Numbers of assessors n = 421.

On average the largest portion of consumers (44 %) were willing to pay only £1.00 - £1.49 per 100 g of blueberries (Table 3.3) and nearly 14 % preferred not to specify their willingness to pay a specific price. Overall the data indicated that barriers to consumption of blueberries were price dependent with females were more likely to consume blueberries (Table 3.2) and prepared to pay more than £2.00 per 100 g for premium UK blueberries (Table 3.3). Consumers aged 45 - 60 years were willing to pay a higher price for premium UK blueberries (Table 3.2); but only 2 % of them would pay £2.00 - £2.49 per 100 g compared with around 19 % of younger age groups (Table 3.3). Health status correlated with a willingness to pay a higher price for premium blueberries (Table 3.2). 20 % of the groups reporting good and fair health status would pay £2.00 - £2.49 per 100 g, while only 0.7 % of the poor health status group accepted this price (Table 3.3). Single people and those reporting a pessimistic outlook were less likely to pay £2.00 - £2.49 per 100 g for blueberries.

Table 3.3. Summary statistic for an acceptable price for premium high quality UK grown blueberries per 100 g with socioeconomic breakdown (% of respondents, n = 421).

	Willingness to pay [%]			
	£1.00 - £1.49	£1.50 - £1.99	£2.00 - £2.49	> £2.50
Total	44.3	17.7	21.1	3.0
Gender				
male	11.2	3.7	6.1	0.8
female	33.0	14.0	15.0	2.2
Age				
18 - 24	12.6	4.9	9.7	1.5
25 - 44	12.6	6.1	9.5	1.5
over 45	19.0	6.7	2.0	0.0
Domestic status				
married/co-habiting	26.5	9.4	13.6	0.8
single	17.8	8.1	7.3	2.2
Optimism	32.1	13.8	16.5	1.8
Pessimism	12.2	3.9	4.6	1.2
Health status				
good	25.1	11.5	12.2	1.9
fair	18.0	6.2	8.2	1.1
poor	1.2	0.0	0.7	0.0

In addition to the collection of data regarding consumer attitudes to blueberry purchase and consumption, respondents were asked to describe the ideal blueberry in terms of appearance, aroma and taste (Figure 3.3). Among responses the most frequently mentioned attributes were sweet (15 %), juicy (11 %) and firm (9 %). Only 123 respondents (29 %) answered the question, presumably the remainder had no clear expectations of ideal blueberries. Due to frequency of mention of appearance attributes (62 %), this suggests that consumers found it easier to describe the ideal blueberries in terms of appearance rather than aroma and taste (38 %). Juiciness and firmness were frequently mentioned together suggesting that the degree of firmness is very important to customers. Consumers did not use highly specific attributes with respect to flavour, instead using terms like flavourful and fresh. There were no significant ($p < 0.05$) differences in terms of attributes between the genders. Overall, cultivars that are sweet, not too soft and still juicy were suggested as an ideal blueberry.

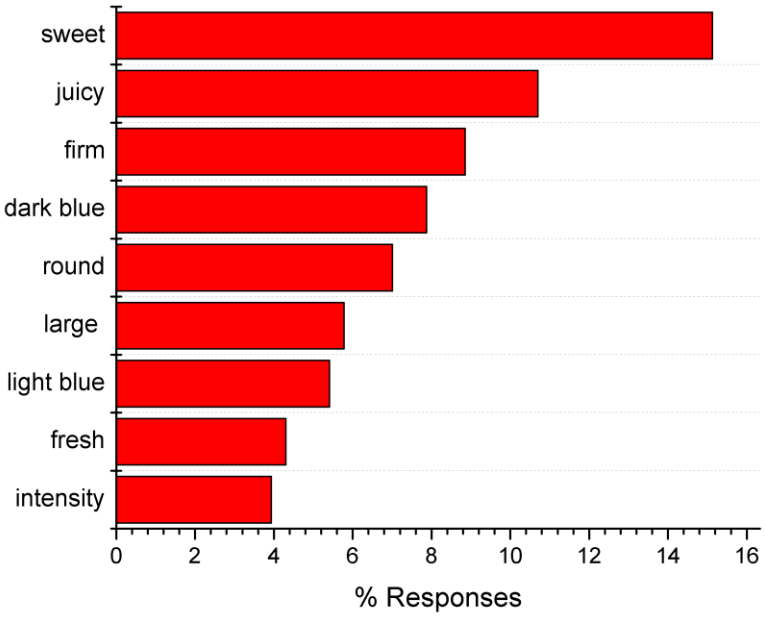


Figure 3.3. Percentage distribution of the most frequently elicited descriptors for "the ideal blueberry".

A further question investigated the impact of food neophobia. Neophobia is defined as a rejection to unfamiliar food. The study of neophobia and neophilia based on the research of Henriques et al. (2009), where a positively correlation to fear and anxiety and negatively with food familiarity and sensation seeking were measured. The willingness of trying new food can be increased by tasting, nutritional or usage information (Tuorila et al., 2001). These statements are modified according to blueberry consumption. The selected statements 2, 3 and 6 associated to neophobia; 1, 4 and 5 to neophilia (Table 3.4).

Table 3.4. Characterisation of neophobic and neophilic modified from Tuorila et al. (2001). The order of the statements in the questionnaire (Apendix A) refers to the numbers in the table.

Neophobic	Neophilic
2. I do not trust new fruits. 3. If I am not familiar with a fruit, I won`t try it. 6. I am very particular about the fruits I will eat.	1. I am constantly sampling new and different fruits. 4. I like fruits from different countries. 5. I like to taste new fruits.

Table 3.5 illustrates the results of the scoring on a five-point scale from strongly agree to strongly disagree. The low mean score indicated that the participants were open-minded to novel foods (neophilic) and significant different ($p < 0.05$) to neophobic. Neophobia could not show any correlation to age, gender, optimist or pessimists, health status and ethnicity. Contrary to previous studies, where correlations were shown between food neophilia and optimism or ethnicity (Raudenbush and Frank, 1999). The health consciousness depended on the gender; females tended to choose healthier food and be more interested in nutrition information (Kiefer et al., 2005) than males. The marital status affects more men than women (Roos et al., 1998). In consent with Henriques et al. (2009), younger people were more neophillic than consumers over 45 years, but no correlation of neophobia to pessimism was found.

Table 3.5. Food neophobia and neophilia scores on a five-point scale from strongly agree to strongly disagree with socioeconomic breakdown.

	Neophilic	Neophobic
Total	2.1	4.0
Gender		
females	2.0	4.1
males	2.0	4.2*
Age		
18 - 24	2.0	4.2*
25 - 44	2.1	4.1
over 45	5.0*	4.0
Ethnicity		
UK	2.2	4.0
Rest of Europe	1.9	4.2*
Asia	2.2	3.7
Domestic status		
married/ co-habiting	2.0	2.0*
single	2.0	2.0*
Optimism	2.1	4.1
Pessimism	2.3	3.4
Health status		
good	2.0	4.1
fair	2.3	3.8
poor	2.1	3.3

* superscript indicates significant differences at $p < 0.05$ within each group.

3.2.3 Summary

Responses to the questionnaire were received from 421 consumers at three sites. Blueberries were generally consumed for health benefits. Consumers aged 18 – 24 years were least likely to purchase blueberries and males less than females. Married/ cohabiting were more likely to purchase than single. Of 10 fruits blueberries were ranked in the lower half by all consumers but this was not related to neophobia. Females especially cohabiting were more likely to consume blueberries and be prepared to pay a premium for UK fruit. The ideal blueberry was: large, sweet, firm but juicy, blue and fruity in character. On average consumers were prepared to pay £1.00 - £1.50 per 100 g.

3.3 Free-choice profiling

3.3.1 Introduction

FCP supplies information about how people judge blueberries; the frequency distribution, sensory characteristics, range of perception and the product space. It additionally generates a vocabulary for DP. The selection of participants depended on the interests of the product and their availability.

FCP generates attributes without the requirement for intensive training in advance. It is an inexpensive and rapid method, based on short introduction (Meilgaard et al. 1999). The technique allows the participants to use as many attributes as they need for the description of the product without the requirement for an explanation of the exact meaning (Williams and Langron, 1984). It is assumed that the perceptions of the panelists are similar and that they only differ in the descriptive terms used. This sensory evaluation technique supplied important information on perceived sensory characteristics, determined the relationships between sensory characteristics and consumer preferences (Jack and Piggott, 1991). Furthermore the socio-economic groups like gender, age, education level and nationality can be crucial for the choice of the panelist.

GPA was used to evaluate data from FCP to summarise individual evaluations into a consensus space (Oreskovich et al., 1991, Deliza and MacFie, 2005, Lachnit et al., 2003). The aim was to evaluate how consumers describe sensory characteristics of blueberry cultivars, to identify the relationship between blueberry cultivars and sensory characteristic and to provide information about the use of descriptors for DP.

3.3.2 Results and Discussion

3.3.2.1 Lexicon

The attributes were divided by appearance, aroma and taste. The 25 assessors used the most descriptors to characterise appearance, followed by taste and aroma (Table 3.6). The dominant characters were large, sweet aroma and sweet taste.

Table 3.6. Frequency of the elicited descriptor for appearance, aroma and taste in blueberries by 25 assessors.

Attributes	No. of responses	Attributes	No. of responses	Attributes	No. of responses
Appearance		*Aroma*		*Taste*	
large	16	sweet	18	sweet	19
round	13	intensive	14	intensive	13
purple	12	fruity	12	sour	13
blue	11	rich in aroma	9	bitter	8
small	11	sharp	8	thick	6
juicy	10	sour	8	fruity	5
oval-shaped	10	earthy	5	tangy	5
matt	6	musty	5	viscous	5
soft	5	citric	3	acidic	3
black	3	grassy	3	bland	3
ripe	3	woody	3	rich in flavour	3
sheen	3	acidic	2	smooth	3
smooth	3	flowery	2	aftertaste	2
squishy	3	heavy	2	orange	2
firm	2	like wine	2	powdery	2
pale	2	over-ripe	2	sharp	1
big bloom	1	berry	1	berry	1
big stem spot	1	bitter	1	blackcurrant	1
brown spots	1	black currant	1	chunky	1
brown stem	1	blueberry like	1	creamy	1
defrosting	1	ginger aroma	1	heavy	1
dented	1	orange	1	like wine	1
flat	1	pear note	1	medicinal	1
marks on fruit	1	pungent	1	musty	1
moist	1	soft	1	raspberry	1
plum colored	1			refreshing	1
plump	1			solid particles	1
puckered	1			sticky	1
structured bloom	1				
wizened	1				

In terms of appearance and taste most of the participants described them with 6 - 10 attributes, while using 0 - 5 attributes to describe aroma (Figure 3.4). The 25 assessors used a total of 127 terms for appearance, 107 for aroma and 104 for taste (Table 3.6). A difficult interpretation occurred for terms that were only used by one

assessor. A variety of terms were used by more than one assessor. Only a minority group used more than 10 attributes. Following discussions with the assessors it became clear that some attributes had the same meaning such as sharp and sour aroma. Fruity taste was less mentioned than fruity aroma. The terms sweet, sour, intensity, large and round were used by most of the assessors. Attributes were selected for DP on the basis of the most frequently used descriptors.

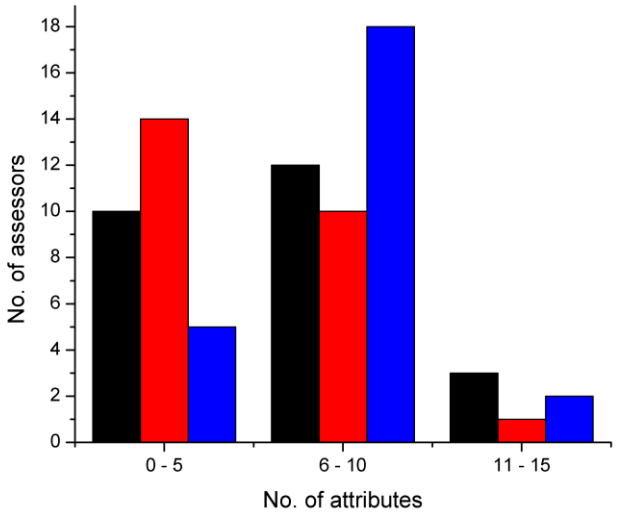


Figure 3.4. Frequency distribution of attributes to describe thawed blueberry samples. The descriptors for appearance refers to ■, for aroma refers to ■ and for taste refers to ■. Numbers of assessors n = 25.

3.3.2.2 Procrustes analysis of variance

25 assessors scored six blueberry cultivars on a 10 cm anchored-scale from absence to intensive against their own developed vocabulary. The untrained panel obtained no training regarding the usage of the scale. The assessors scored non-significant different ($p > 0.05$) between the replicates. GPA was used to analyse the data in order to normalise the different usage of the scale by different assessors. The individual data sets were averaged after rescaling and rotation to obtain a consensus matrix. The consensus matrix was plotted by PCA to reduce the dimensionality of the data matrix with a minimum loss of information. The PCA plot illustrated the blueberry cultivars and attributes in a product space. The most mentioned were used to visualize the relationships between attributes and blueberry cultivars.

3.3.2.2.1 ***Procrustes analysis of within each attributes class***

The two dimensional consensus spaces present the interrelationship among the six blueberry cultivars evaluated. Hereby the position of the samples and distances to each other were used to evaluate the data. Samples were considered similar if they located together in the two dimensional PCA plot, while samples that were more distantly located were considered different (Naes and Risvik, 1996). Samples that appear close to the centre of the plot cannot be compared. It is suggested that such samples were scored differently by the individual assessors. A large percentage of consensus variance for samples indicated an agreement regarding sample scores by the assessors; while high within variance indicated a low agreement with others due to different scoring. Attributes with large distances to the center had a large value and contained a high correlation factor. Products in an extreme position in the same direction as a given attribute had large values for that attribute, products lying in the opposite direction had low values (Dijksterhuis, 1997).

Several Procrustes analyses were conducted to evaluate the data from the FCP. An initial analysis considered all of the attributes together and this was followed by specific analyses of the individual attribute classes appearance, aroma and taste.

Overall view

The two-dimensional consensus space of six blueberry cultivars is shown in Figure 3.5. The first two principal components amounted for 59 % of total variance. 38 % of total variance was explained by the first dimension with positive scores for AU and LI and negative scores for SP and OZ. Sour taste and bitter taste contributed to positive scores, while sweet taste provided a negative contribution. 21 % of the variance was explained by the second dimension with positive scores for OZ and negative scores for SP. Sweet taste and fruity aroma contributed to positive scores, while sour aroma and round appearance provided a negative contribution.

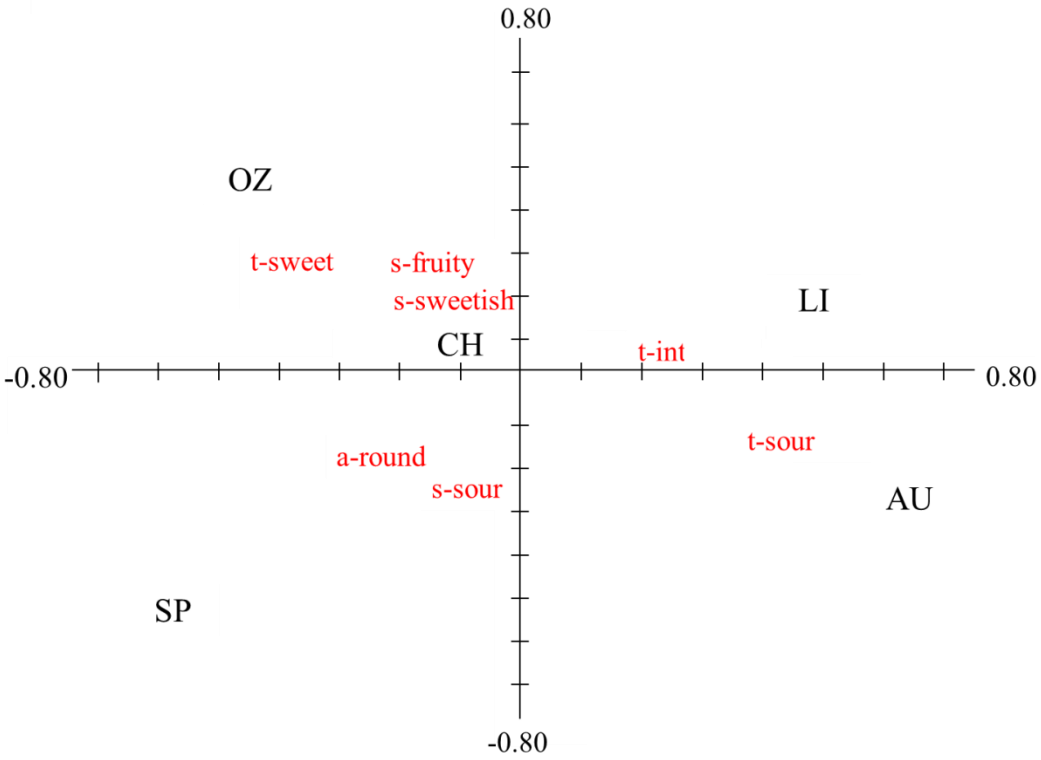


Figure 3.5. Two-dimensional consensus space using Generalised Procrustes Analysis of six thawed blueberry cultivars of all attributes classes. The sensory descriptors were scored on a 10 cm anchored-scale from absence to intensive; a- refers to appearance attributes, s- to aroma attributes and t- to taste attributes. For identification of the blueberry cultivars codes refer to Table 2.2.

The distribution of consensus- and within variance over six varieties of blueberries of all attribute classes is shown in Figure 3.6. SP and AU had the highest consensus variance, followed by LI and OZ. These cultivars were the most consistently scored cultivars by the different assessors. The lowest agreement is shown for DA and CH based on the small amount of consensus variance. The within variance was very similar across all cultivars.

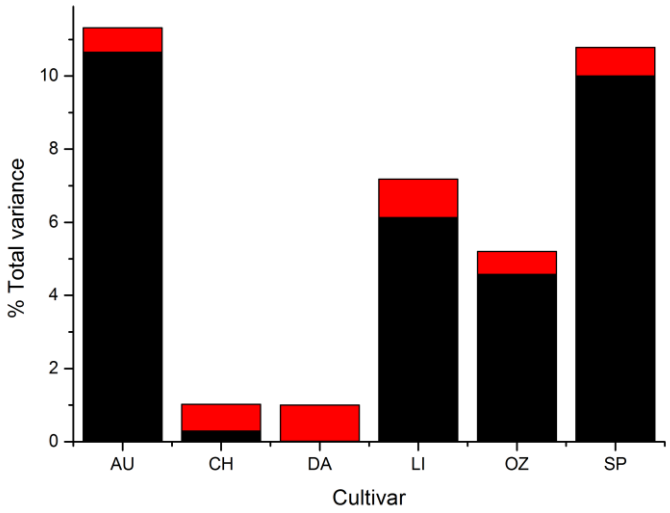


Figure 3.6. Percentages of consensus- and within variance distributed over the six thawed blueberry cultivars of all attribute classes. Consensus variance refers to ■ and within variance refers to ■. For identification of the blueberry cultivars codes refer to Table 2.2.

Appearance

After the overall view of the six varieties of blueberries the individual consideration followed in Figure 3.7. When considering appearance attributes in isolation, the first two principal components amounted for 65 % of the total variance. 46 % of the total variance was explained by the first dimension with positive scores for LI and CH and negative scores for SP. Small size contributed to positive scores and large size and round shape provided a negative contribution. 19 % of the total variance was explained by the second dimension with positive scores for AU and negative scores for LI. Large contributed to positive scores, while small provided a negative contribution.

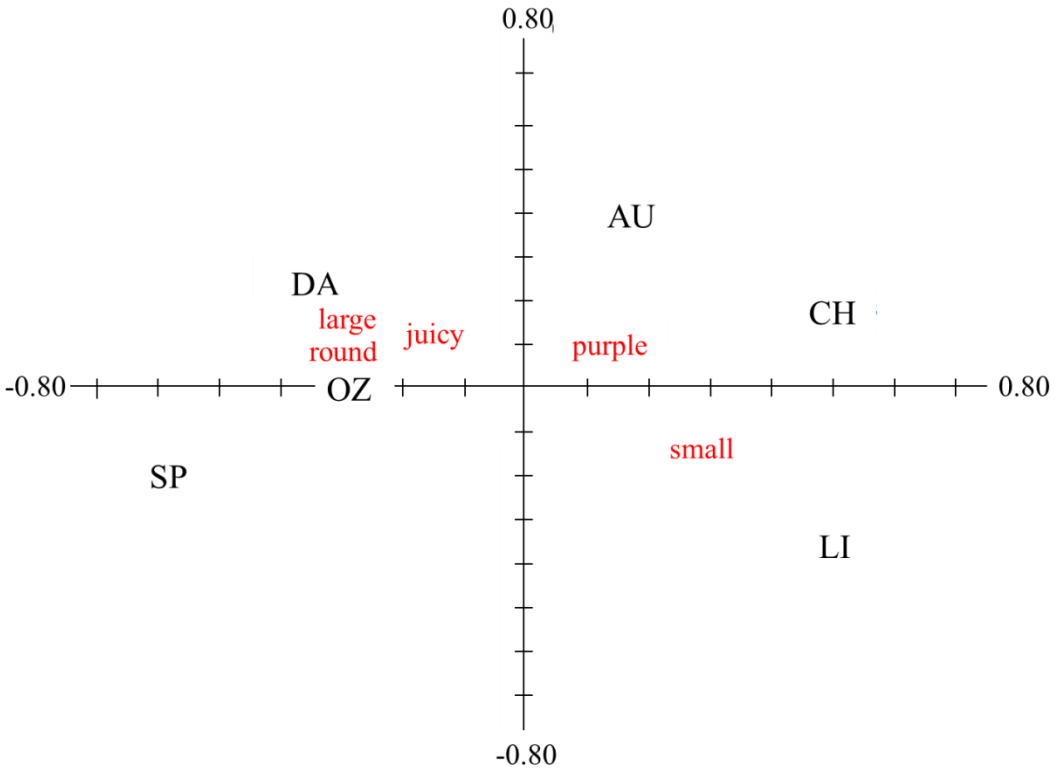


Figure 3.7. Two-dimensional consensus space using Generalised Procrustes Analysis of six thawed blueberry cultivars in terms of appearance. The sensory descriptors were scored on a 10 cm anchored-scale from absence to intensive. For identification of the blueberry cultivars codes refer to Table 2.2.

The distribution of consensus- and within variance over six varieties of blueberries in terms of appearance is shown in Figure 3.8. SP exhibited the highest consensus variance, followed by CH and LI. These cultivars were the most consistently scored cultivars by the different assessors. The lowest agreement was observed in AU based on the small amount of consensus variance. The within variance was similar across all cultivars.

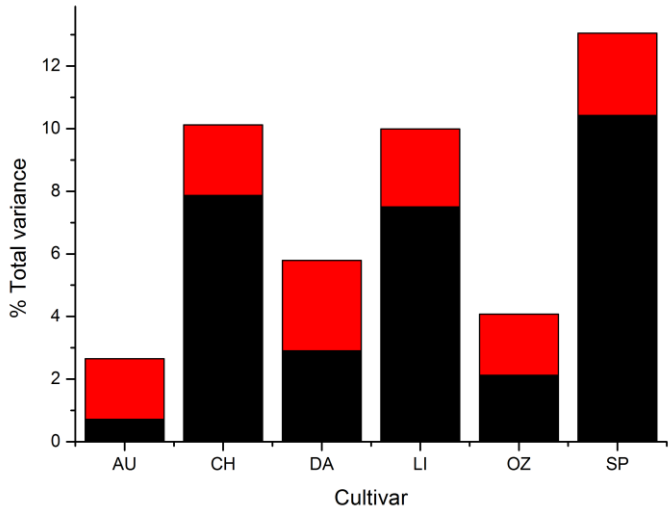


Figure 3.8. Percentages of consensus- and within variance distributed over the six thawed blueberry cultivars in terms of appearance. Consensus variance refers to ■ and within variance refers to ■. For identification of the blueberry cultivars codes refer to Table 2.2.

Aroma

The first two principal components amounted for 71 % of total sample variance. 38 % of the total variance was explained by the first dimension with positive scores for LI and negative scores for SP (Figure 3.9). Fruity contributed to positive scores and sour provided a negative contribution. 33 % of the total variance was explained by the second dimension with positive scores for CH and large negative scores for OZ. Sour contributed to small positive scores, while sweet provided a strong contributor to blueberry characteristic.

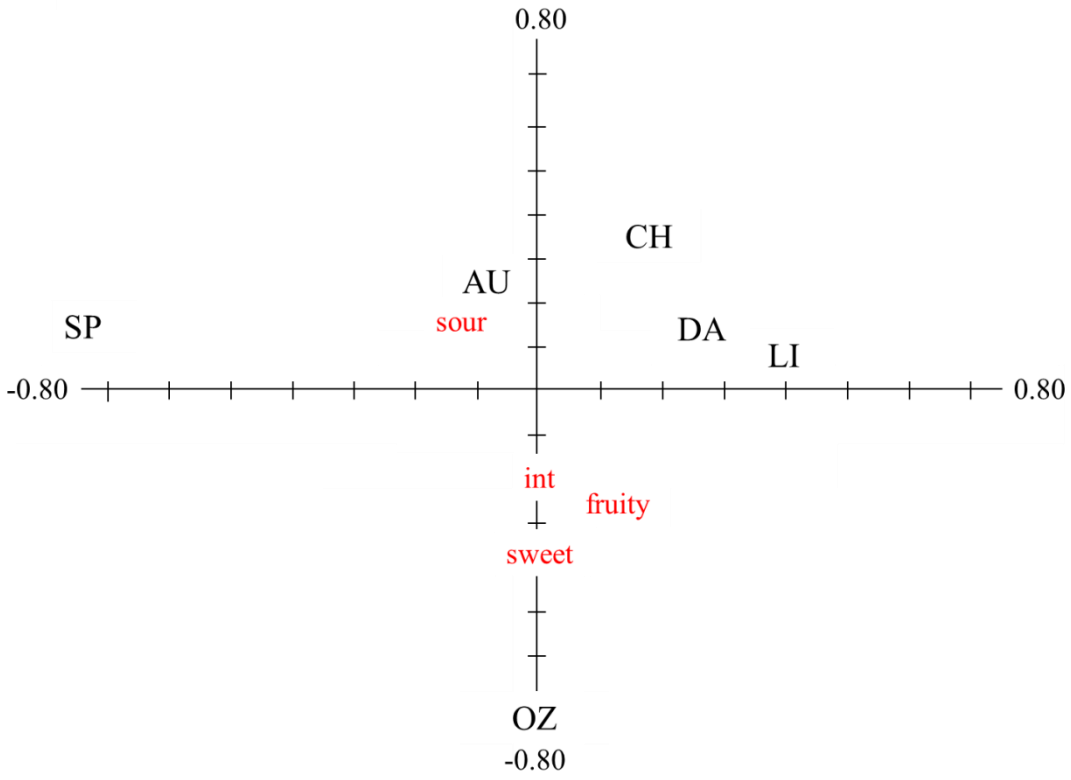


Figure 3.9. Two-dimensional consensus space using Generalised Procrustes Analysis of six thawed blueberry cultivars in terms of aroma. The sensory descriptors were scored on a 10 cm anchored-scale from absence to intensive. For identification of the blueberry cultivars codes refer to Table 2.2.

The distribution of consensus- and within variance over six varieties of blueberries in terms of aroma is shown in Figure 3.10. SP exhibited the highest consensus variance. This cultivar was the most consistently scored cultivar by the different assessors. The remaining cultivars exhibited only a small consensus variance suggesting that only SP displayed a notable aroma. The result corresponded with the assessors discussions that assessors had difficulties in perception of blueberry aroma. The within variance is over the remaining cultivars very similar.

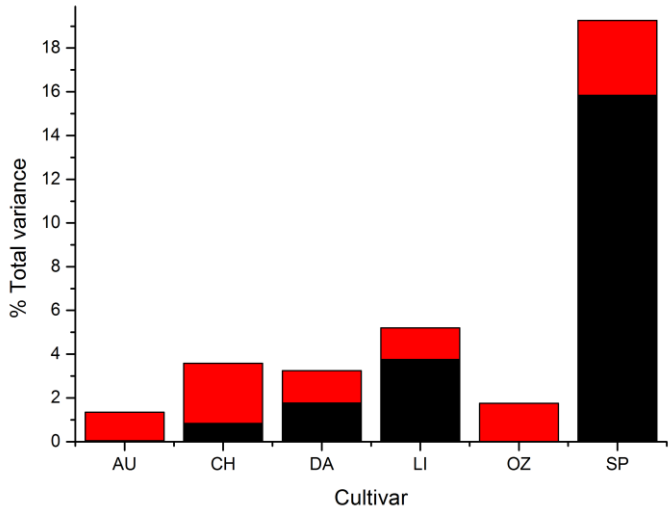


Figure 3.10. Percentages of consensus- and within variance distributed over the six thawed blueberry cultivars in terms of aroma. Consensus variance refers to ■ and within variance refers to ■. For identification of the blueberry cultivars codes refer to Table 2.2.

Taste

The first two principal components amounted for 79 % of the total variance, 63 % of the total variance was explained by the first dimension with positive scores for SP and OZ and negative scores for AU and LI (Figure 3.11). Sweetness contributed to positive scores and sour, bitter and intensive flavours provided a negative contribution. 16 % of the total variance was explained by the second dimension with positive scores for SP and small negative scores for OZ and DA. Sweet, rich and intense provided small negative contributions while watery provided a weakly positive score.

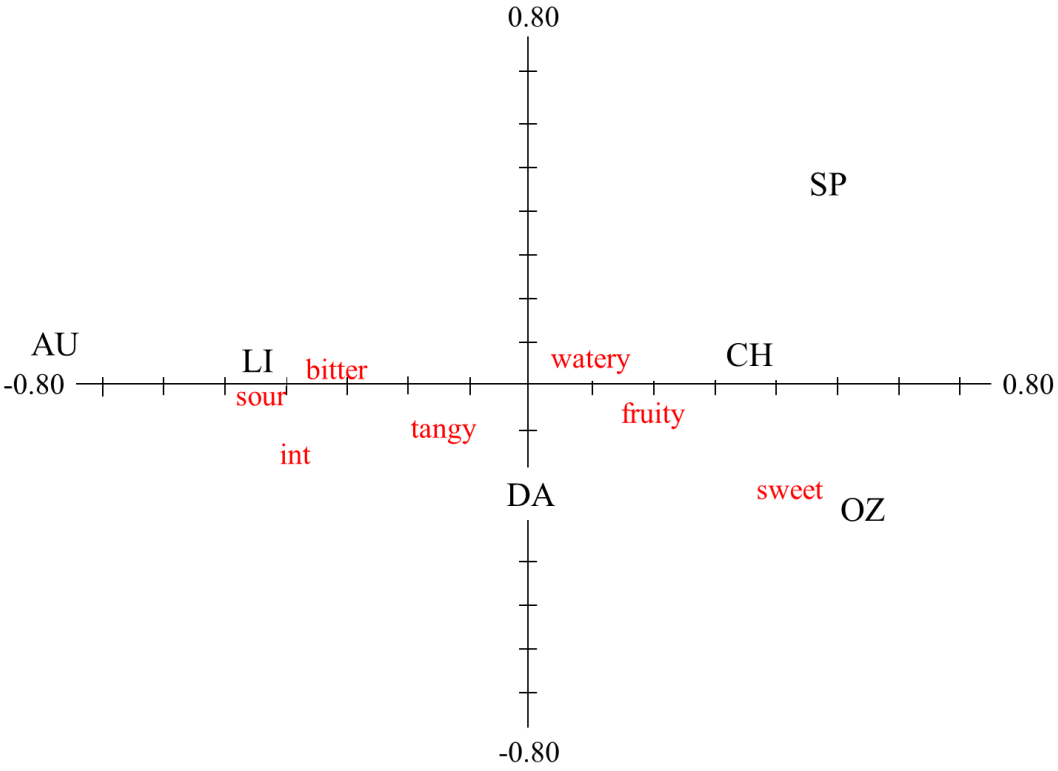


Figure 3.11. Two-dimensional consensus space using Generalised Procrustes Analysis of six thawed blueberry cultivars in terms of taste. The sensory descriptors were scored on a 10 cm anchored-scale from absence to intensive. For identification of the blueberry cultivars codes refer to Table 2.2.

The distribution of consensus- and within variance over six varieties of blueberries in terms of taste is shown in Figure 3.12. AU has shown the highest consensus variance. This cultivar was the most consistently scored cultivar by the different assessors. DA provided a small consensus variance and a large within variance presumably due to less agreement between the assessors.

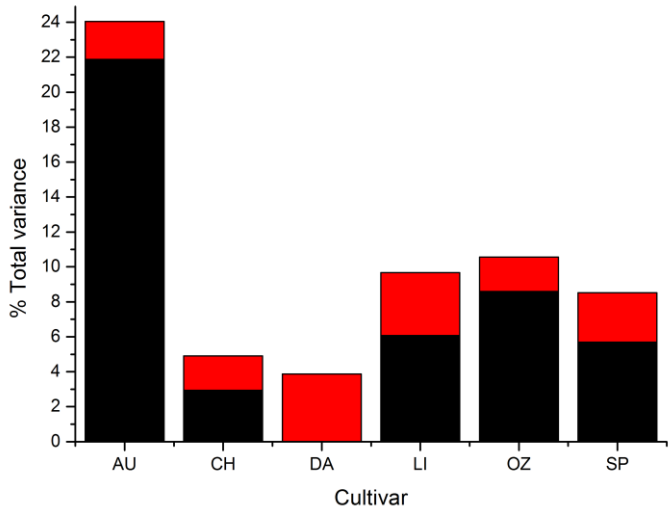


Figure 3.12. Percentages of consensus- and within variance distributed over the six thawed blueberry cultivars in terms of taste. Consensus variance refers to ■ and within variance refers to ■. For identification of the blueberry cultivars codes refer to Table 2.2.

3.3.2.2.2 ***Procrustes analysis of judges***

Overall view

The distribution of the residual variance (within variance) over the 25 judges is shown in Figure 3.13. Judge number 14 represented the highest differentiation from the other judges based on the highest within variance. The highest agreement was between judge numbers 1, 5, 8, 11, 13, 19 and 20, the other judges lie in between.

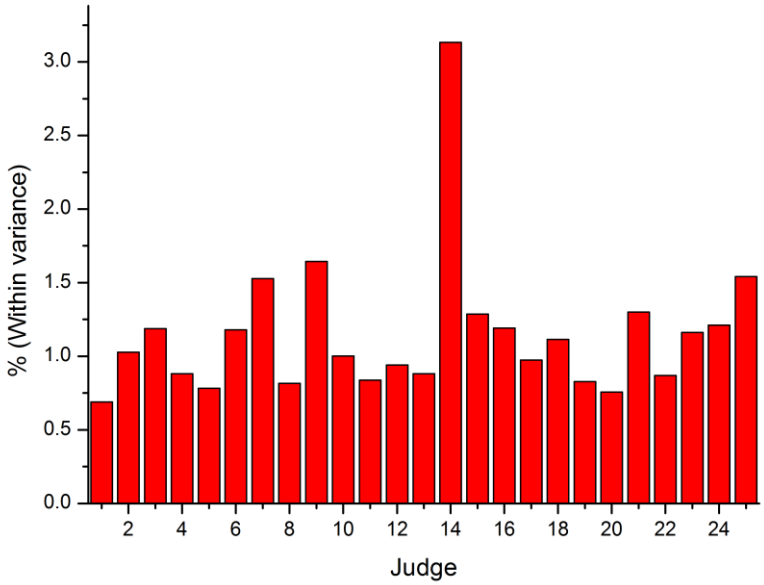


Figure 3.13. Distribution of the residual (within) variance over the 25 judges scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive of all attribute classes analysed with Generalized Procrustes Analysis.

The assessor plot (Figure 3.14) showed a strong consensus without any clusters. The assessor points are spread in the graphic. Assessor 14 differs most from the other assessors based on distribution of the residual variance (Figure 3.13), but did not appear as an outlier on the assessor plot (Figure 3.14). An examination of the individual scoring in terms of appearance, aroma and taste will follow.

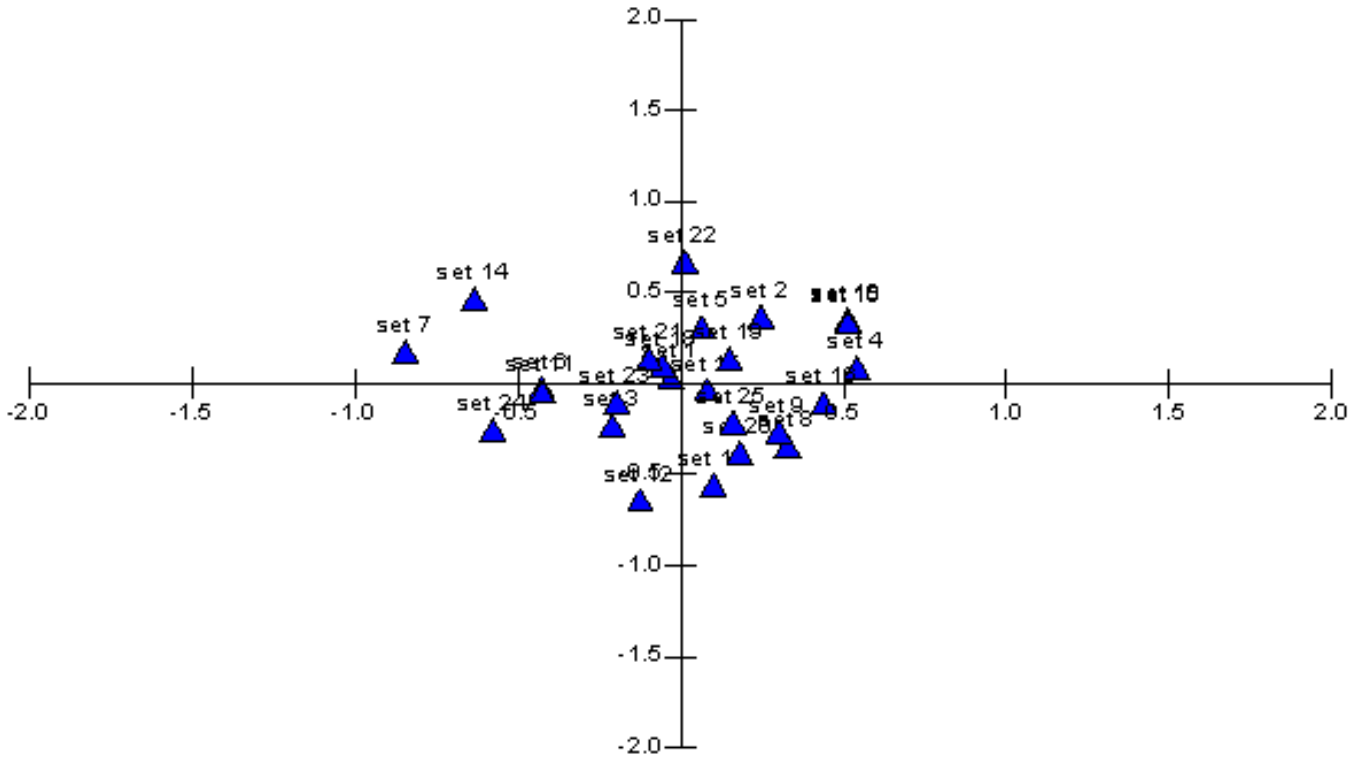


Figure 3.14. Distribution of the 25 assessors scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive in all attribute classes. X- and y-axis correspond to the eigenvalues of assessors calculated with Generalized Procrustes Analysis. ▲ with the corresponding number refers to assessors (set).

Appearance

After the overall view of the judges the individual consideration followed. The distribution of the residual variance (within variance) over the 25 judges according to the scoring in appearance is shown in Figure 3.15. Judge number 22 represented the highest differentiation from the other judges based on the highest within variance. The highest agreement was between judge numbers 5, 8, 11 and 20; the other judges lie in between.

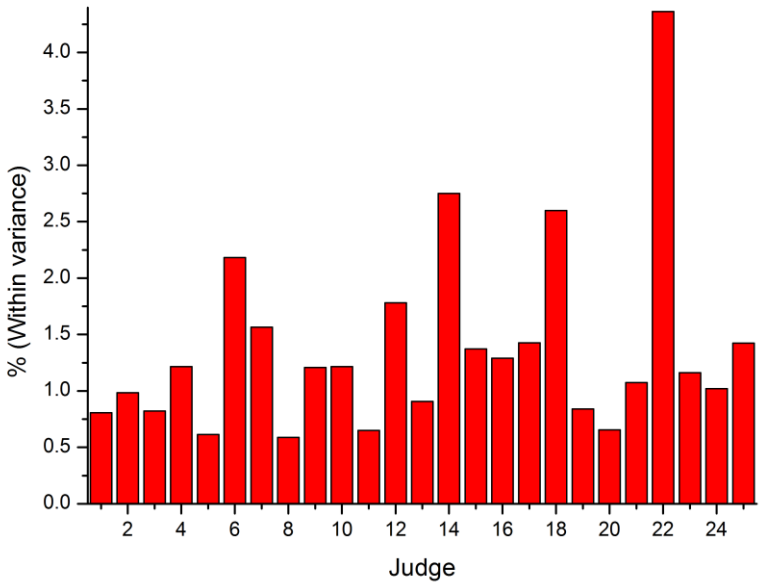


Figure 3.15. Distribution of the residual (within) variance over the 25 judges scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive in appearance calculated with Generalized Procrustes Analysis.

The assessor plot based on the scoring in appearance does not show any differences to the overall assessor plot (Figure 3.16). Assessor 22 differs most from the other assessor based on distribution of the residual variance (Figure 3.15), but did not appear as an outlier on the assessor plot (Figure 3.16).

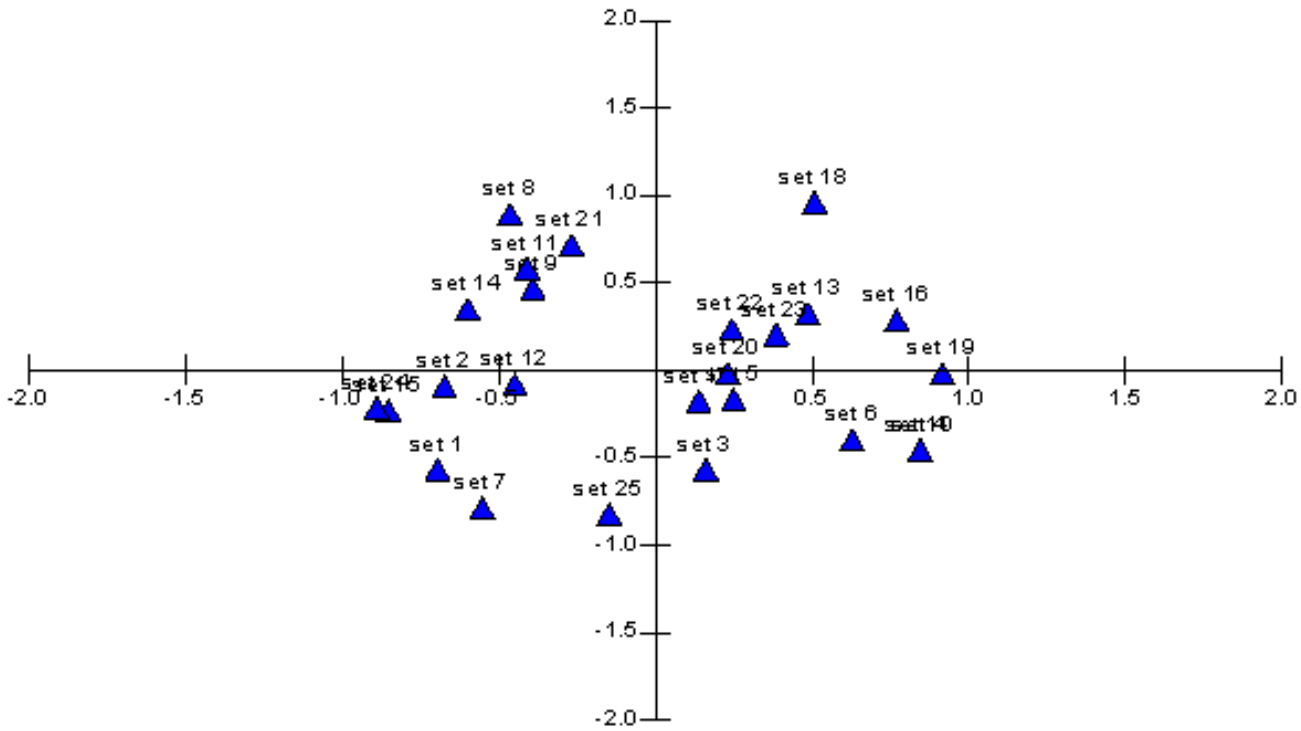


Figure 3.16. Distribution of the 25 assessors scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive in appearance. X- and y-axis correspond to the eigenvalues of assessors calculated with Generalized Procrustes Analysis. ▲ with the corresponding number refers to assessors (set).

Aroma

The distribution of the residual variance (within variance) over the 25 judges according to the scoring in aroma is shown in Figure 3.17. Similar to the overall view, judge number 14 represented the highest differentiation from the other judges based on the highest within variance. The highest agreement was between judge numbers 1, 3, 4, 11, 17 and 20, the other judges lie in between.

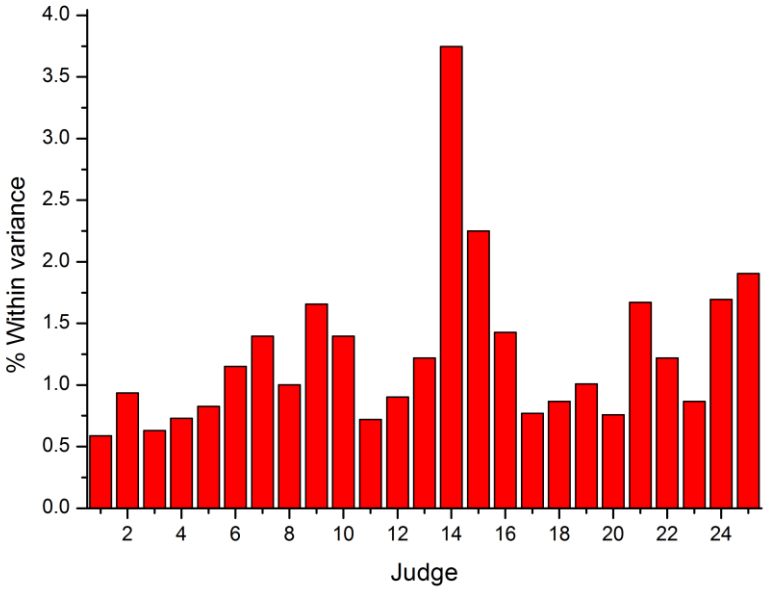


Figure 3.17 Distribution of the residual (within) variance over the 25 judges scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive in aroma calculated with Generalized Procrustes Analysis.

The assessor plot (Figure 3.18) showed no clusters in scoring of aroma. Assessor 14 differs most from the other assessor based the distribution of the residual variance (Figure 3.17), but did not appear as an outlier on the assessor plot (Figure 3.18)

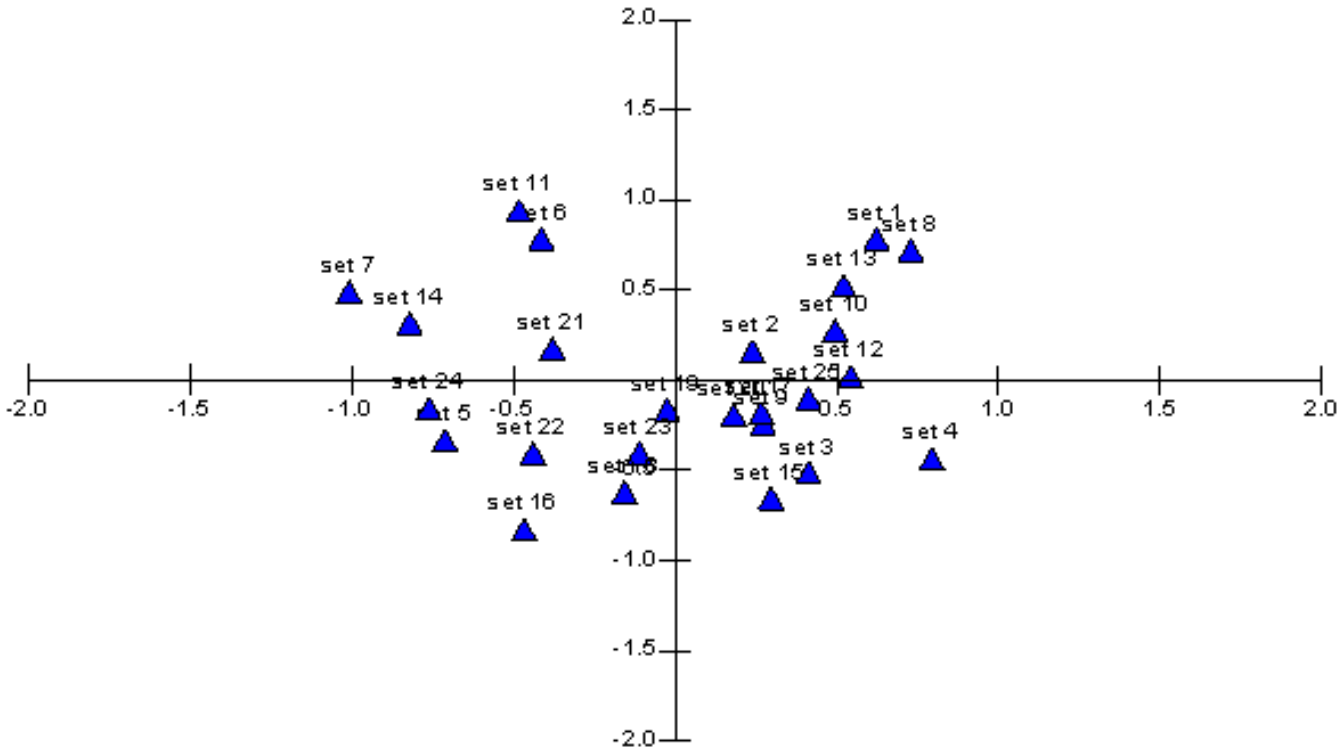


Figure 3.18. Distribution of the 25 assessors scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive in aroma. X- and y-axis correspond to the eigenvalues of assessors calculated with Generalized Procrustes Analysis. ▲ with the corresponding number refers to assessors (set).

Taste

A large percentage of residual variance (within variance) indicated that this judges had a small agreement with the consensus space (Figure 3.19). Similar to overall view and aroma, judge number 14 represented the highest differentiation from the other judges based on the highest within variance. The highest agreements have judges' number 1, 12, 13, 19 and 22; the other judges lie in between.

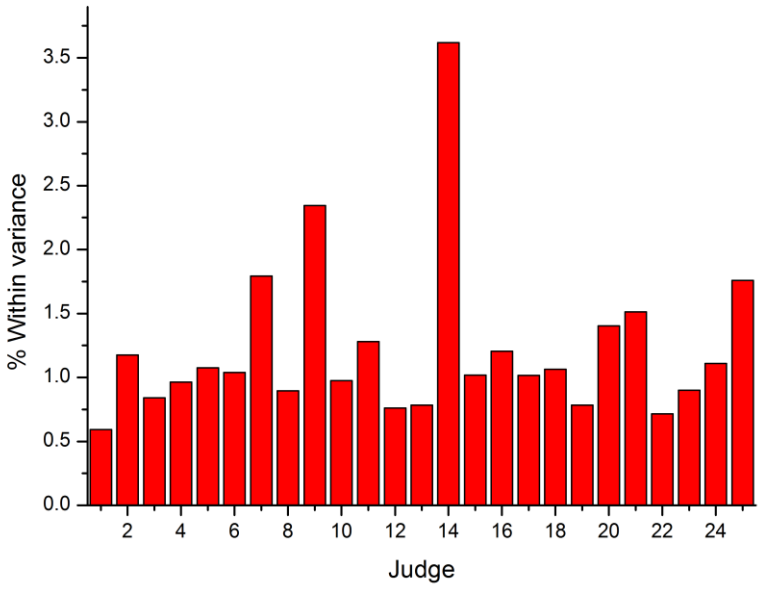


Figure 3.19. Distribution of the residual (within) variance over the 25 judges scored of six thawed blueberry cultivars on a 10 cm anchored-scale from absence to intensive in taste calculated with Generalized Procrustes Analysis.

The assessor plot (Figure 3.20) showed no clusters in scoring of aroma. Assessor 14 differs most from the other assessor based the distribution of the residual variance (Figure 3.19), but did not appear as an outlier on the assessor plot (Figure 3.20).

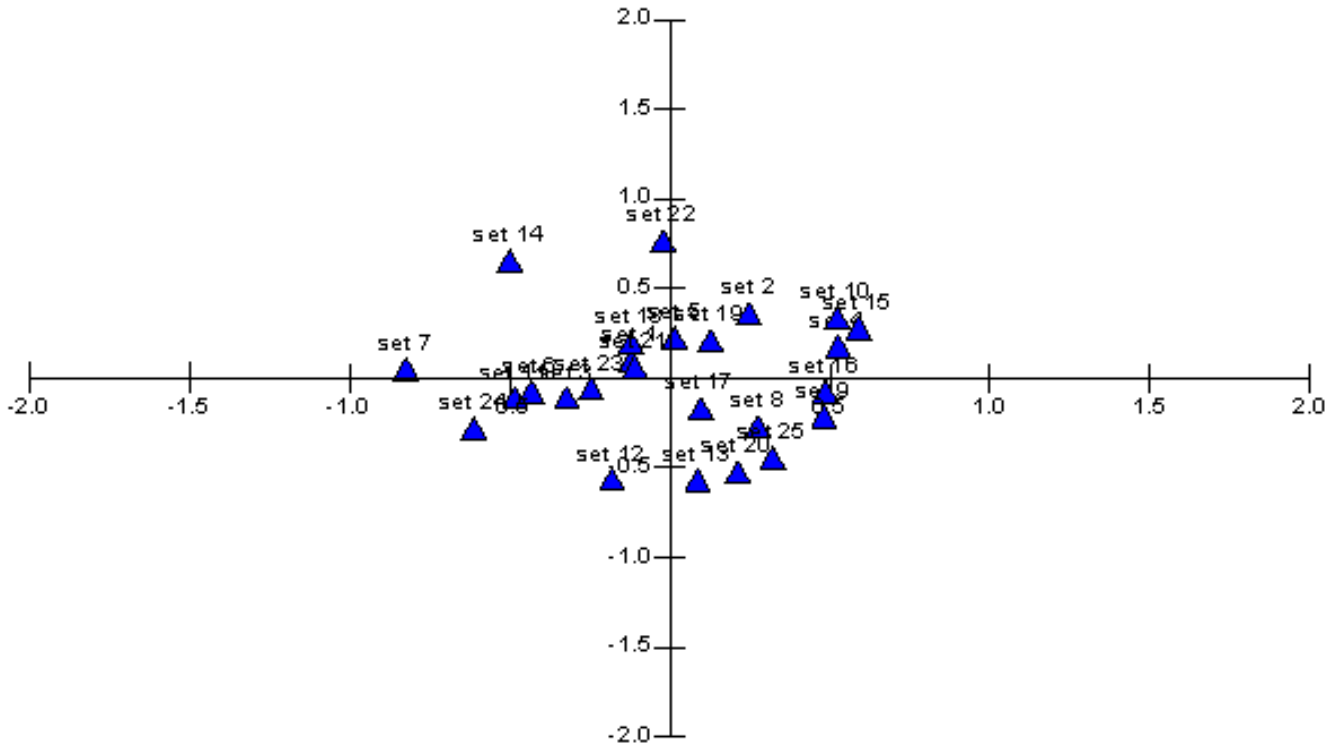


Figure 3.20. Distribution of the 25 assessors scored of six thawed blueberry cultivars on a nine-point scale in taste. X- and y-axis correspond to the eigenvalues of assessors calculated with Generalized Procrustes Analysis. ▲ with the corresponding number refers to assessors (set).

3.3.3 Summary

FCP supplied important information of the interrelationship among the six blueberry cultivars and their sensory characteristics. This sensory test can differentiate and detect relationships between the cultivars. Furthermore FCP is a useful supplement for conventional sensory profiling based on the information provided regarding the use of descriptors. The generated vocabulary with juicy vs. firm, size, red, blue, matt vs. shiny, round vs. oval by appearance, sweetish, fruity, acid and intensity by aroma and sweet, sour, tangy, bitter, fruity, acid, intensity by taste is apply for DP.

The GPA analysis of the FCP data gave the following result; only CH and DA were located in the product space close to the center with the expectation of the scoring in appearance, presumably of low agreements between the panels. In terms of appearance LI and CH were judged as the smallest crop in contrary to SP and DA. The assessors struggled in aroma probably because of their unfamiliarity. Further reasons for this low agreement could be that blueberries are weak in aroma. The provided thawed blueberry cultivars presumably reduced the aroma profile. The chosen descriptors based on frequency usage by assessors and were assumed to be the most important attributes and this was confirmed following discussions with assessors. In agreement with the GPA OZ had the highest scores in sweet and fruity taste, while AU got the highest scores in sour taste. In consideration to the weak aroma profile in blueberry cultivars OZ had the highest scores in sweetish and fruity aroma.

Table 3.7 illustrates the highest mean scores of blueberry cultivar of the selected descriptors as a recommendation for growers. The chosen descriptors were the most frequently used and most important attributes due to discussions with assessors.

Table 3.7. The highest mean scores (n = 2) of highbush blueberry cultivars in selected attributes presented with crosses. The thawed blueberry cultivars grown in 2010 were scored on a 10 cm anchored-scale from absence to intensive. For identification of the blueberry cultivars codes refer to Table 2.2.

Category	Attributes	AU	CH	DA	LI	OZ	SP
Appearance	large			x			
	firm		x				
	round						x
	small				x		
Aroma	sweetish					x	
	fruity					x	
	sour						x
	intensive					x	
Taste	sweet					x	
	intensive	x					
	sour	x					
	bitter				x		
	fruity					x	

GPA illustrated that the lowest agreement between assessors was in terms of aroma. In summary the assessor plot of all attribute classes showed a similar distribution to the attribute classes with agreements on their scoring with the expection in terms of appearance. Judge number 14 represented the highest differentiation from the other judges in all attribute classes, aroma and taste, presumably because it used more terms.

3.4 Descriptive profiling

3.4.1 Introduction

DP defined the intensity of the attributes of blueberry cultivars by a trained panel and established the character based on a generated lexicon from FCP consisting of 17 attributes covering appearance (6), aroma (4) and taste (7). Reference points were required to reduce variability, it depending on the analysing product. For tasting participants used individual booths in order to prevent mutual interaction (Stone and Sidel, 2004). The developed attributes of the trained panel are very complex and difficult to understand for regular consumer. DP is a time-consuming technique, based on a training of a panel (Meilgaard et al. 1999).

Data were analysed using PCA to evaluate the dimensionality and provide an overview of dominant pattern and major trends as visualised in two dimensional spaces. The sensory scoring were analysed for cultivars and consumer effects with ANOVA. The significance of these effects was evaluated using Fisher's protected least significance. The cluster analysis of consumers was applied to estimate the similarity of scoring using Euclidean distances.

The aim was to analyse the sensory properties by descriptive profiling to identify sensory characteristics of different blueberry cultivars and to generate a multivariate product space to describe the relationships between the cultivars.

3.4.2 Method of descriptive profiling of three different years

The DP of blueberry cultivars in three different growing years used a consensus vocabulary to generate a multivariate product space to describe the relationships between the cultivars. Table 3.8 illustrates information regarding the growing location, cultivation conditions and the years when DP was performed. DP was designed to treat a taste panel, thereby reference points were required to reduce variability that was set using a series of sensory standards.

Table 3.8. The used blueberry cultivars for descriptive profiling in three different harvesting years. For identification of the blueberry cultivars codes refer to Table 2.2.

Cultivars	Growing location	Years	Growing conditions
AU	Blairgowrie	2010, 2011, 2012	Polytunnel
BE	Dundee	2010, 2011, 2012	Open Field
BL	Dundee	2010, 2011, 2012	Open Field
BG	Muirhead	2011	Open Field
BR	Blairgowrie	2011, 2012	Polytunnel
CH	Blairgowrie	2010, 2011, 2012	Polytunnel
CHT	Blairgowrie	2012	Polytunnel
DA	Blairgowrie	2010, 2011, 2012	Polytunnel
EL	Blairgowrie	2011, 2012	Polytunnel
LI	Blairgowrie	2010, 2011, 2012	Polytunnel
OZ	Blairgowrie	2010, 2011, 2012	Polytunnel
RE	Blairgowrie	2011, 2012	Polytunnel
TO	Blairgowrie	2012	Polytunnel
PU	Blairgowrie	2012	Polytunnel
SP	Blairgowrie	2010, 2011, 2012	Polytunnel

3.4.3 Results and Discussions

Initial analysis was conducted using one-way ANOVA to define significant differences between cultivars (Table 3.9). The attributes blue, red, sweetish aroma and acid aroma were not significantly different ($p > 0.05$) between cultivars in the three growing years; while fruity and intensive aroma were non-significant ($p > 0.05$) in 2011 and 2012. In agreement to Silva (2005), colour and flavour were non-significant ($p > 0.05$) in highbush and rabbiteye blueberry cultivars. Fruity taste was also not significantly different ($p > 0.05$) between cultivars only in 2010. The assessors scored non-significant different ($p > 0.05$) between the replicates. PCA were used to evaluate data from DP to identify variability in sensory attributes for blueberry cultivars.

Table 3.9. Analysis of variance applied to the sensory scores of descriptive profiling. Eight blueberry cultivars grown in 2010, twelve in 2011 and fourteen in 2012 were scored on a nine-point scale by trained assessors. Values in bold are significant ($p < 0.05$).

Category	Attributes	Cultivars		
		2010	2011	2012
Appearance	large	**<0.001**	**<0.001**	**<0.001**
	oval	**<0.001**	**<0.001**	**<0.001**
	shiny	**<0.001**	**<0.001**	**0.010**
	firm	**<0.001**	**0.013**	**<0.001**
	blue	0.084	0.693	0.950
	red	0.052	0.059	0.990
Aroma	sweetish	0.221	0.481	0.702
	fruity	**0.001**	0.188	0.281
	acid	0.081	0.467	0.328
	intensity	**0.007**	0.260	0.343
Taste	sour	**<0.001**	**<0.001**	**<0.001**
	tangy	**0.005**	**0.027**	**<0.001**
	bitter	**0.019**	**0.032**	**0.009**
	fruity	0.147	**0.045**	**0.002**
	acid	**0.001**	**0.014**	**<0.001**
	intensity	**0.016**	**0.032**	**<0.001**
	sweet	**0.004**	**<0.001**	**<0.001**

Principal component analysis within three different years

The data from the DP were analysed by PCA in three different growing years. The position of the samples in the PCA plots and relative distances to each other were used to evaluate the data. Samples were considered similar if they located together in the two dimensional PCA plot, while samples that were more distantly located were considered different (Naes and Risvik, 1996). Analysis of loading plots allowed identification of the attributes that acted as the drivers of sample variability. Attributes with large negative or positive values on the loading axes had a strong influence in describing differences between samples. Samples in an extreme position in the same direction as a given attribute on a specific axis had large values for that attribute, samples lying in the opposite direction had low values (Graffelman and van Eeuwijk, 2005). The two dimensional consensus spaces presented the interrelationship among the blueberry cultivars.

3.4.3.1 Principal component analysis of blueberry cultivars grown in 2010

The principal component product space of eight varieties of blueberries grown in 2010 is shown in Figure 3.21A. The first two PCs accounted for 54 % of the total sample variance. 33 % of total variance was explained by the first PC. LI, AU and BE are described as firm and bitter and sour flavoured cultivars, while OZ and SP are described as sweet cultivars (Figure 3.21B). 21 % of variance was explained by the second PC. AU is described as oval and tangy, acid, intensive and sour flavoured cultivar, BL is described as sweetisch and intensive aromatic cultivar.

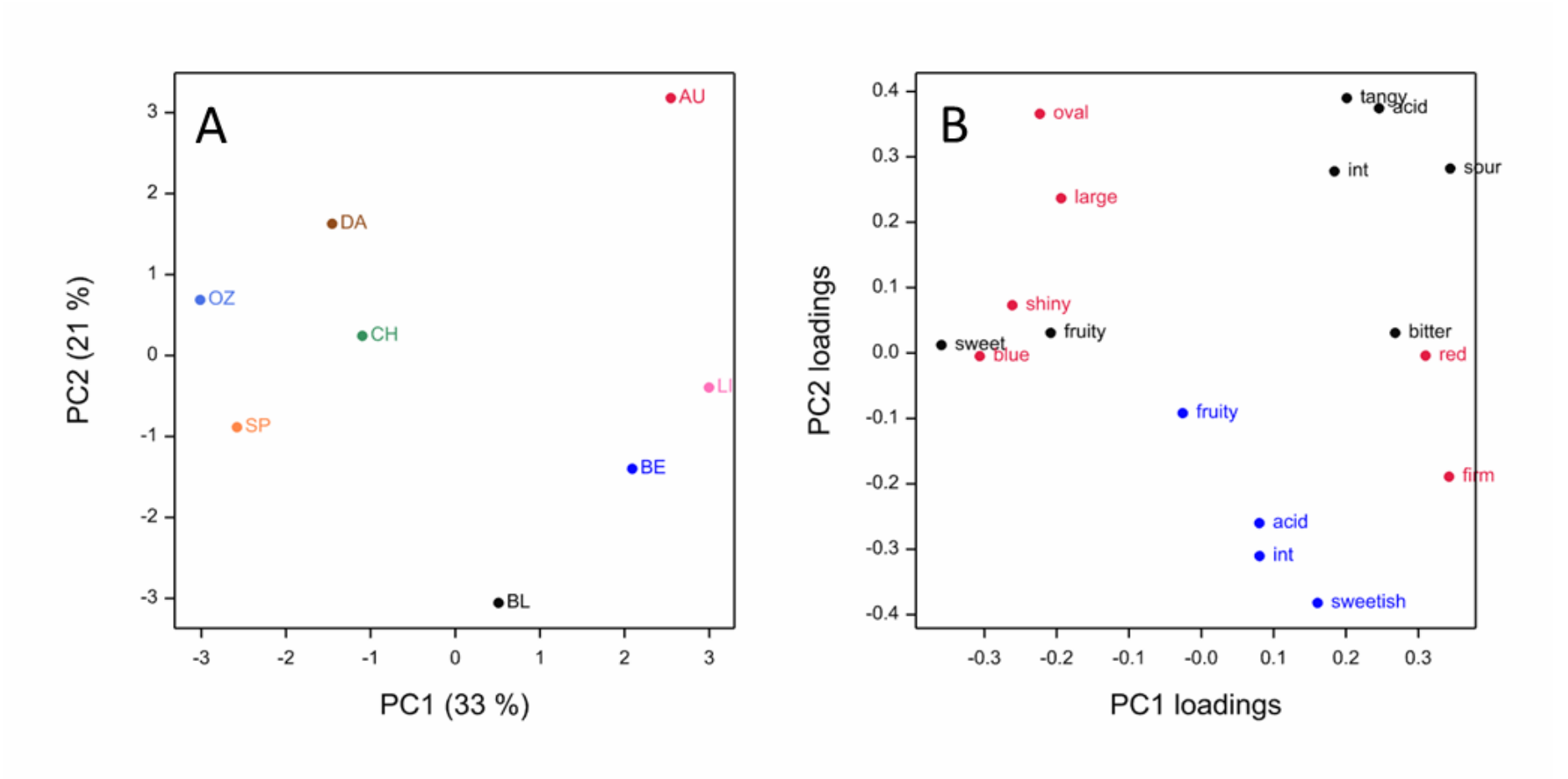


Figure 3.21. Principal component analysis (PCA) of the sensory data from the descriptive profiling of eight thawed blueberry cultivars grown in 2010 scored on a nine-point scale. PCA scores plot (A) and the corresponding PCA loading plot (B, the appearance descriptors refers to ■, the aroma descriptors refers to ■ and the taste descriptors refers to ■). For identification of the blueberry cultivars codes refer to Table 2.2. Int, intensity.

The five highest correlated sensory attributes with extreme positions in the loading plot in the first two PCs in blueberry cultivars grown in 2010 are shown in Figure 3.22. Pearson correlation coefficients r determined the relationships between descriptors. Large size exhibited a negative correlation to firmness (r = -0.853) (Figure 3.22A) and it has previously been reported that larger blueberry cultivars are softer because they contain a higher amount of free water. The free water binds the breakdown products of pectin and hemicellulose (Yu et al., 2012). The selected taste descriptors sour, acid, tangy, intensity exhibited positive correlations (Figure 3.22B-E). Intensity, tangy, acid and sour were highly correlated in the blueberry cultivars, tangy to intensity (r = 0.759), sour (r = 0.777) and acid (r = 0.795) and further acid to sour (r = 0.915). The assessors explained in discussions, that they could not differentiate between the taste descriptors tangy, sour, acid and bitter. In agreement to previous studies; consumers confound sourness with acid, acidic, citric, sharp and bitterness (Luby and Finn, 1986, Ormsby, 2005). Correlations between sensory and chemical data in fruits exhibited a better correlation of titratable acids to flavour intensity than sugars (Lee et al., 2004, Harker et al., 2002, Baldwin et al., 1998).

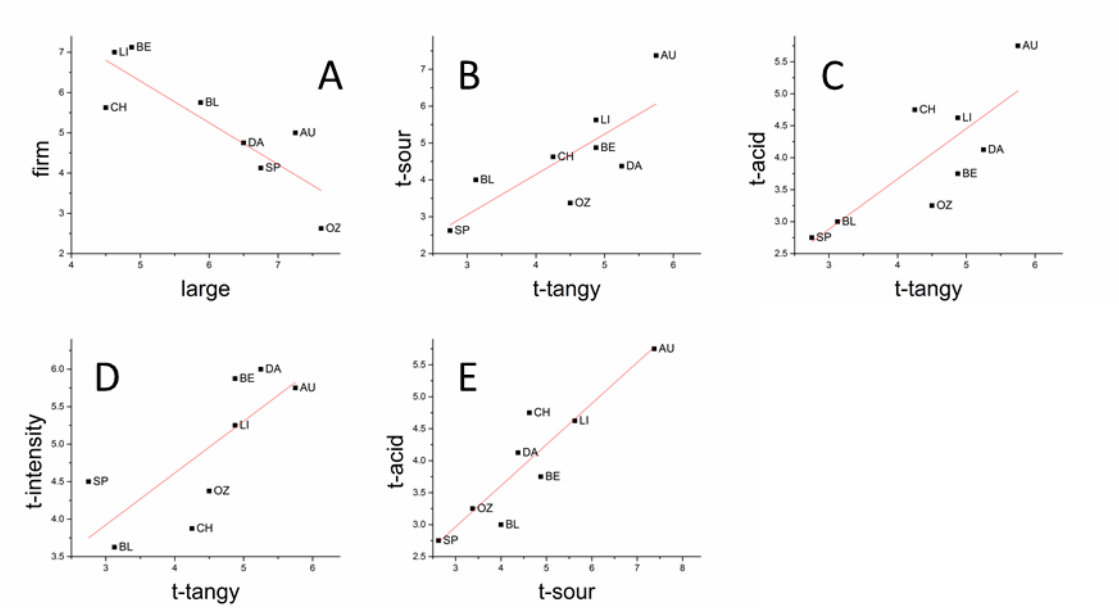


Figure 3.22. Relationship between sensory attributes in eight blueberry cultivars grown in 2010 to present the highest correlated sensory attributes. The sensory attributes were scored on a nine-point scale assessed on thawed blueberries. Correlations between large and firm (r = -0.851) (A), the tangy and sour taste descriptors (r = 0.777) (B), the tangy and acid taste descriptors (r = 0.795) (C), the tangy and intensive taste descriptors (r = 0.759) (D), the sour and acid taste descriptors (r = 0.915) (E); t refers to taste attributes. For identification of the blueberry cultivars codes refer to Table 2.2.

Assessor performance

The cluster analysis determined the similarities of the scoring by the judges using Euclidean distances (Figure 3.23). The x-axis describes the degree of similarity and the y-axis presents the assessors (n = 8). The same numbers on the y-axis presented the same assessor scoring of different cultivars. The assessors scored similar (75 %) to each other, without any outlier. The resulting consistency in scoring of blueberry cultivars indicate that the data can be considered reliable and suggests that assessor training has been of an adequate standard.

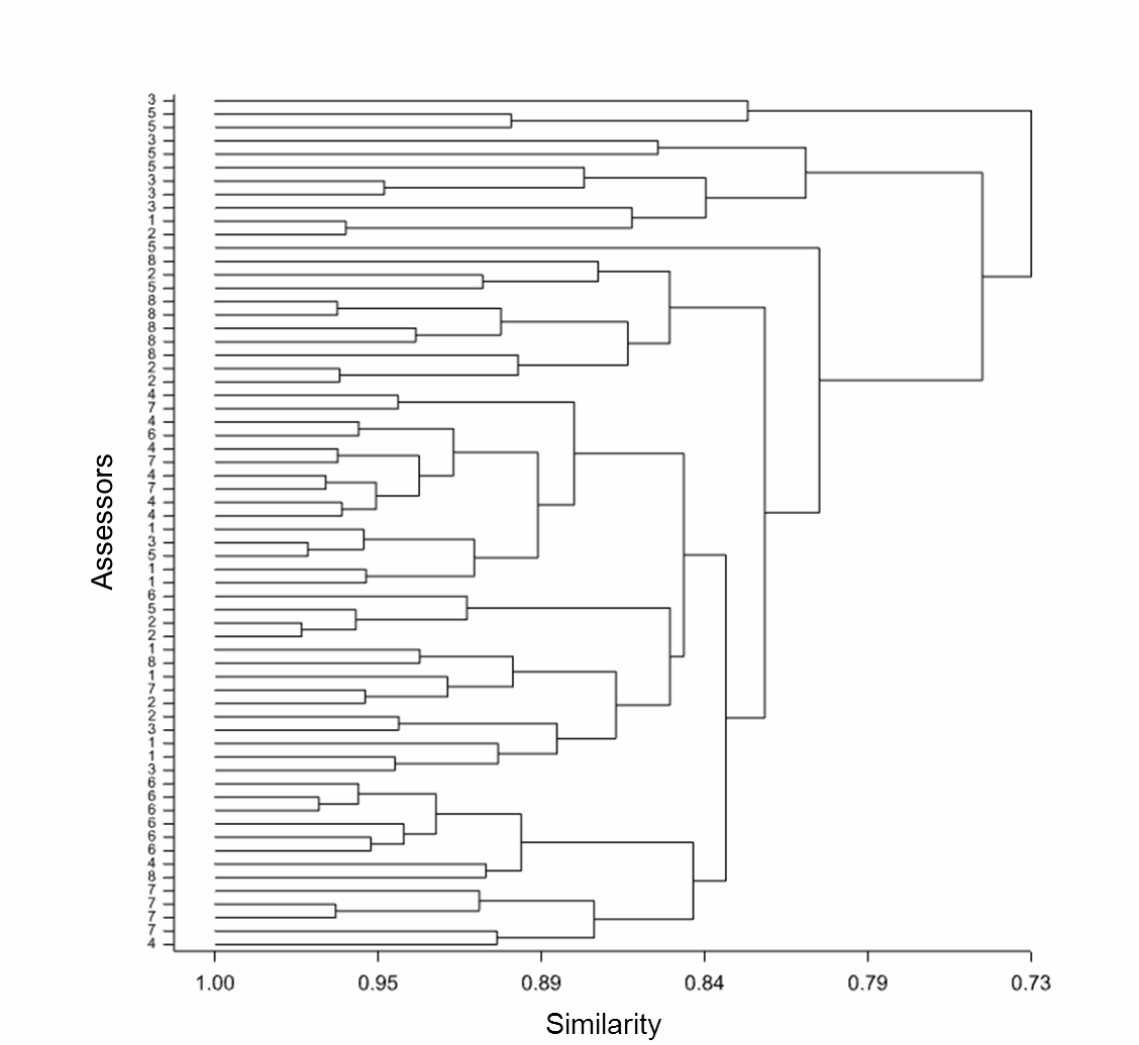


Figure 3.23. Dendogram of hierarchical cluster analysis over the eight judges based on the attribute scoring of eight thawed cultivars grown in 2010 using Euclidean distances to determinate their similarities. The same numbers on the y-axis indicate the same assessor scored of different blueberry cultivars.

3.4.3.2 Principal component analysis of blueberry cultivars grown in 2011

The principal component product space of twelve varieties of blueberries grown in 2011 is shown in Figure 3.24A. The first two PCs accounted for 51 % of the total sample variance. 30 % of total variance was explained by the first PC. BL is described as bitter, tangy and intensive taste flavoured cultivars, while BG is described as large and oval cultivars (Figure 3.24B). 21 % of variance was explained by the second PC. EL is described as sour and acid flavoured cultivars, while BL and RE are described as sweet and fruity flavoured cultivars.

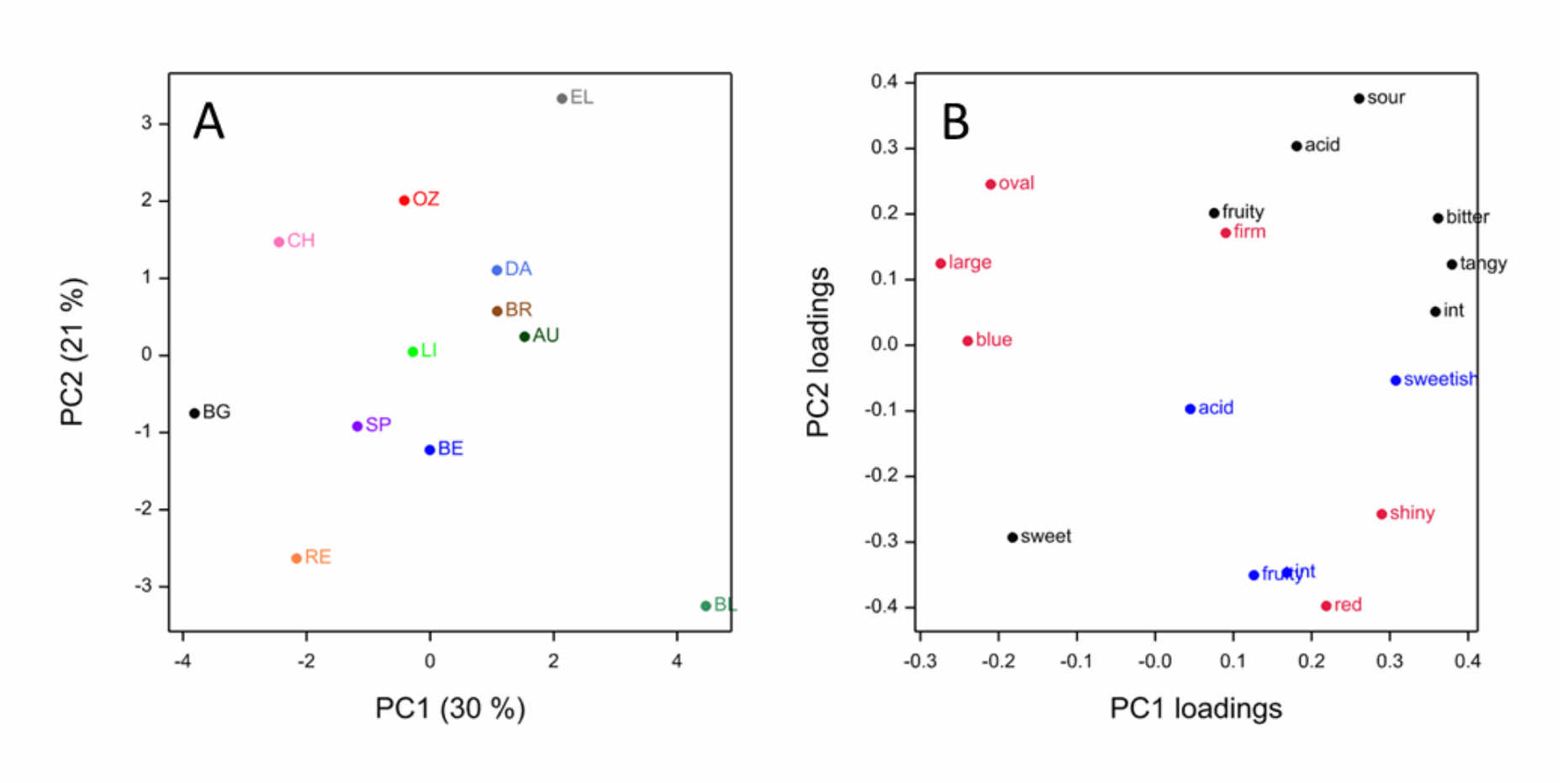


Figure 3.24. Principal component analysis (PCA) of the sensory data from the descriptive profiling of twelve fresh blueberry cultivars grown in 2011 scored on a nine-point scale. PCA scores plot (A) and the corresponding PCA loading plot (B, the appearance descriptors refers to ■, the aroma descriptors refers to ■ and the taste descriptors refers to ■). For identification of the blueberry cultivars codes refer to Table 2.2.

The five highest correlated sensory attributes with extreme positions in the loading plot in the first two PCs in blueberry cultivars grown in 2011 are shown in Figure 3.25. The Pearson's correlations coefficient (r) determined the relationships between attributes for blueberry cultivars in 2011.

Cultivars grown in 2010 and 2011 have shown similar correlations. The selected taste sour, acid, tangy, intensity descriptors exhibited positive correlations (Figure 3.22A-D). The taste attributes intensity, tangy, bitter and sour were highly correlated in the blueberry cultivars, tangy to bitter (r = 0.722), bitter to intensive (r = 0.705) and sour to bitter (r = 0.715). Similar to the previous harvesting year the assessors explained in discussions, that they could not differentiate between the following taste descriptors tangy, sour, acid and bitter. The sour taste descriptor exhibited an expected negative correlation to sweet taste (r = -0.721) (Figure 3.25E). Correlations between sensory and chemical data have shown an inverse relationship between acid content and sweet scoring in sea buckthorn berries (Tiitinen et al., 2005).

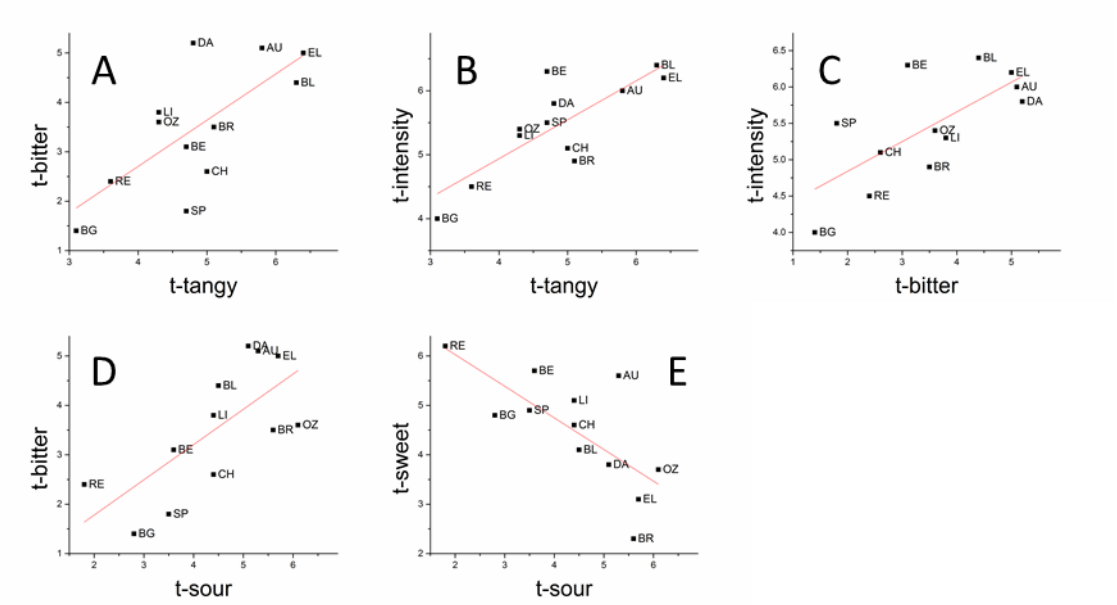


Figure 3.25. Relationship between sensory attributes in twelve blueberry cultivars grown in 2011 to present the highest correlated sensory attributes. The sensory attributes were scored on a nine point scale assessed on fresh blueberries. Correlations between the tangy and bitter taste descriptors (r = 0.722) (A), the tangy and intensive taste descriptors (r = 0.811) (B), the bitter and intensive taste descriptors (r = 0.705) (C), the sour and bitter taste descriptors (r = 0.713) (D) and the sour and sweet taste descriptors (r = -0.721) (E); t refers to taste attributes. For identification of the blueberry cultivars codes refer to Table 2.2.

Assessor performance.

The cluster analysis determined the similarities of the scoring by the judges using Euclidean distances (Figure 3.26). The x-axis describes the degree of similarity and the y-axis shows assessors (n = 8). The same numbers on the y-axis presented the same assessor scoring of different cultivars. The assessors scored similar (81 %) to each other, without any outlier. The resulting consistency of scoring of blueberry cultivars indicated that the data can be considered reliable, suggested due to a well-received training. Despite different assessors between the harvesting years 2010 and 2011, the assessors' performance was similar.

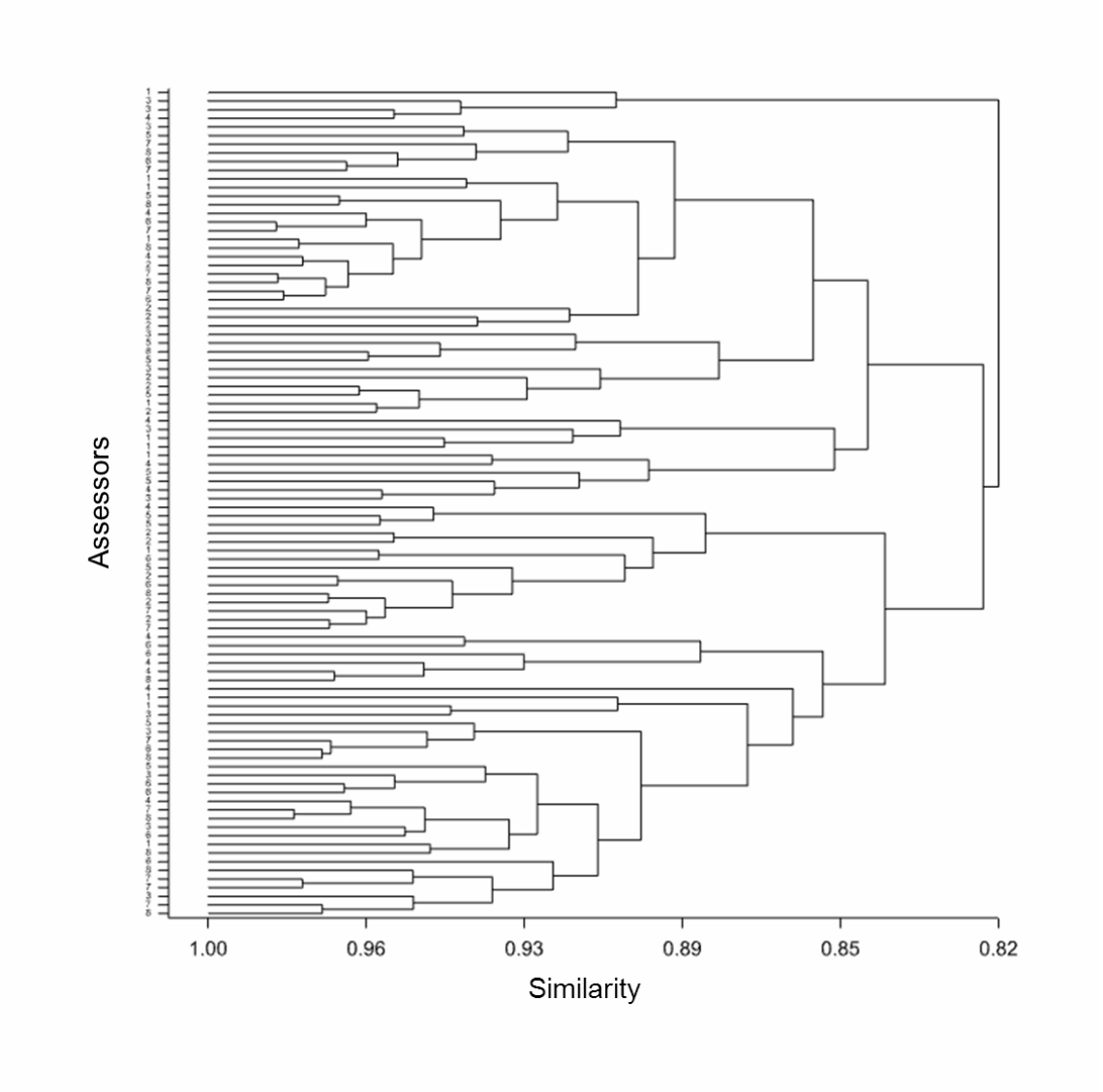


Figure 3.26. Dendogram of hierarchical cluster analysis over the eight judges based on the attribute scoring of twelve fresh cultivars grown in 2011 using Euclidean distances to determinate their similarities. The same numbers on the y-axis indicate the same assessor scored of different blueberry cultivars.

3.4.3.3 Principal component analysis of blueberry cultivars grown in 2012

The principal component product space of fourteen varieties of blueberries grown in 2012 is shown in Figure 3.27A. The first two principal components accounted for 56 % of the total sample variance. 30 % of total variance was explained by the first PC. TO, BL and RE are described as oval and fruity and intensive flavour cultivars, while BR and CH are described as sweetish and acid aromatic cultivars (Figure 3.27B). 26 % of variance was explained by the second PC. BE is described as intensive and sweetish aromatic cultivars and LI and SP are described as firm and sour and acid flavour cultivars.

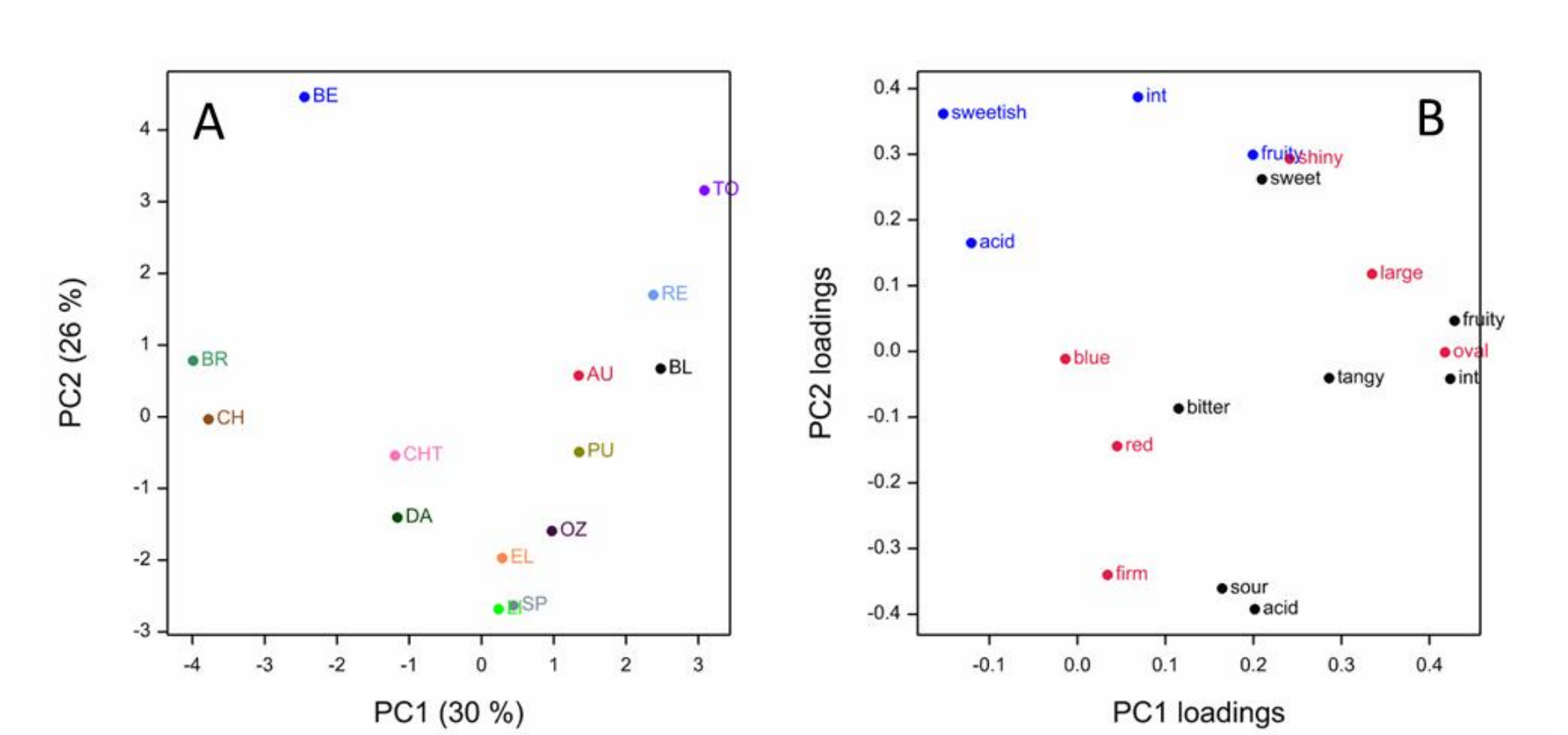


Figure 3.27. Principal component analysis (PCA) of the sensory data from the descriptive profiling of fourteen fresh blueberry cultivars grown in 2012 scored on a nine-point scale. PCA scores plot (A) and the corresponding PCA loading plot (B, the appearance descriptors refers to ■, the aroma descriptors refers to ■ and the taste descriptors refers to ■). For identification of the blueberry cultivars codes refer to Table 2.2.

The five highest correlated sensory attributes with extreme positions in the loading plot in the first two PCs in blueberry cultivars grown in 2012 are shown in Figure 3.28. Pearson correlation coefficients determined the relationships between descriptors. Cultivars grown in 2010 and 2011 and have shown similar correlations. The taste attributes intensity, bitter and sour were highly correlated in the blueberry cultivars, sour to bitter (r = 0.948) and intensity (r = 0.872) and intensity to bitter (r = 0.801) (Figure 3.28A-C). Similar to the harvesting years 2010 and 2011, assessors explained in discussions, that they could not differentiate between the following taste descriptors tangy, sour, acid and bitter. Further sweetish aroma descriptor was highly correlated to fruity aroma (r = 0.876) and negatively correlated sour taste descriptors (Figure 3.28D-E). It was suggested that the perception of fruity aroma is influenced by sweetness. Therefore sugars might have an impact on fruitiness.

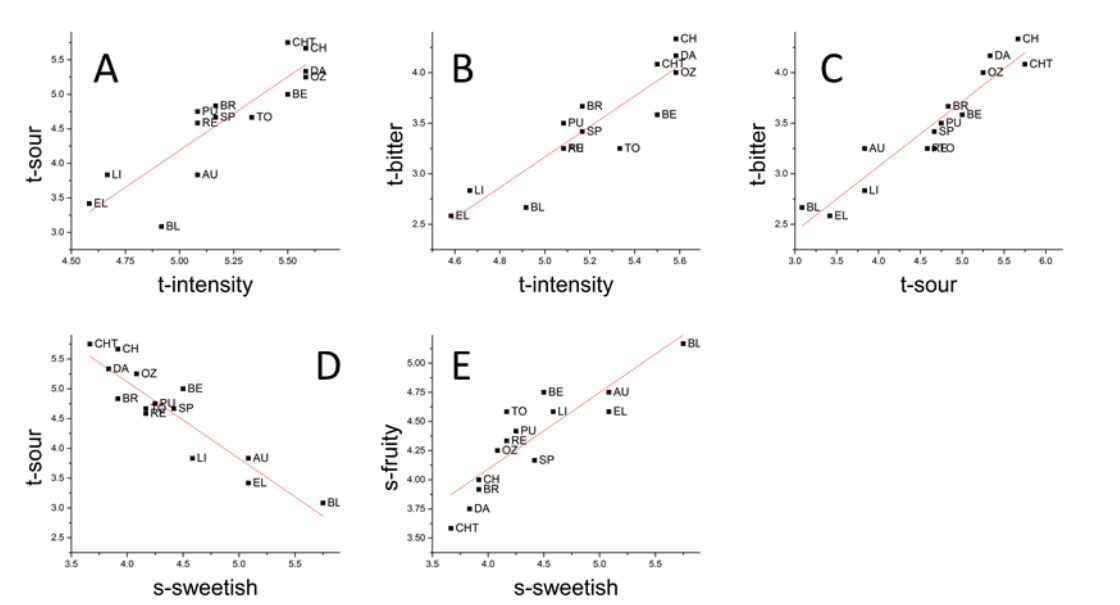


Figure 3.28. Relationship between sensory attributes in fourteen blueberry cultivars grown in 2012 to present the highest correlated sensory attributes. The sensory attributes were scored on a nine point scale assessed on fresh blueberries. Correlations between the intensive and sour taste descriptors (r = 0.872) (A), bitter and intensive taste descriptors (r = 0.907) (B), the bitter and sour taste descriptors (r = 0.948) (C), the sweetish aroma to sour taste descriptors (r = -0.919) (D) and the sweetish and fruity aroma descriptors (r = 0.876) (E), t refers to taste attributes, s refers to aroma attributes. For identification of the blueberry cultivars codes refer to Table 2.2.

Assessor performance.

The cluster analysis determined the similarities of the scoring by the judges using Euclidean distances (Figure 3.29). The x-axis describes the degree of similarity and the y-axis shows assessors (n = 8). The same numbers on the y-axis presented the same assessor scoring of different cultivars. The assessors scored similar (81 %) to each other, without any outlier. As in previous years this suggests reliable data facilitated by quality assessor training facilitated by quality assess training.

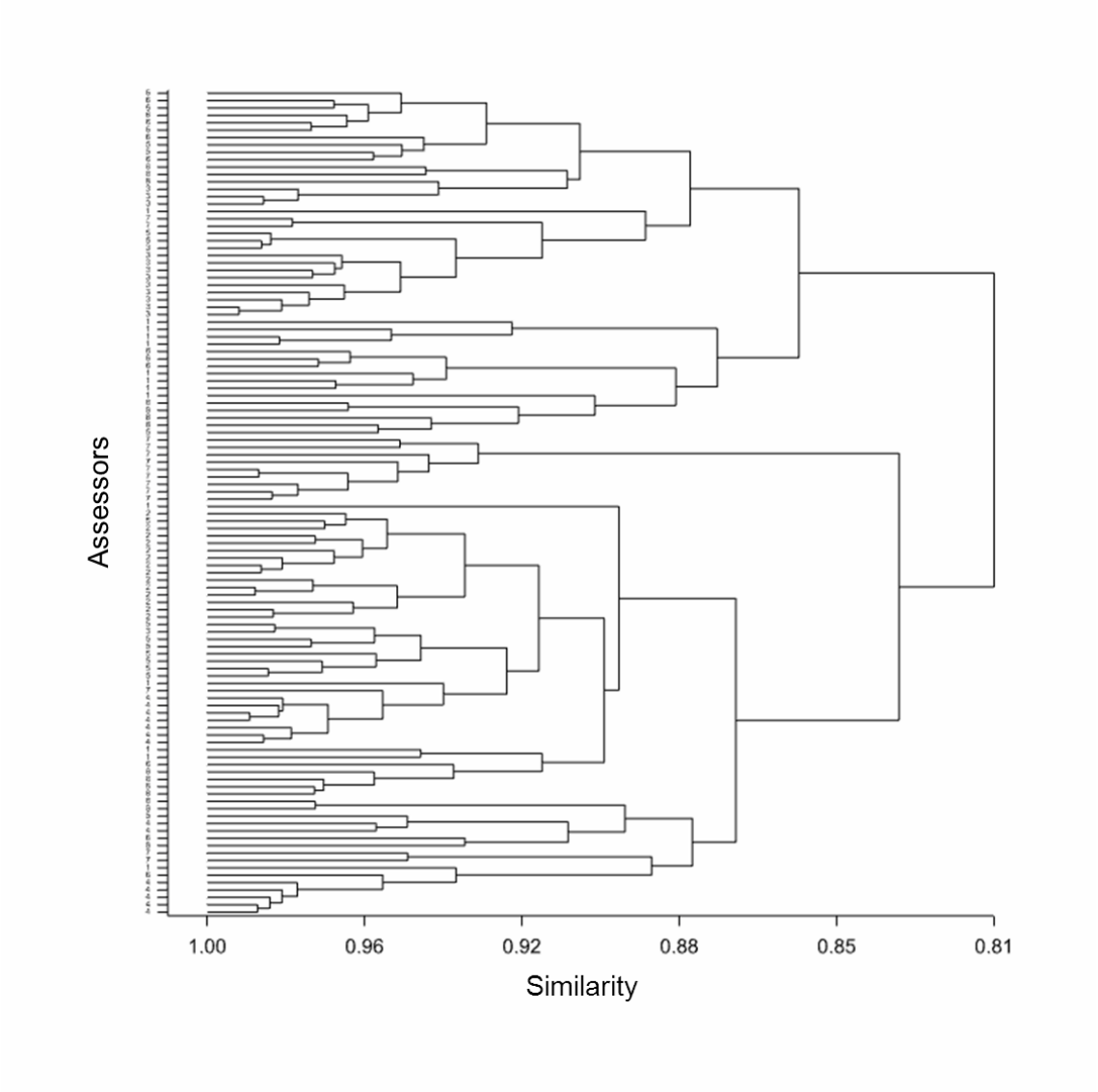


Figure 3.29. Dendogram of hierarchical cluster analysis over the eight judges based on the attribute scoring of fourteen fresh cultivars grown in 2012 using Euclidean distances to determinate their similarities. The x-axis indicate the degree of similarities, the same numbers on the y-axis indicate the same assessor scored of different blueberry cultivars.

3.4.4 Summary

DP was carried out with 8 panellists trained on 17 attributes and able to rank fruit in sweetness, sourness, and flavour intensity. Scale usage was improved by DP training. Aroma attributes were generally not significantly different ($p > 0.05$) among the cultivars. This corresponds with the discussion of the assessors after the sessions. The cultivars in the three different years have shown the following result with respect to thawed cultivars in 2010, and fresh in 2011 and 2012. Sweet cultivars were negatively correlated to sourness and acidity. The most significant attributes were in terms of appearance large, oval, shiny, and firm and in taste sourness, sweetness over the three growing years. In summary the first and second PCs had mainly high scores of sour, bitter, tangy and acid taste in the three growing years. OZ were scored high in sweetiness in 2010, in contrary to 2012. The sweetest cultivar of these both years were correlated low scored in sourness and acidity. CH and DA were lower scored in sourness, acidity and tangyness in 2012 in comparison to 2010 and 2011. Differences were shown in shinyness of AU and OZ. Common to cultivars grown in 2010 and 2012 LI had high scores in PC2, sour taste contributed to high scores. Additionally a comparison of the fresh blueberry cultivars within the different growing years showed the following similarities. EL was characterised in the growing years 2011 and 2012 as sour, acid and tangy. CH got similar high scores in sweet taste and size and low scores in sourness and acidity. Firm and oval obtained the highest mean scores and acid the lowest in both years.

3.5 Hedonic testing

3.5.1 Introduction

The previous sections (3.3 and 3.4) established a two-phase descriptive sensory analysis using initially FCP to understand the range of descriptors used by consumers to differentiate the fruit and subsequently conventional profiling by trained assessors using a consensus vocabulary to generate a multivariate product space describing relationships between the cultivars. In order to persuade the consumer to buy premium highbush blueberries the product has to meet consumer expectations. Preference mapping is a useful tool to understand underlying perceptions by scoring for consumer acceptability. Preference maps on fresh blueberries were performed at the University of Strathclyde to obtain information about consumer preferences. The preference testing started with a questionnaire about gender, age, occupation and sport exercise, then the consumer rated the preference in appearance, taste and overall (Apendix C). The hedonic testing was performed twice first with nine cultivars (BE, BL, BR, CH, DA, LI, OZ, RE, SP) and secondly three (AU, BG, EL) based on the cultivar availability. These two sessions couldn't be compared, because of different consumers, different cultivars and different numbers of cultivars. The partial least squares regression (PLS) method was applied to find the relationship between the hedonic and descriptive datasets, the x-variables (predictors) were the sensory profiles, and the y-variables (responses) the consumer preference scores. PLS produce robust interactions of independent variables; even with large data sets and is applicable for non-linear relationships and non-Gaussian distributions within the variables (Starkweather, 2011). Cross validation was performed to test the robustness of the model. The hierarchical cluster analysis was applied to understand the acceptance of certain cultivars by particular consumers using Euclidean distances. One-way ANOVA from independent variables calculated the significant differences ($p < 0.05$) of each group from independent variables in the data set. The aim was to identify consumer preferences for a range of highbush blueberry cultivars and to relate the consumer preference to sensory characteristics using descriptive analysis. Furthermore the clusters were characterised according to socioeconomic

data obtained from a consumer questionnaire to analyse the impact of these variables to consumer preferences.

3.5.2 Results and Discussion

3.5.2.1 Analysis of variance of the consumer data

Consumers scored significant differences between the blueberry cultivars ($p < 0.05$). Table 3.10 presents the mean liking scores for individual cultivars. CH scored highest for overall preference although preference was not significantly ($p > 0.05$) greater than any of the other cultivars with the exception of BL. Similar to the overall score, consumers preferred the taste of CH and had the lowest preference for BL. The remaining cultivars were scored statistically similar to CH and BL, except LI in taste scored significant different ($p < 0.05$) to BL. In terms of appearance assessors preferred RE and SP and least liked BR. The remaining cultivars were scored non-significant different ($p > 0.05$) to either RE, SP or BR. In general the consumer gave the highest scores in terms of appearance within the blueberry cultivars and the lowest scores in terms of taste. The consumer scored significant differences ($p < 0.05$) within the cultivars in overall, appearance and taste. The mean scores over the cultivars ranged between extremes; in overall from 2.78 to 5.78, in appearance 3.71 to 6.78 and taste 5.56 to 3.11.

Table 3.10. Mean liking scores (n = 56) for blueberry cultivars grown in 2011 rated of 56 consumers on a seven-point scale from dislike extremely to like extremely for all attribute classes, appearance and taste. The highest mean preferences scores within each column is indicated by ■ and the lowest mean preferences scores within column is indicated by ■. For identification of the blueberry cultivars codes refer to Table 2.2.

	Mean liking scores[A]		
	Overall	Appearance	Taste
SP	4.27 [a,b]	5.09 [a]	4.08 [a,b]
DA	4.68 [a,b]	4.9 [a,b]	4.29 [a,b]
OZ	4.46 [a,b]	4.36 [a,b]	3.94 [a,b]
CH	4.75 [a]	4.52 [a,b]	4.73 [a]
BL	3.73 [b]	4.50 [a,b]	3.87 [b]
BR	4.3 [a,b]	4.11 [b]	4.04 [a,b]
BE	4.57 [a,b]	4.89 [a,b]	4.33 [a,b]
LI	4.48 [a,b]	4.79 [a,b]	4.61 [a]
RE	4.52 [a,b]	5.23 [a]	4.14 [a,b]

[A] Means with different letters in a column indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

3.5.2.2 Hierarchical cluster analysis

The cluster analysis of consumers was applied to understand the preference for certain cultivars by particular consumers. First the overall preference was discussed, followed by the preference in appearance and taste. The cluster analysis was applied on the 9 cultivar x 56 consumers matrix of consumer data. The x-axis describes the degree of similarity and the y-axis shows assessors (n = 56). The same numbers on the y-axis presented the same assessor scoring for different cultivars. The differences among the means were compared between different clusters using one-way ANOVA.

Overall

The hierarchical cluster analysis performed on the consumer liking of blueberry cultivars indicated that three distinct consumer clusters adequately captured the variation in preferences for the nine blueberry cultivars (Figure 3.30). The mean preference scores within each cluster for each cultivar are shown in Table 3.11. The preferences of cultivars were significantly different ($p < 0.05$) within each cluster and the preferences of cultivars were significantly different ($p < 0.05$) among the clusters, except CH, DA, LI and OZ. Cluster 1 represented 19.7 % of consumers. The largest

range of scores was obtained in cluster 1, the lowest in cluster 2. BR had the lowest average preference scoring on a scale from 1 to 7 in cluster 1, while in cluster 3 (33.9 % of consumers) BR was the most liked cultivar. LI got the highest scores in cluster 1 and the lowest scores in cluster 2 (46.4 % of consumer). BE had the highest average scores in cluster 2, the remaining cultivars were in the middle region. On a seven-point scale ranges from 5 to 7 were liked and from 1 to 3 were disliked (Appendix C), scores of 4 was neither liked nor disliked.

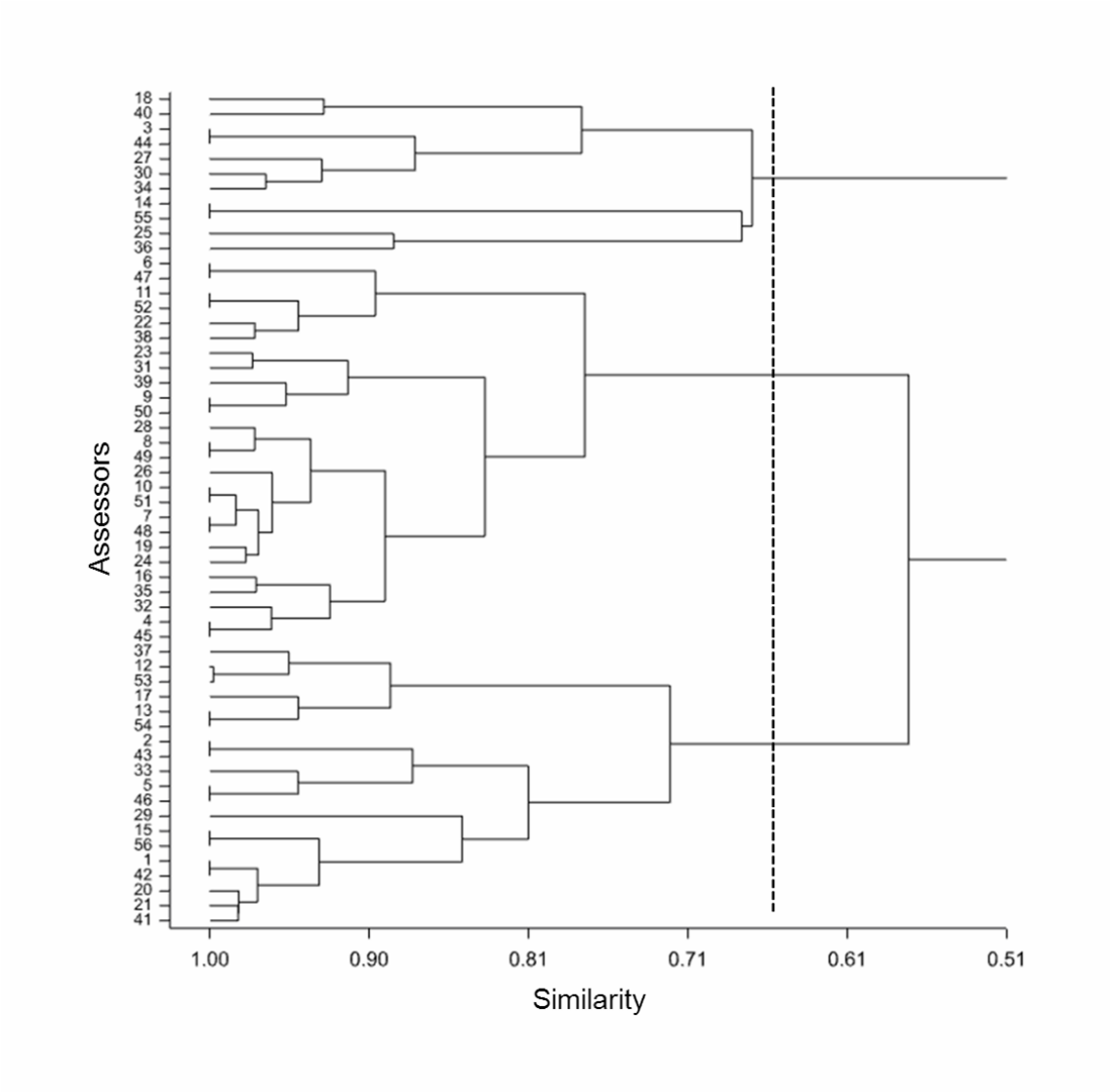


Figure 3.30. Dendogram of hierarchical cluster analysis over the 56 consumsers based on the overall preference scoring of nine blueberry cultivars grown in 2011 rated on a seven-point scale from dislike extremely to like extremely using Euclidean distances to determinate their similarities. The x-axis indicate the degree of similarities, the same numbers on the y-axis indicate the same assessor scored of different blueberry cultivars.

Table 3.11. Mean preference overall scores ± standard deviations within each cluster for blueberry cultivars grown in 2011 rated of 56 consumers on a seven-point scale from dislike extremely to like extremely for all attribute classes, appearance and taste. 19.7 % of the consumers contributed to cluster 1, 46.4 % to cluster 2 and 33.9 % to cluster 3. The highest mean preferences scores within each cluster is indicated by ■ and the lowest mean preferences scores within each cluster by ■. Values in bold are significant ($p < 0.05$). For identification of the blueberry cultivars codes refer to Table 2.2.

Cultivars	Cluster 1 (19.7 %)	Cluster 2 (46.4 %)	Cluster 3 (33.9 %)	p-value
BE	$3.45 \pm 1.86^{a,2,3,4}$	$5.54 \pm 0.9^{b,1}$	$3.89 \pm 0.74^{a,2,3,4}$	**< 0.001**
BL	$2.91 \pm 1.22^{a,3,4}$	$4.54 \pm 0.71^{b,3,4}$	$3.11 \pm 1.33^{a,4}$	**< 0.001**
BR	$2.09 \pm 1.14^{a,4}$	$4.44 \pm 1.58^{b,3,4}$	$5.37 \pm 1.83^{b,1}$	**< 0.001**
CH	$4.73 \pm 1.62^{a,1,2}$	$5.04 \pm 1.15^{a,1,2,3}$	$4.37 \pm 1.61^{a,2,3}$	0.298
DA	$3.91 \pm 2.02^{a,2,3}$	$5.15 \pm 1.26^{a,1,2,3}$	$4.43 \pm 1.47^{a,1,2}$	0.062
LI	$5.45 \pm 1.75^{a,1}$	$4.00 \pm 1.90^{a,4}$	$4.58 \pm 1.43^{a,1,2}$	0.070
OZ	$4.27 \pm 1.90^{a,1,2,3}$	$4.69 \pm 1.54^{a,2,3,4}$	$4.26 \pm 1.79^{a,2,3}$	0.649
RE	$4.27 \pm 1.90^{a,1,2,3}$	$5.38 \pm 1.10^{b,1,2}$	$3.47 \pm 1.26^{a,3,4}$	**< 0.001**
SP	$4.91 \pm 2.34^{a,1,2}$	$4.88 \pm 0.82^{a,1,2,3}$	$3.11 \pm 2.10^{b,4}$	**0.002**
p-value	**0.001**	**0.000**	**0.000**	

^{a,b} Means with different letter superscripts within row indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

^{1,2,3,4} Means with different number superscripts within column indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

Appearance

Similar to the hierarchical cluster analysis in the overall view, three distinct consumer clusters were observed with respect to preferences for blueberry appearance across the nine cultivars (Figure 3.31). The mean preference scores within each cluster for each cultivar are shown in Table 3.12. The preferences of cultivars were not significantly different ($p > 0.05$) within the each cluster, except cluster 2 and the preferences of cultivars were significantly different ($p < 0.05$) among the clusters, except BR. In general consumers of cluster 3 had the highest preference scores and scored the blueberry cultivars significant higher ($p > 0.05$) than consumer of cluster 1. The largest range of scores was obtained in cluster 2, the lowest in cluster 3. DA had the lowest average preference scoring on a scale from 1 to 7 in cluster 1 and SP got the highest scores. RE got the highest preference scores in cluster 2, while BE and CH got the highest average preference scores in cluster 3.

Similar to cluster 2, cluster 3 got the lowest scores of BR. No cultivars were scored lower than 4 on a seven-point scale that implies that no cultivars were disliked.

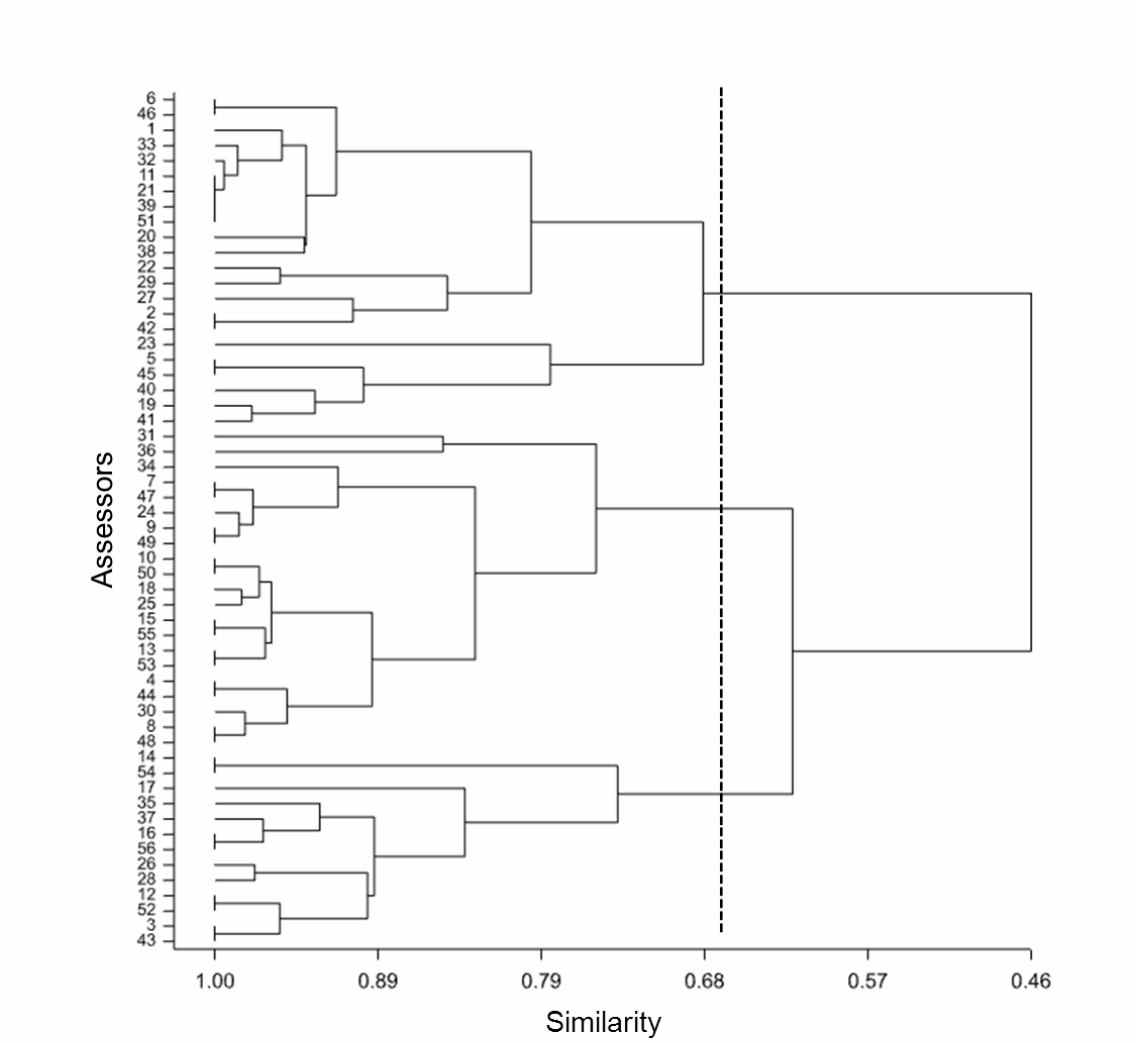


Figure 3.31. Dendogram of hierarchical cluster analysis over the 56 consumers based on the preference scoring in appearance of nine blueberry cultivars grown in 2011 rated on a seven-point scale from dislike extremely to like extremely using Euclidean distances to determinate their similarities. The x-axis indicates the degree of similarities, the same numbers on the y-axis indicates the same assessor scored of different blueberry cultivars.

Table 3.12. Mean preference scores ± standard deviations within each cluster for blueberry cultivars grown in 2011 rated of 56 consumers on a seven-point scale from dislike extremely to like extremely for appearance. 39.3 % of the consumers contributed to cluster 1, 37.5 % to cluster 2 and 23.2 % to cluster 3. The highest mean preferences scores within each cluster is indicated by ■ and the lowest mean preferences scores within each cluster is indicated by ■. Values in bold are significant ($p < 0.05$). For identification of the blueberry cultivars codes refer to Table 2.2.

Cultivars	Cluster 1 (39.3 %)	Cluster 2 (37.5 %)	Cluster 3 (23.2 %)	p-value
BE	$4.50 \pm 1.41^{a,1}$	$4.50 \pm 1.17^{a,3,4}$	$6.31 \pm 0.85^{b,1}$	**< 0.001**
BL	$3.95 \pm 1.29^{a,1}$	$4.00 \pm 1.05^{a,4}$	$6.23 \pm 0.60^{b,1}$	**< 0.001**
BR	$4.05 \pm 1.62^{a,1}$	$3.86 \pm 1.15^{a,4}$	$4.69 \pm 2.18^{a,1}$	0.335
CH	$3.86 \pm 1.04^{a,1}$	$4.14 \pm 1.49^{a,4}$	$6.31 \pm 1.18^{b,1}$	**< 0.001**
DA	$3.86 \pm 0.71^{a,1}$	$5.43 \pm 0.81^{b,2}$	$5.92 \pm 1.19^{b,1}$	**< 0.001**
LI	$4.00 \pm 1.45^{a,1}$	$4.81 \pm 0.93^{b,2,3}$	$6.08 \pm 1.12^{c,1}$	**< 0.001**
OZ	$4.00 \pm 1.57^{a,1}$	$3.95 \pm 1.28^{a,4}$	$5.62 \pm 1.76^{b,1}$	0.005
RE	$4.00 \pm 1.48^{a,1}$	$6.10 \pm 0.70^{b,1}$	$6.00 \pm 1.63^{b,1}$	< 0.001
SP	$4.55 \pm 1.14^{a,1}$	$5.33 \pm 0.86^{b,2}$	$5.69 \pm 1.93^{b,1}$	0.029
p-value	0.592	**0.000**	0.147	

[a,b] Means with different letter superscripts within row indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

[1,2,3,4] Means with different number superscripts within column indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

Taste

Similar to the hierarchical cluster analysis in the overall view and in terms of appearance, three distinct consumer clusters were an adequate number for nine blueberry cultivars (Figure 3.32). The mean preference scores within each cluster for each cultivar are shown in Table 3.13. The preferences of cultivars were significantly different ($p < 0.05$) within each cluster and the preferences of cultivars were significantly different ($p < 0.05$) among the clusters, except BL, CH and LI. The largest range of scores was obtained in clusters 2 and 3, the lowest in cluster 1. BR had the lowest average preference scoring and BE had the highest scoring on a scale from 1 to 7 in cluster 1, SP and LI got the highest average preference scoring in cluster 2 and OZ the lowest. In contrary to cluster 1 BR had the highest scores in cluster 3 and in contrary to cluster 2 SP had the lowest scores in cluster 3. Similar to the appearance view no cultivars in cluster 1 were scored lower than 4 on a seven-point scale that implies that no cultivars were disliked in this cluster.

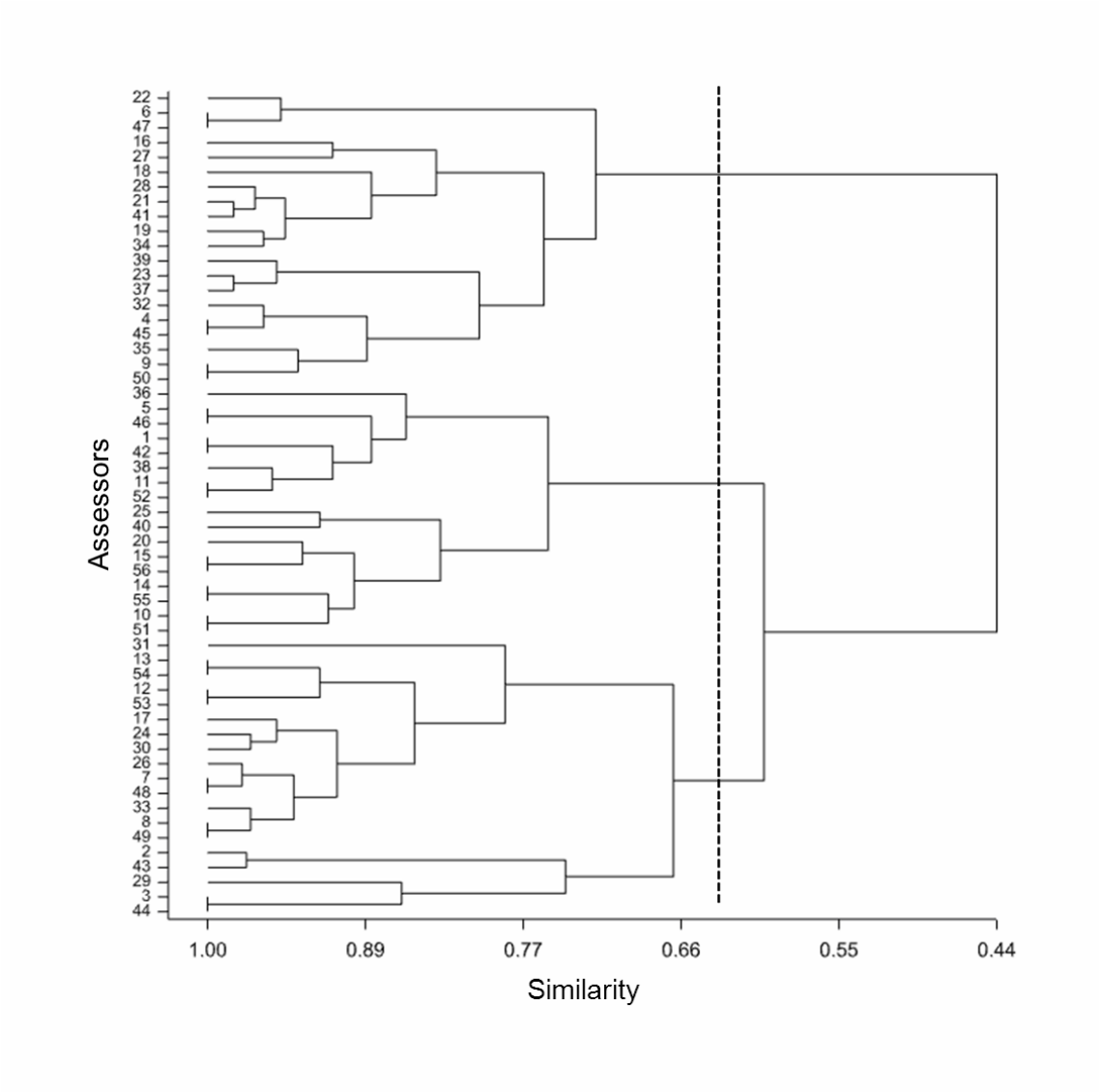


Figure 3.32. Dendogram of hierarchical cluster analysis over the 56 consumers based on the preference scoring in taste of nine blueberry cultivars grown in 2011 rated on a seven-point scale from dislike extremely to like extremely using Euclidean distances to determinate their similarities. The x-axis indicates the degree of similarities, the same numbers on the y-axis indicates the same assessor scored of different blueberry cultivars.

Table 3.13. Mean preference scores ± standard deviations within each cluster for blueberry cultivars grown in 2011 rated of 56 consumers on a seven-point scale from dislike extremely to like extremely for taste. 35.7 % of the consumers contributed to cluster 1, 30.4 % to cluster 2 and 33.9 % to cluster 3. The highest mean preferences scores within each cluster is indicated by ■ and the lowest mean preferences scores within each cluster indicated by ■. Values in bold are significant ($p < 0.05$). For identification of the blueberry cultivars codes refer to Table 2.2.

Cultivars	Cluster 1 (35.7 %)	Cluster 2 (30.4 %)	Cluster 3 (33.9 %)	p-value
BE	5.60 ± 1.05^{a,1}	3.47 ± 1.74^{b,3}	3.47 ± 1.68^{b,4,5,6}	**< 0.001**
BL	3.85 ± 1.14^{a,4}	3.00 ± 1.22^{a,3,4}	3.11 ± 1.63^{a,5,6}	0.114
BR	3.80 ± 1.91^{a,4}	3,00 ± 1.73^{a,3,4}	5.21 ± 1.40^{b,1}	**< 0.001**
CH	4.50 ± 1.19^{a,2,3,4}	4.53 ± 1.59^{a,2,3}	5.16 ± 1.26^{a,1,2}	0.245
DA	5.00 ± 1.86^{a,1,2,3}	3.53 ± 1.28^{b,3,4}	4.21 ± 1.72^{a,b,2,3,4}	**0.033**
LI	4.20 ± 1.88^{a,3,4}	4.59 ± 1.54^{a,1}	5.05 ± 1.31^{a,1,2}	0.261
OZ	4.30 ± 1.72^{a,3,4}	2.06 ± 0.75^{b,4}	4.68 ± 1.89^{a,1,2,3}	**< 0.001**
RE	5.30 ± 1.45^{a,1,2}	2.88 ± 1.76^{b,3,4}	4.05 ± 1.27^{c,3,4,5}	**< 0.001**
SP	5.30 ± 1.13^{a,1,2}	4.59 ± 1.37^{a,1}	2.58 ± 1.30^{b,6}	**< 0.001**
p-value	0.000	0.000	0.000	

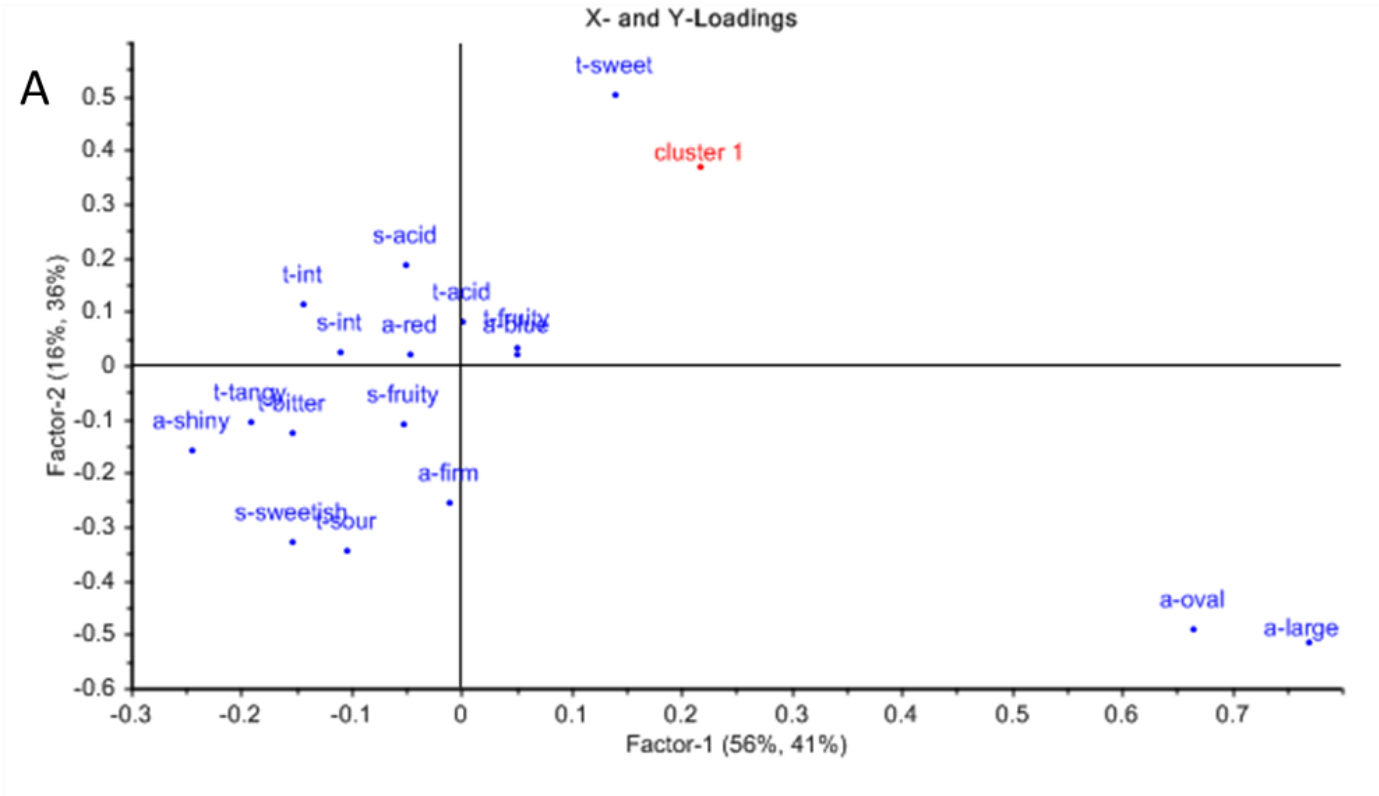
^{a,b} Means with different letter superscripts within row indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

^{1,2,3,4,5,6} Means with different number superscripts within column indicate significant differences at $p < 0.05$ (Fisher's protection of least significant difference).

3.5.2.3 Relationships between sensory data and consumer preferences

The hierarchical cluster analysis was performed to group consumer preferences. In order to identify the relationship between the cluster of the consumer preferences and sensory profiling PLS were applied. The advantages of using PLS is that it produces robust interactions of independent variables taking into account the multiplicity of factors that may contribute to a given sensory perception. The x-variables (predictors) were the sensory data, and the y-variables (responses) the clusters of the consumer preferences. Figure 3.33 illustrates the interaction between cluster of consumer preferences and sensory data from DP. A statistical analysis revealed that the model cannot be trusted for several reasons; the wide divergence between predicted (blue) and measured (red) plots and low coefficient of determination R^2 (R^2 = 0.215). The low R^2 values obtained indicated the lack of robustness of the models generated.

The PLS-1 plot was applied to analyse clusters separately. Again, a statistical analysis revealed that the model cannot be trusted because of the low R^2 (R^2 = 0.159) in cluster 1 and the not applicable R^2 in clusters 2 and 3. The reason for the limited robustness may be the low variety between blueberry cultivars. Given the poor fit of the PLS models, PCA was used to identify trends between consumer preferences andsensory data. PCA was applied to give a data overview, detect outliers, groups, trends and estimate the relationships between variables; while PLS does modeling and prediction to indicate the model robustness (Moertsell et al, 2001).


X- and Y-Loadings
A
t-sweet
cluster 1
s-acid
t-int
t-acid
s-int
a-red
a-blue
t-tangy
t-bitter
s-fruity
a-shiny
a-firm
s-sweetish
t-sour
a-oval
a-large
Factor-2 (16%, 36%)
Factor-1 (56%, 41%)
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.3
-0.2
-0.1
0
0.1
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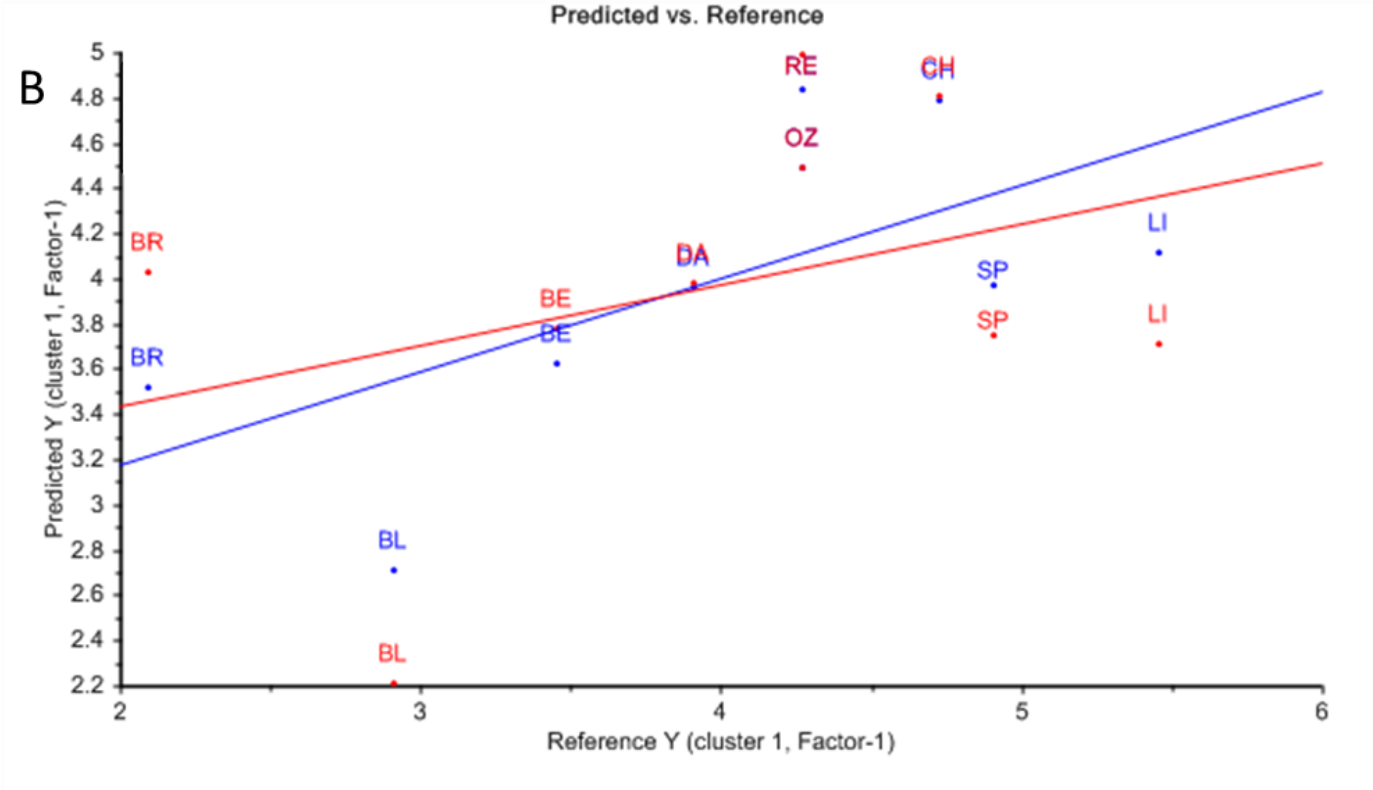


Figure 3.33. Result of PLS showing the relationship between descriptive and consumer preference data of nine blueberry cultivars grown in 2011. For descriptive profiling the trained panel (n = 8) evaluated the blueberry cultivars on a nine-point scale, for preference testing consumers (n = 56) evaluated the blueberry cultivars on a seven-point scale from dislike extremely to like extremely. (A) x- and y-loading plot fom PLS; blue indicate the sensory data from descriptive profiling and red indicates cluster1 resulting from consumer preferences scoring and (B) indicate the predicted (blue) vs measured (red) plot from PLS. For identification of the blueberry cultivars codes refer to Table 2.2.

Principal Component Analysis

The principal component product space of nine blueberry cultivars has shown the overall preferences in Figure 3. The position of the samples in the PCA plots and relative distances to each other were used to evaluate the data. The evaluation of PCA was further described under 3.4.3. The sensory attributes related well with the consumer clusters. In consideration that attributes in the same positions of the product space as the clusters have the same impact on the preference (Greenhoff and MacFie, 1994).

The first two principal components accounted for 53 % of total sample variance, 32 % of total variance was explained by the first PC and 21 % of the variance was explained by the second PC. Consumers in cluster 1 preferred the sensory characteristics of CH and least liked BL. This group of consumers appeared to prefer large, oval, blue and fruity taste cultivars and disliked sensory attributes of BL. Consumers in cluster 2 preferred RE and disliked BR, LI and OZ. It could be concluded that this consumer group preferred sweet cultivars, while consumers in cluster 3 preferred BR, LI and OZ, the suggested a preference for sour and acid cultivars. The PCA could not be applied for the preferences in appearance and taste due to insufficient available variables.

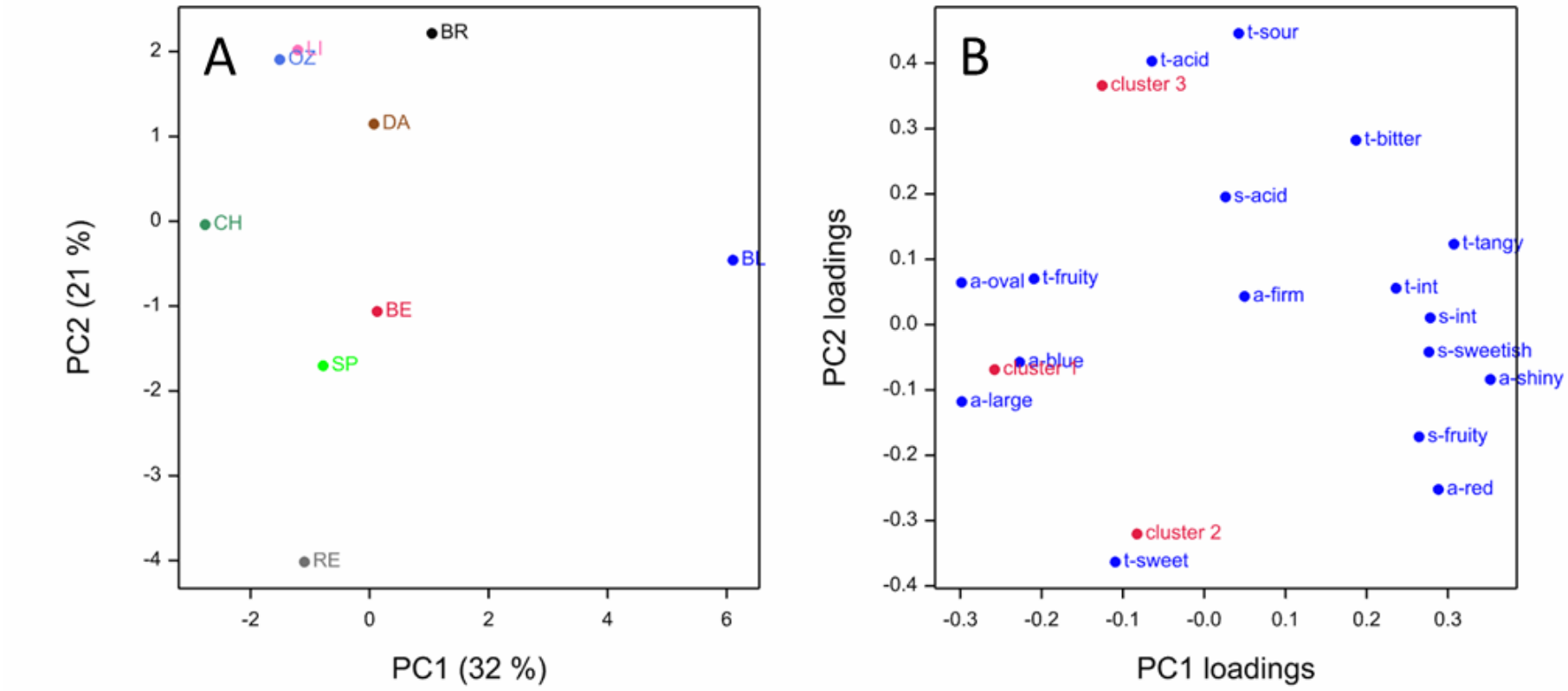


Figure 3.34. Principal component analysis (PCA) of consumer clusters and sensory data profiling of nine blueberry cultivars grown in 2011. For descriptive profiling the trained panel (n = 8) evaluated the blueberry cultivars on a nine-point scale, for preference testing consumers (n = 56) evaluated the blueberry cultivars on a seven-point scale from dislike extremely to like extremely. PCA scores plot (A) and the corresponding PCA loading plot (B, the sensory attributes refers to ■, the cluster resulting from preference testing refers to ■). For identification of the blueberry cultivars codes refer to Table 2.2.

3.5.2.4 Preference clusters related to socioeconomic variables

In the previous section (3.5.2.2) the consumers have been segmented by similarity of preference scoring. In this section consumers within the subgroups were characterised to analyse a possible relationship between socioeconomic groups and product preferences to determine possible drivers like age or life style to blueberry preferences in overall, appearance and taste. The evaluation was done according to the studies of Lawler and Delahunty (2000). PCA were applied instead of PLS. PLS could not be applied because of the previously discussed reasons (3.4.3).

Overall

The socioeconomic data of the three consumer overall clusters are shown in Table 3.14. One-way ANOVA analysed the significant differences in gender, age, occupation and sport exercise. It can be deduced from Table 3.11 and Table 3.14 that consumers who were older than 34 and exercise once a week (cluster 1) tend to like cultivars like CH and dislike BL, while younger consumers, who exercise more than once a week (cluster 2) tend to prefer RE. Females who exercise occasionally preferred BR, LI and OZ (cluster 3). Figure 3.34 exhibited the relationships between sensory characteristics and consumer preferences of blueberry cultivars. Consumer over 35 years, who exercise once a week (cluster 1) preferred oval, large, blue and fruit taste cultivars like CH; BL was the least liked cultivar. Younger consumers, who exercise more than once a week (cluster 2) exhibited the opposite trends of female consumers who exercise occasionally (cluster 3). Consumers of cluster 2 preferred the sweeter cultivars like RE, while consumers of cluster 3 preferred sour and acid cultivars like BR, LI and OZ.

Table 3.14. Proportion of consumers (n = 56) in the three clusters for overall liking with socioeconomic breakdown. Values are stated as percentage of total consumers within each clusters and values in bold indicate high percentage in comparison to other percentages in the same socioeconomic group.

	Total n	Cluster 1 (19.7 %)	Cluster 2 (46.4 %)	Cluster 3 (33.9 %)
Age (years)				
under 25	6	10	11.1	10.5
26 – 34	21	20	**51.9**	26.3
35 – 44	13	**40**	11.1	31.6
over 45	16	30	25.9	31.6
Gender				
female	31	45.5	53.8	**63.2**
male	25	54.5	46.2	36.8
Occupation status				
professional	12	22.2	18.8	26.7
office	10	33.3	12.5	20
student	16	11.1	34.4	26.7
sales	18	33.3	34.4	26.7
Sport				
every day	4	9.1	7.7	5.3
more than once a week	21	27.3	**46.2**	31.6
once a week	12	**36.4**	19.2	15.8
fortnightly	4	9.1	3.8	10.5
occasionally	15	18.2	23.1	**36.8**

Appearance

The socioeconomic data of the three consumer clusters are shown in Table 3.15. One-way ANOVA showed that occupation and similar to overall view gender had no significant impact ($p > 0.05$) on the consumer references. It can be deduced from Table 3.12 and Table 3.15 that consumers who were older than 45 tend to like more BE and CH (cluster 3) than consumers aged 26 – 34 years (cluster 1).

Table 3.15. Proportion of consumers (n = 56) in the three clusters for liking in terms of appearance with socioeconomic breakdown. Values are stated as percentage of total consumers within each clusters and values in bold indicate high percentage in comparison to other percentages in the same socioeconomic group.

	Total n	Cluster 1 (39.3 %)	Cluster 2 (37.5 %)	Cluster 3 (23.2 %)
Age (years)				
under 25	6	18.2	14.3	0
26 – 34	21	**40.9**	**38.1**	30.8
35 – 44	13	9.1	33.3	23.1
over 45	16	31.8	14.3	**46.2**
Gender				
female	31	54.5	**61.9**	46.2
male	25	45.5	38.1	53.8
Occupation status				
professional	12	19	28.6	**42.9**
office	10	9.5	19	21.4
student	16	28.6	14.3	21.4
sales	18	**42.9**	**38.1**	14.3
Sport				
every day	4	9.1	10.5	13.3
more than once a week	21	27.3	31.6	33.3
once a week	12	13.6	31.6	20
fortnightly	4	13.6	0	13.3
occasionally	15	**36.4**	26.3	20

Taste

The socioeconomic data of the three consumer clusters are shown in Table 3.16. ANOVA showed that similar to appearance sport exercise had no significant impact on the consumer references ($p > 0.05$). It can be deduced from Table 3.13 and Table 3.16 that consumers who were older than 34 and females tend to like oval cultivars like BR and dislike SP, while younger consumers tend to like SP (cluster 2). Consumers who exercise quite regularly liked OZ (cluster 1 and 3), while consumer who occasionally exercise dislike OZ (cluster 2).

Table 3.16. Proportion of consumers (n = 56) in the three clusters for liking in terms of taste with socioeconomic breakdown. Values are stated as percentage of total consumers within each clusters and values in bold indicate high percentage in comparison to other percentages in the same socioeconomic group.

	Total n	Cluster 1 (%)	Cluster 2 (%)	Cluster 3 (%)
Age (years)				
under 25	6	14.3	17.6	0
26 – 34	21	42.9	**35.3**	33.3
35 – 44	13	0	23.5	**50**
over 45	16	**42.9**	23.5	16.7
Gender				
female	31	45	**64.7**	**57.9**
male	25	55	35.3	42.1
Occupation status				
professional	12	28.6	25	31.6
office	10	14.3	18.8	21.1
student	16	19	25	26.3
sales	18	38.1	31.3	21.1
Sport				
every day	4	10	6.3	5
more than once a week	21	**60**	18.8	30
once a week	12	5	18.8	**45**
fortnightly	4	0	18.8	5
occasionally	15	25	**37.5**	15

3.5.3 Summary

The consumer test was carried to range the consumer preferences of commercial available blueberry cultivars grown in 2011 on a seven point scale. The similar scoring within blueberries suggested that consumers had difficulties to distingh between the provided cultivars similar to the results in FCP and DP. Nevertheless different socioeconomic groups preferred different cultivars. The linkeage between hedonic test and descriptive profiling were done to verify what sensory characterics are important in blueberry cultivars in order to obtain information about consumer expectations. The applied PLS could not produce a robust model due to the small differences between the cultivars. Therefore PCA was applied to determine domestic

patterns with the results that younger consumers tended to preferred sweeter cultivars, while females preferred more sour cultivars.

3.6 Conclusions

Current consumers do not have a clear perception of premium blueberry cultivars. Furthermore there was no knowledge of how blueberries perform in UK soil. In order to persuade the consumer to pay the premium for high quality UK grown blueberries it is necessary to deliver a product that meets consumer expectations.

An online questionnaire was applied to a broader cross-section of the UK buying public. The online questionnaire found out that females, especially married women are more likely to purchase blueberries, therefore consumer preference tests should be carried out for the target group in the supermarket. Information of preparation suggestions would give the consumers more variations and promotion about the health properties may enhance the consumption of blueberries. Due to the possible influence of texture to the flavour perception, texture attributes should be included in further sensory studies. The limited diversity of blueberry cultivars made it difficult to identify the important sensory characteristics to deliver fruits that meet consumer expectations. Additional sensory tests should be conducted on blueberries grown in open fields and polytunnels. In recent years breeding efforts were focused on appearance (size, colour) and yield. It is suggested that more distinctive flavour and aroma intensive blueberry cultivars would get a higher acceptance by consumers. The assessors had difficulties to scoring blueberry cultivars in terms of aroma probably because of their unfamiliarity. Further reasons for this low agreement could be that blueberries are weak in aroma. The provided thawed blueberry cultivars presumably reduced the aroma profile.

In general blueberries grown in 2010 were distingushable from blueberries grown in 2011 and 2012, whereas blueberries grown in the harvesting years 2011 and 2012 tasted similar. Blueberries grown in 2010 were freeze-thawed with no shelf life component, while the remaining two harvesting years were fresh blubeberries. It was

suggested that the processing method had a larger influence on sensory profiling than genotypes.

The consumer test demonstrated different liking between consumer clusters. Therefore it is important to determine the target group to provide blueberry cultivars that meet consumer expectations, because different consumer groups preferred different sensory characteristics of blueberries.

Chapter 4

Genetic and environmental determinants of fruit metabolites

4.1 Introduction

Like the majority of berry fruits, blueberry fruit undergo profound changes in metabolite profiles throughout development. Such changes are influenced by a combination of genetics and environment, and determine the phytochemical composition of ripe fruit upon which many of the fruits sensory attributes are dependent. While a number of studies have examined the impact of G x E interactions on blueberry fruit quality the vast majority of these have been conducted in the USA and very little is known regarding these influences under UK climatic conditions (Connor et al., 2002a, Finn et al., 2003). Fruit phytochemicals, that are relevant to sensory and health promoting properties were therefore examined in a number of UK blueberry cultivars harvested in different years. Phytochemical profiling was conducted to estimate the concentration of a range of components with potential sensory (sugar, organic acid, flavour volatiles, anthocyanins) or health promoting (polyphenols) properties in three growing seasons. The analysed metabolites were divided into primary (sugars, organic acids, lipids) and secondary metabolites (polyphenols, anthocyanins, terpenes) that were expected to influence the sensory characteristics of blueberries (Silva et al., 2005). T-AO capacity, T-Phen, T-Anth, organic acids and sugars were quantified using a range of spectrophotometric and HPLC techniques. Furthermore individual polyphenols and

anthocyanins were quantified by LC-MS^2, lipids by GC-MS and flavour volatiles by head space SPME-GC-MS. Data were analysed using PCA to evaluate the genetic and environmental impact on metabolite profiles of blueberry cultivars. PCA was used to identify the impact of genetic (cultivar) and environmental (growing location) variables on fruit phytochemical composition with the position of the samples in PCA plots and relative distances to each other used to evaluate data. Analysis of loading plots allowed identification of the phytochemicals that acted as the drivers of sample variability. The evaluation of PCA was further described under 3.4.3. Box and whisker plots of selected compounds were used to confirm the location of the genotypes in the product space. Pairwise correlation analysis was performed to identify metabolites that exhibited positive or negative correlation across samples. One-way ANOVA from independent variables was used to calculate the significant differences ($p < 0.05$) of each group from independent variables in the data set.

The aim was to determine the magnitude of environmental and genetic influences on the phytochemical composition of blueberry cultivars and to identify the relationships between individual compounds. Furthermore the obtained data were used to identify phytochemical drivers of sensory properties as described in chapter 3.

4.2 Results and Discussion

4.2.1 Genetic diversity in blueberry cultivars

The genetic element of phytochemical diversity in blueberry cultivars was evaluated. Every harvesting year was analysed separately in order to determine similarities or differences in the metabolites within the cultivars grown under similar environmental conditions.

4.2.1.1 Genetic diversity in blueberry cultivars 2010

The principal component product space of eight blueberry cultivars in the growing year 2010 is shown in Figure 4.1A. The first two PCs accounted for 39 % of total sample variance. 23 % of total variance was explained by the first PC with a high positive score for BL and high negative scores for OZ and LI. The loading plot indicated that lipids, C_6 flavour volatiles and a range of polyphenols contributed to positive scores in this PC while terpenoids provided a negative contribution (Figure 4.1B). 16 % of the variance was explained by the second PC, which highlighted the outlying nature of DA. A range of polyphenols contributed to positive scores in this PC with high levels observed in DA and a range of anthocyanins contributed negative scores with high levels observed in AU and OZ (Figure 4.1).

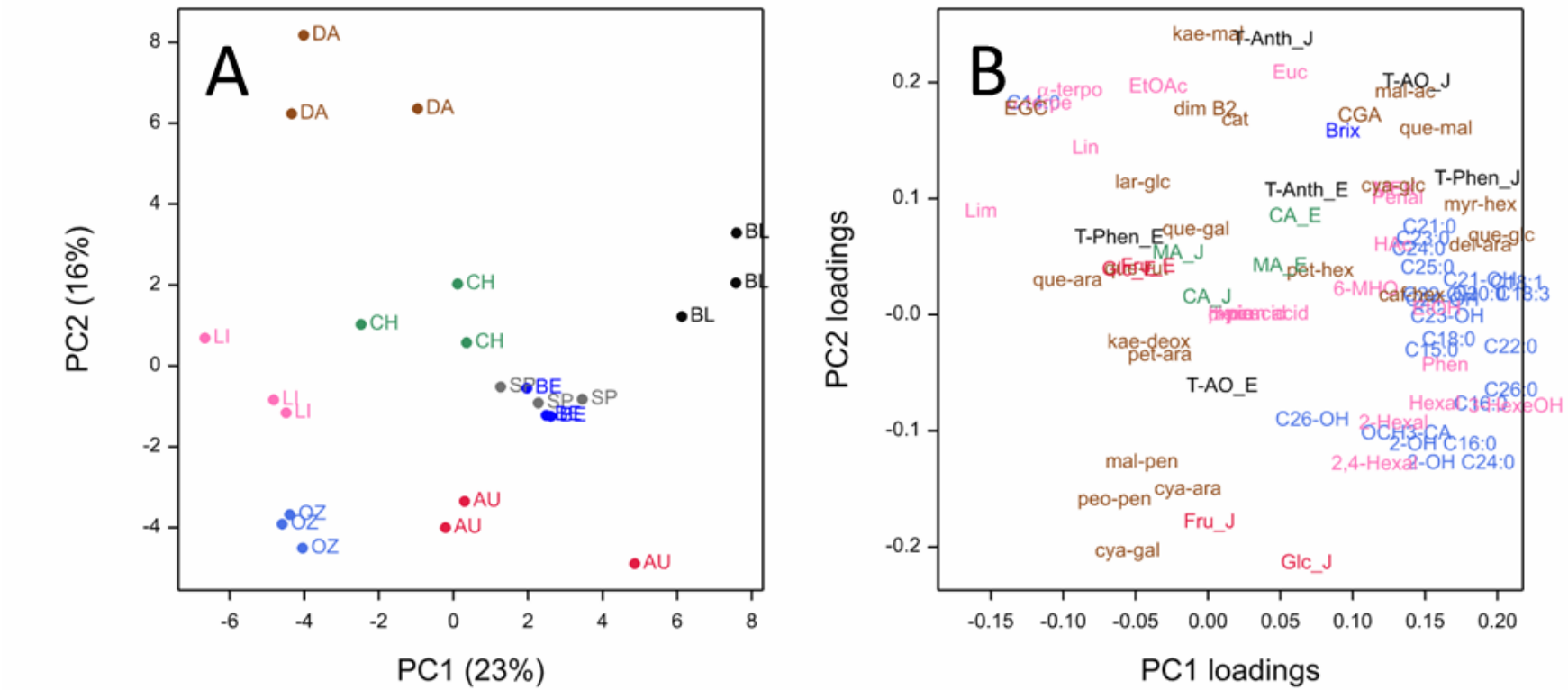


Figure 4.1. Principal component analysis (PCA) of metabolites (flavour volatiles and lipids measured by GC-MS, individual polyphenols and anthocyanins by LC-MS^2, sugars and organic acids by HPLC, brix by refractometer and antioxidant compounds by UV-photometer) from blueberry cultivars grown in 2010. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ volatiles, ■ lipids, ■ individual polyphenols and anthocyanins, ■ antioxidant components, ■ sugars, ■ organic acids, ■ brix). The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Compounds with extreme positions in the loading plot were selected to provide further information about the specific levels within the cultivars. A range of lipids, flavour volatiles, polyphenols and anthocyanins were selected to confirm their contribution to the location of cultivars in the PCA. The polyphenols que-hex, myr-hex and CGA were present at high concentrations in BL and low levels in LI and OZ as would be expected from their positions on the PCA plot (Figure 4.2A-C). Similarly, the C22:0, C18:0 and C18:1 were present at high levels in BL but lower levels in LI and OZ as expected (Figure 4.2D-F). However, while the C_6 flavour volatiles Hexal, 3-HexeOH and 2-Hexal had as expected high concentrations in BL, levels were also high in OZ but low in LI suggesting that other compounds were contributing to the position of theses cultivars on PC1 (Figure 4.2G-I). The terpenoids Lin and α-terpe were unlikely to contribute significantly with the exception of Lim which was present at high concentrations in LI and OZ (Figure 4.2J-L).

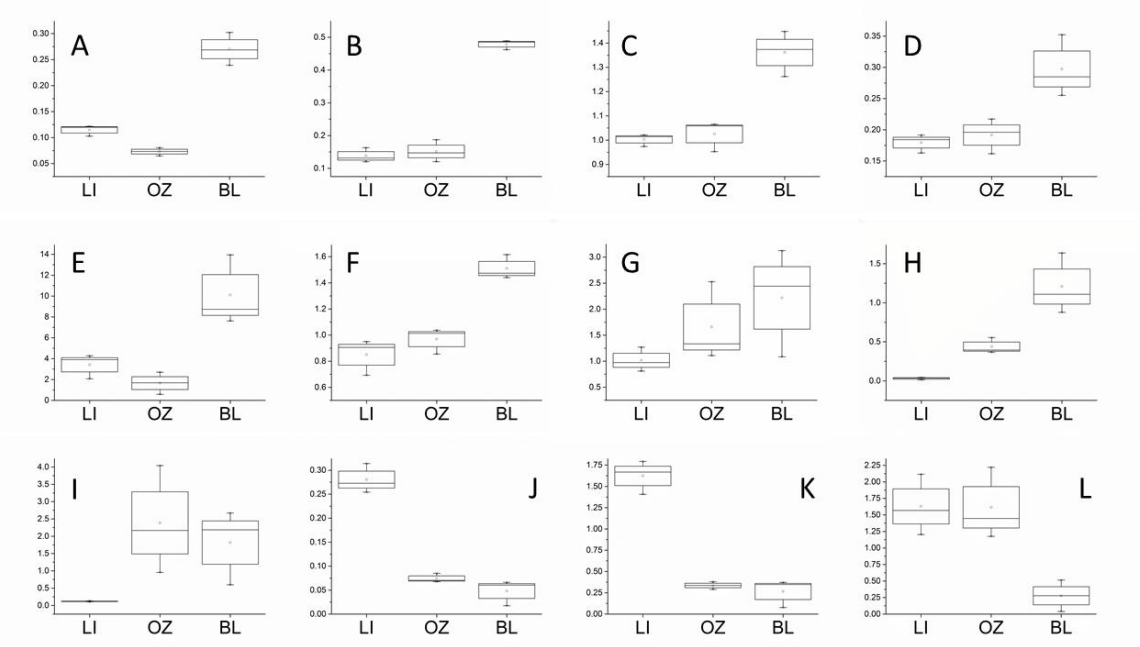


Figure 4.2. Box and whisker plots of selected phytochemicals in the blueberry cultivars grown in 2010 to confirm the location in PC1. A, que-mal; B, myr-hex; C, CGA; D, C22:0; E, C18:1; F, C18:0; G, Hexal; H, 3-HexeOH; I, 2-Hexal; J, α-terpe; K, Lin; L, Lim, lim. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

To define the impact of specific compounds on the location of cultivars in the second PC the level of selected polyphenols with strong positive scores and anthocyanins with strong negative scores was examined. The levels of cat, CGA and que-mal were present at high levels in DA compared with AU and OZ suggesting that these compounds contributed significantly to the score of DA on PC2 (Figure 4.3A-C). The anthocyanins cya-ara, cya-gal and peo-pen were as expected present at high concentrations in OZ and low concentrations in DA. However, in AU which shared a similar score on PC2 to OZ they were present at intermediate levels (Figure 4.3D-F).

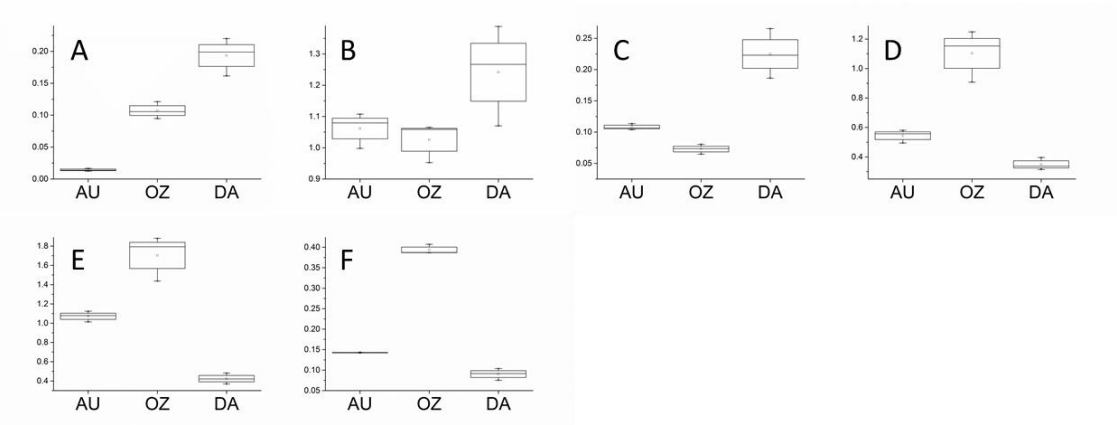


Figure 4.3. Box and whisker plots of selected phytochemicals in the blueberry cultivars grown in 2010 to confirm the location in PC2. A, cat; B, CGA; C, que-mal; D, cya-ara; E, cya-gal; F, peo-pen. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

4.2.1.2 Genetic diversity in blueberry cultivars 2011

The principal component product space of twelve varieties of blueberries grown in 2011 is shown in Figure 4.4A. The first two principal components accounted for 37 % of the total sample variance. 25 % of total variance was explained by the first PC with strong positive scores for BG and BE and strong negative scores for CH. Lipids and anthocyanins contributed to positive scores and flavour volatiles provided a negative contribution (Figure 4.4B). 12 % of variance was explained by the second PC with positive scores of OZ and negative scores of RE and SP. T-Anth and T-Phen contents and T-AO capacity and a range of polyphenols and CA contributed to positive scores, while sugars and MA provided a negative contribution.

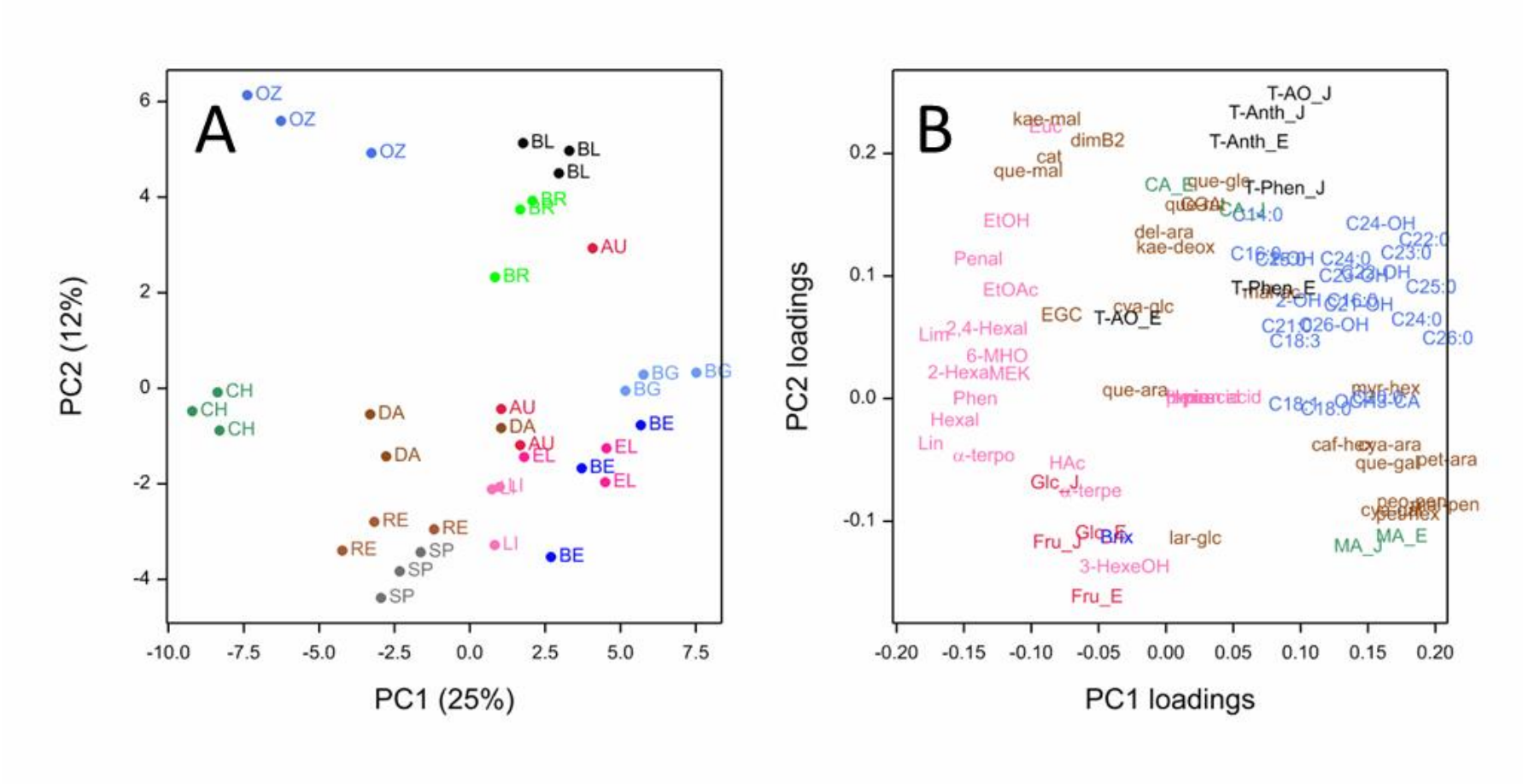


Figure 4.4. Principal component analysis (PCA) of metabolites (flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer) from twelve blueberry cultivars grown in 2011. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ volatiles, ■ lipids, ■ individual polyphenols and anthocyanins, ■ antioxidant components, ■ sugars, ■ organic acids, ■ brix). The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Similar to the cultivars in the harvesting year 2010, lipids had positive scores in the first PC while terpenoids had negative scores (Figure 4.4). A number of compounds from the classes lipids, anthocyanins and flavour volatiles were selected for more detailed analysis given their extreme positions on the PCA loadings plot. The levels of the lipids C14:0, C24:0 and C25:0 and the anthocyanins pet-ara, peo-pen, mal-pen in CH, BG and BE were consistent with their score on PC1 with low levels in the former cultivar and higher levels in BG and BE (Figure 4.5A-F). On the contrary, the flavour volatiles 6-MHO, Hexal and Lim which provided negative scores on PC1 were present at high levels in CH and low levels in BG and BE (Figure 4.5G-I).

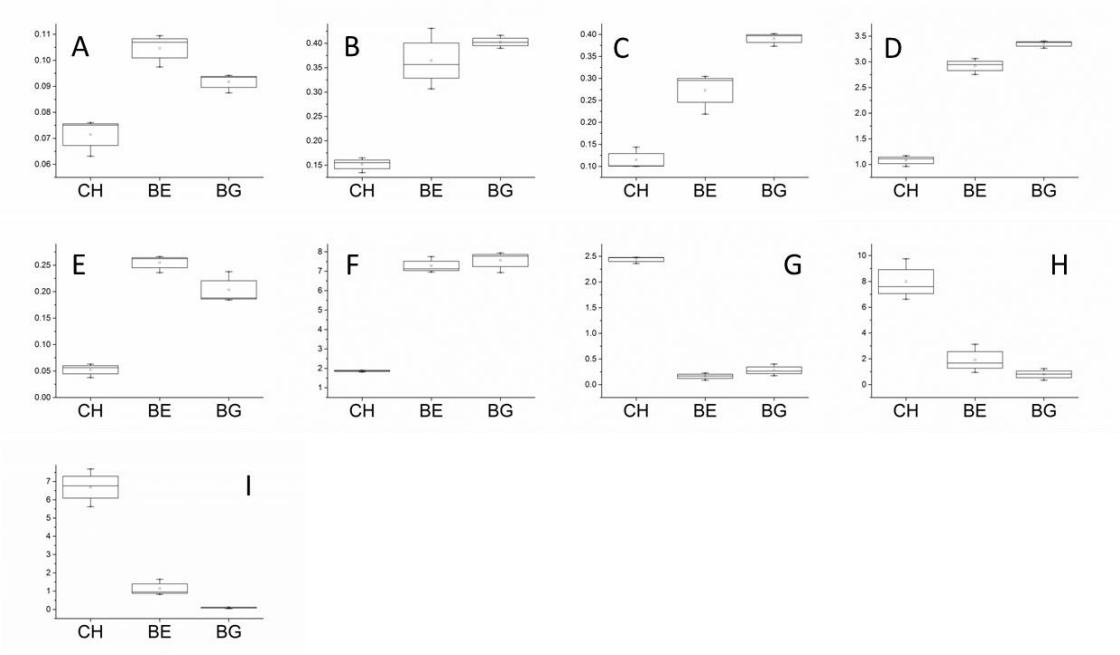


Figure 4.5. Box and whisker plots of selected phytochemicals in the blueberry cultivars grown in 2011 to confirm the location in PC1. A, C14:0; B, C24:0; C, C25:0; D, pen-ara; E, peo-pen; F, mal-pen; G, 6-MHO; H, Hexal; I, Lim. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

In the second PC flavonoids, antioxidant capacity and CA provided positive scores while sugars and MA provided negative scores. Consistent with their location on the PCA plot, levels of que-rut, que-mal and CGA, T-Anth content and CA were present at high levels in OZ and low levels in RE and SP (Figure 4.6A-C, E-F). T-AO_J capacity had as

expected high concentrations in OZ, levels were also high in SP but low in RE suggesting that other compounds were contributing to the position of theses cultivars on PC2 (Figure 4.6D). Also consistent was the finding that MA was present at higher levels in RE and SP than OZ (Figure 4.6I). Levels of Glc and Fru were fairly similar within these blueberry cultivars and therefore probably less significant in their positioning on the PCA plot (Figure 4.6G-H).

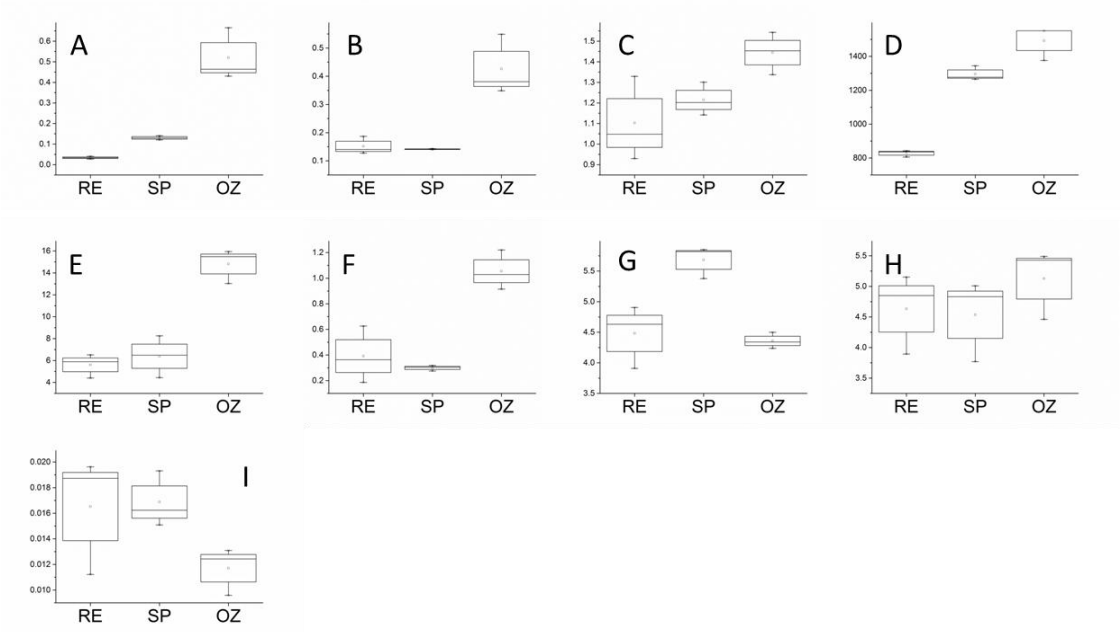


Figure 4.6. Box and whisker plots of selected phytochemicals in the blueberry cultivars grown in 2011 to confirm the location in PC2. A, que-rut; B, que-mal; C, CGA; D, T-AO_J; E, T-Anth_J; F, CA_J; G, Fru_E; H, Glc_J; I, MA_E. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

4.2.1.3 Genetic diversity of blueberry cultivars 2012

The principal component product space of nineteen varieties of blueberries in the growing year 2012 is shown in Figure 4.7A. The first two PCs accounted for 32 % of total sample variance. 19 % of total variance was explained by the first PC with strong positive scores for BG and BL and strong negative scores of SP. Lipids and antioxidant properties contributed to positive scores in this PC while sugars and terpenoids provided a negative contribution (Figure 4.7B). 13 % of variance was explained by the second PC with strong positive scores for PU and negative scores of BR and BL. Flavour volatiles contributed positive scores and anthocyanins provided a negative contribution in this PC.

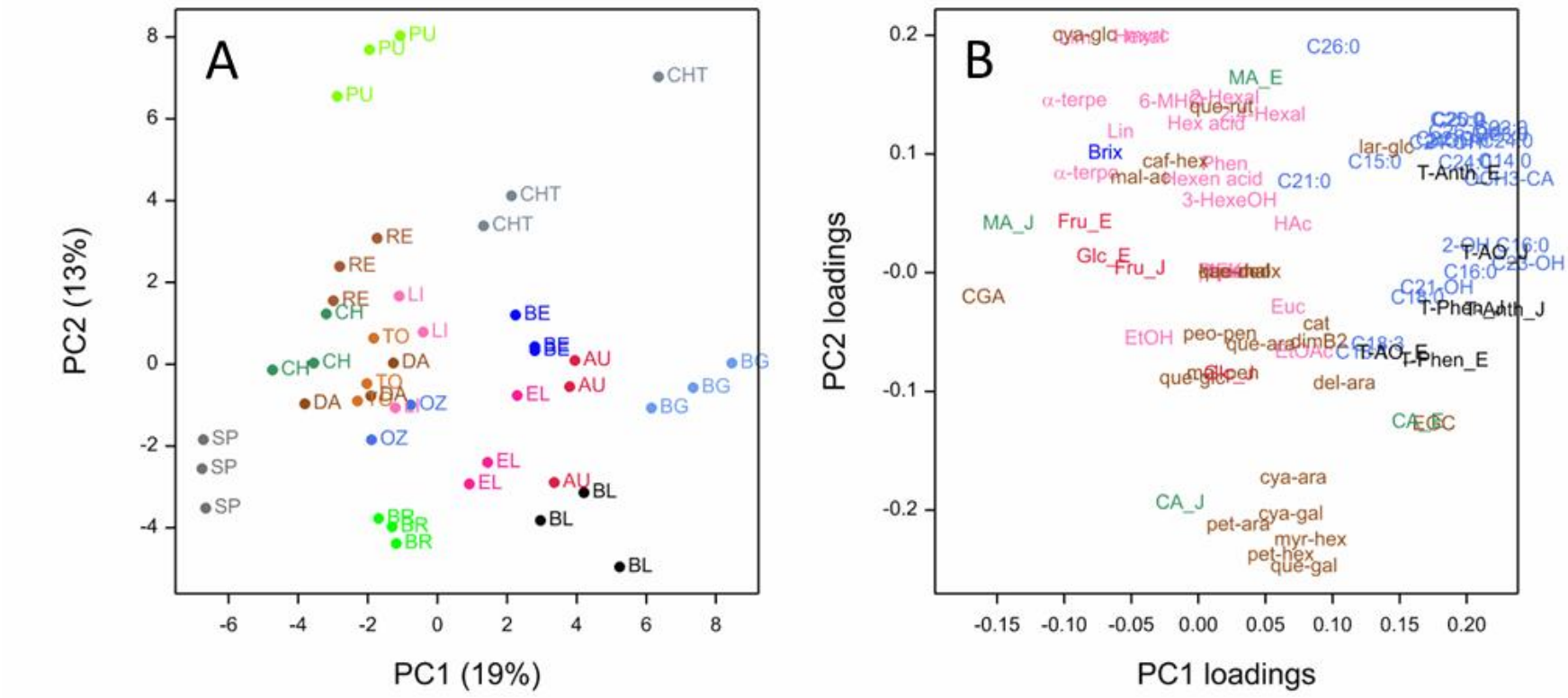


Figure 4.7. Principal component analysis (PCA) of metabolites (flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer) from fifteen blueberry cultivars grown in 2012. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ volatiles, ■ lipids, ■ individual polyphenols and anthocyanins, ■ antioxidant components, ■ sugars, ■ organic acids, ■ brix). The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

A range of lipids, T-AO capacity, terpenoids and sugars were selected to confirm the location of cultivars on the first PC. Consistent with their locations on the PCA plot levels of the lipids C25:0, C16:0 and C20:0 were present at high levels in BG and BL and low levels in SP (Figure 4.8A-C). T-Phen and T-Anth contents and T-AO capacity were also consistent with the positions of the samples on the PCA plot (Figure 4.8D,F), while levels of T-Anth content has as expected low concentrations of SP, levels were also low in BL but high in BG suggesting that other compounds were contributing to the position of these cultivars von PC1 (Figure 4.8E). As expected, the terpenoids Lim, α-terpo and α-terpe that contribute a negative score on PC1 were present at high levels in SP and low levels in BG and BL (Figure 4.8G-I). The distribution of Glc and Fru that also contribute a negative score on this axis was more complex and levels of Glc and Fru were fairly similar within these blueberry cultivars and therefore probably less significant in their positioning on the PCA plot (Figure 4.8J-K).

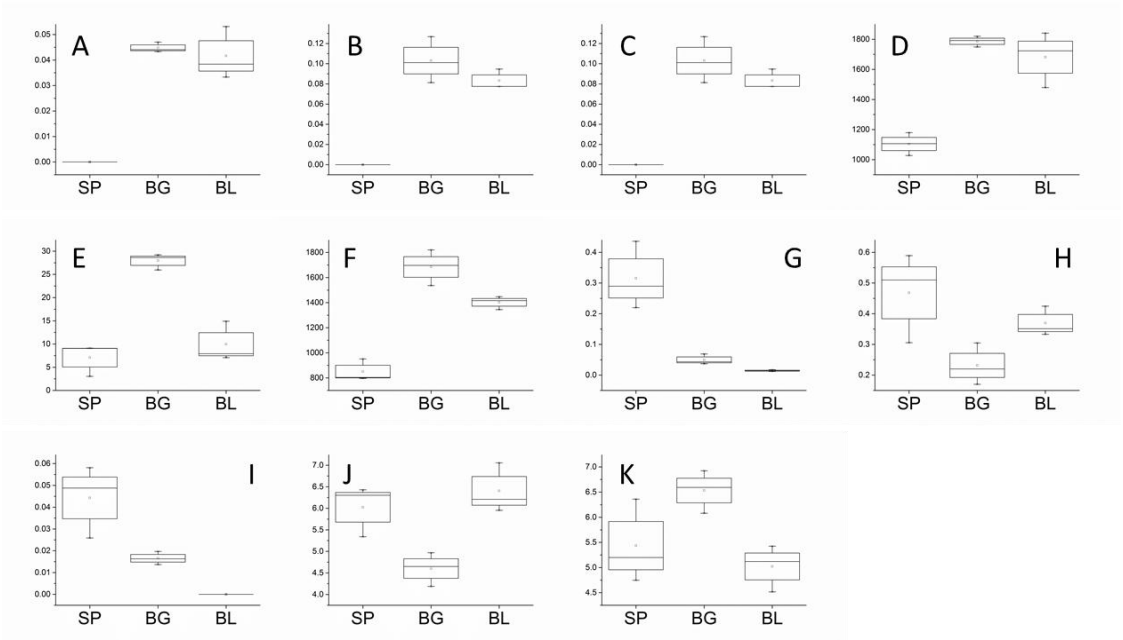


Figure 4.8. Box and whisker plots of selected phytochemicals in the blueberry cultivars grown in 2012 to confirm the location in PC1. A, C25:0; B, C16:0; C, C20:0; D, T-Phen_E; E, T-Anth_J; F, T-AO_J; G, Lim; H, α-terpo; I, α-terpe; J, Glc_E; K, Fru_J. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

In the second PC flavour volatiles contributed positives scores and anthocyanins provided a negative contribution. The flavour volatiles Lim, Hexal and myrc tended to have higher concentrations in PU and lower concentrations in BL and BR consistent with their relative positions on the PCA plot (Figure 4.9A-C). The inverse pattern was observed with the anthocyanins cya-ara, pet-ara and pet-hex that contributed negative scores for PC2 (Figure 4.9D-F).

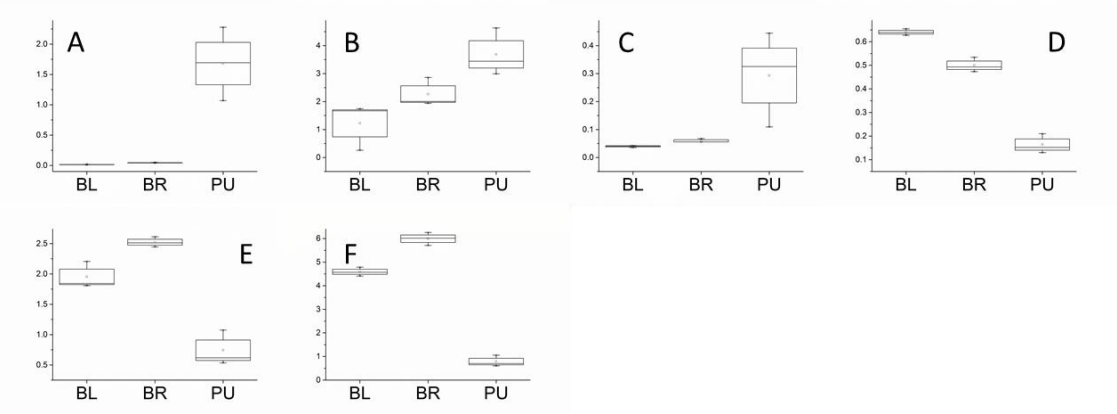


Figure 4.9. Box and whisker plots of selected phytochemicals in the blueberry cultivars grown in 2012 to confirm the location in PC2. A, Lim; B, Hexal; C, myrc; D, cya-ara; E, pet-ara; F, pet-hex. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

4.2.2 Environmental diversity

A clear impact of growing environment was observed by comparison of the phytochemical composition of fruit from identical cultivars grown in the years 2010, 2011 and 2012 (Figure 4.10A). Following PCA analysis of the data, the first two PCs accounted for 40 % of the total sample variance. 29 % of total variance was explained by the first PC which effectively separated 2012 samples from those collected in 2010 and 2011. Lipids, CA and a range of polyphenols contributed extensively to positive scores in the first PC with a range of C_6 flavour volatiles and sugars contributing negative scores (Figure 4.10B). 11 % of variance was explained by the second PC with a range of anthocyanins contributing positive scores and flavour volatiles providing a negative contribution.

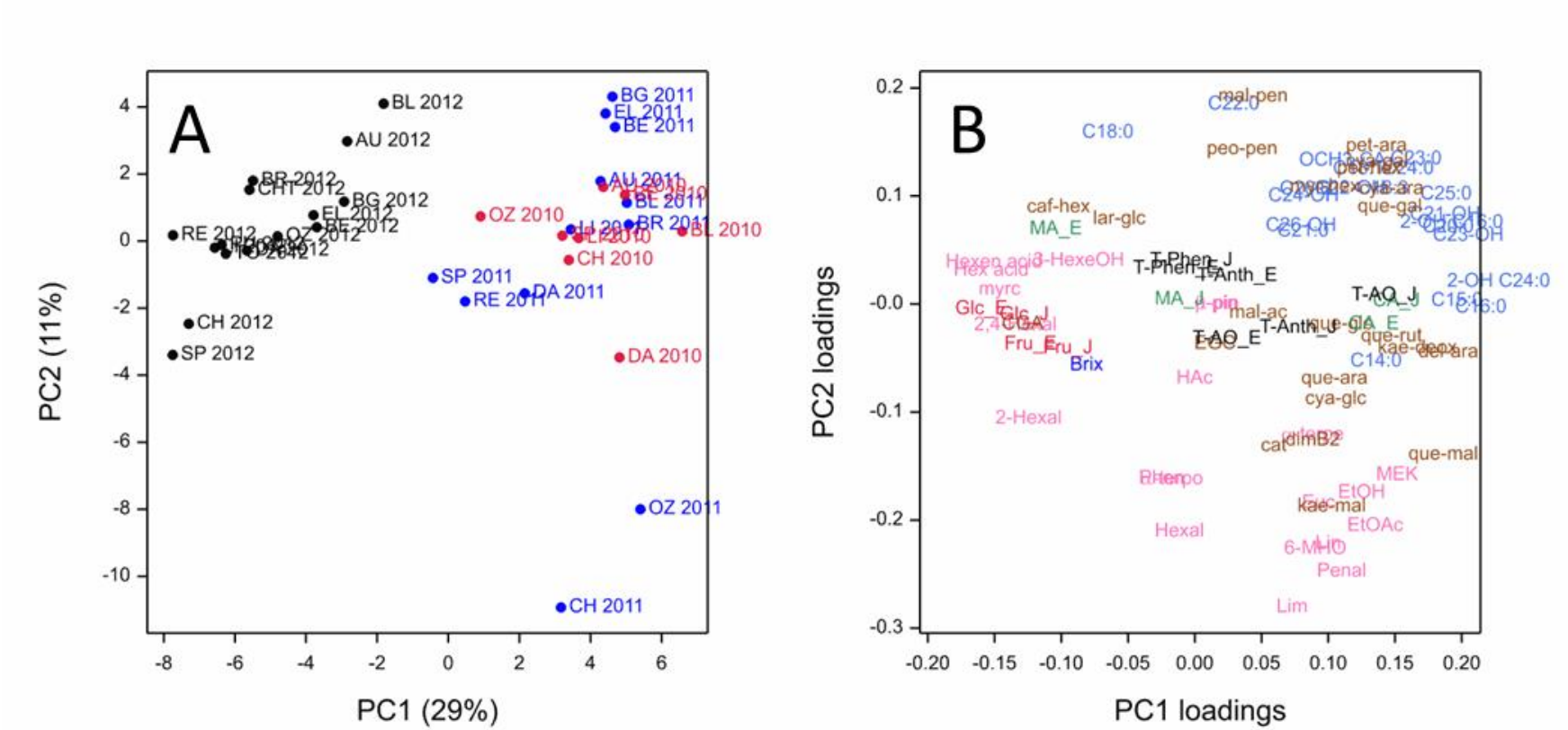


Figure 4.10. Principal component analysis (PCA) of metabolites (flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer) from blueberry cultivars grown in 2010, 2011 and 2012. (A) PCA scores plot of cultivars grown in different years, harvesting year 2010 refers to ■, 2011 refers to ■, 2012 refers to ■ and (B) the corresponding PCA loading plot (B, ■ volatiles, ■ lipids, ■ individual polyphenols and anthocyanins, ■ antioxidant components, ■ sugars, ■ organic acids, ■ brix). The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

For further investigation, compounds with strong positive and negative scores were selected to confirm environmental impact on fruit chemistry in different growing years. The box and whisker plots shows the content of selected lipids, flavour volatiles, CA, anthocyanins, polyphenols and sugars in BG, LI and CH grown in different years to confirm the location in PC1. As expected from the loadings plot and the position of fruit grown in different years on the PCA plot, a range of lipids, polyphenols and CA were present at high concentrations in 2010/11 and low concentrations in 2012 with the exception of CH in which the levels of CA were fairly similar in different growing years and therefore probably less significant in their influence on the positioning of this cultivar on the PCA plot (Figure 4.11A-G). 2-Hexal, Fru and Glc were consistent with the position of the samples on the PCA plot with the exception of CH grown in 2011 in which the 2-Hexal level was very high compared with CH grown in 2010 and 2012 (Figure 4.11H,K,L).

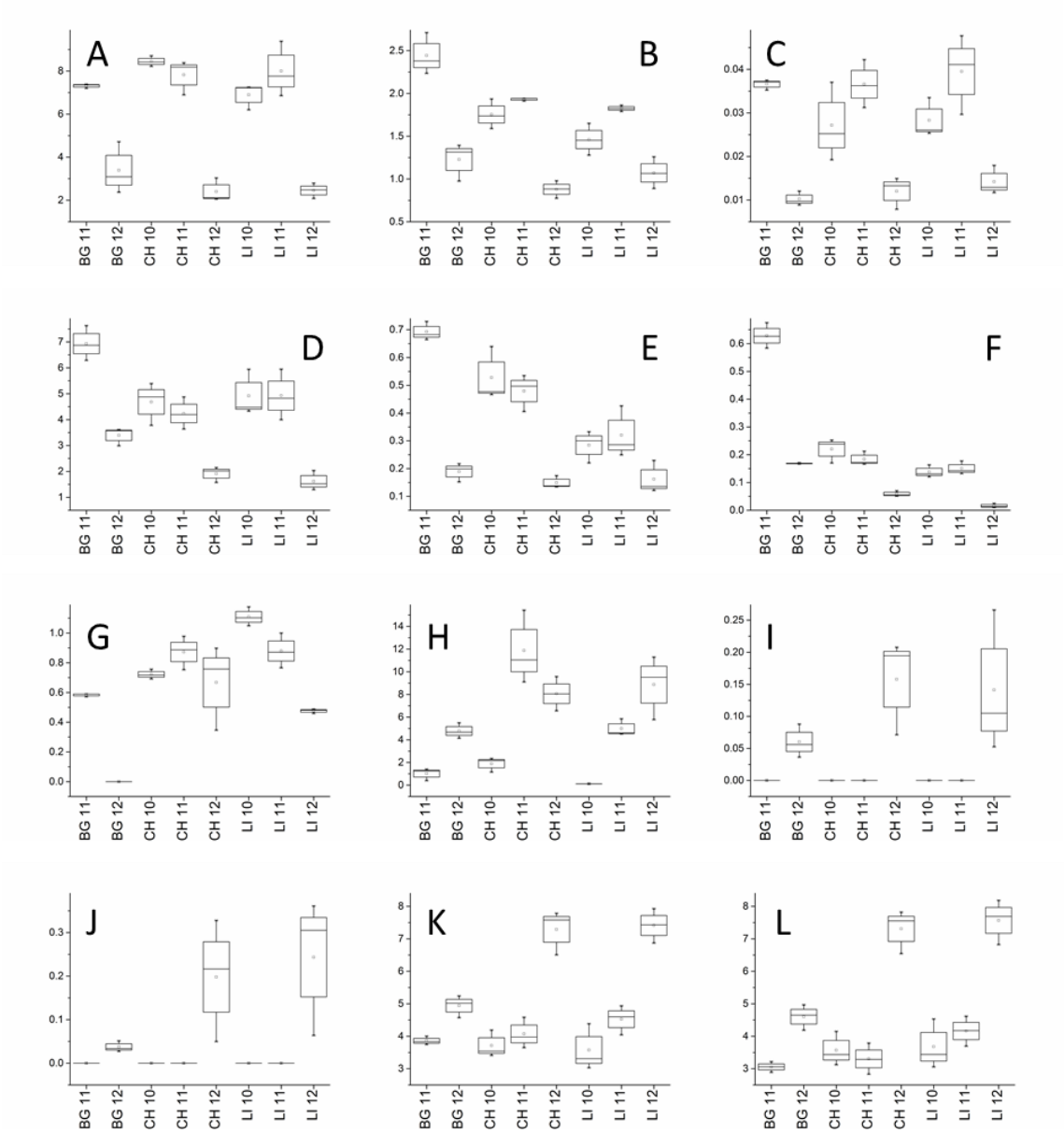


Figure 4.11. Box and whisker plots of selected phytochemicals in blueberry cultivars grown in different years (10, 11, 12 indicated the growing years 2010, 2011 and 2012) to confirm the location in PC1. A, C16:0; B, C20:0; C, C15:0; D, que-hex; E, que-glc; F, myr-hex; G, CA_J; H, 2-Hexal; I, myrc; J, Hex acid; K, Fru_E; L, Glc_E. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

The flavour volatile profile of blueberries grown in 2010 and 2011 differed from blueberry cultivars grown in 2012 (Figure 4.12). The differences among the means were compared between different harvesting years using one-way ANOVA. 2-Hexal and 3-HexeOH were significantly higher ($p < 0.05$) in cultivars grown in 2012 and additionally myrc and Hex acid were only determined in cultivars grown in 2012 (Figure 4.11I-J, Table 4.1). The high levels of 2-Hexal, 3-HexeOH, myrc and Hex acid were emitted in response to insect feeding similar to previous studies in other plants (Turlings and Ton, 2006, Farag et al., 2004, Farine et al., 1996). It has is therefore possible that pests and disease may have had a higher impact than abiotic factors.

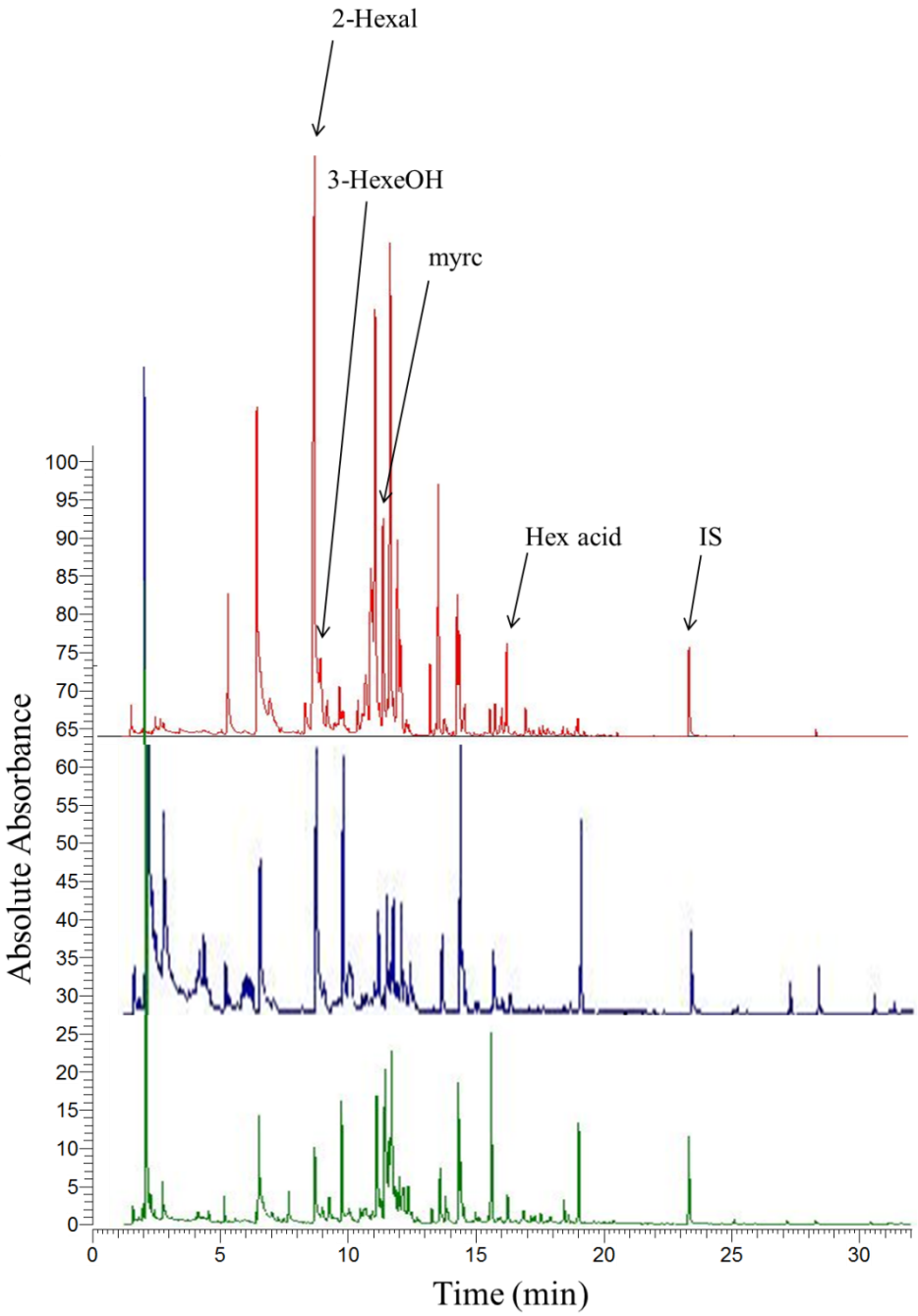


Figure 4.12. Chromatograms of flavour volatiles analysed from CH grown in three different harvesting years. Flavour volatiles were analysed with SPME-GC-MS. CH grown in 2010 refers to ■, CH grown in 2011 refers to ■ and grown in 2012 refers to ■. The list of the phytochemicals including the abbreviations is shown in Appendix D. CH, Chandler; IS, internal standard (4-chlorophenol).

Table 4.1. Selected flavour volatiles estimated in the SPME-GC-MS of blueberry cultivars grown in three different years. The table indicates the mean response factor ± standard deviation (n = 3). The list of the phytochemicals including the abbreviations is shown in Appendix D. NF refers to not found.

	2010	2011	2012
2-Hexal	2.18 ± 1.41^{a}	3.93 ± 3.59^{a}	6.97 ± 2.61^{b}
3-HexeOH	0.50 ± 0.43^{a}	0.21 ± 0.21^{a}	1.56 ± 2.09^{b}
myrc	NF	NF	0.10 ± 0.07
Hex acid	NF	NF	0.22 ± 0.13

^{a,b} different letter superscripts indicate significant differences at $p < 0.05$.

The box and whisker plots shows the content of selected flavour volatiles and anthocyanins and sugars in BG, LI and CH grown in different years to confirm the location in PC2. BG, LI and CH were selected due to their different locations in the PCA plot within the years. As expected from the loadings plot and the position of fruit grown in different years on the PCA plot, a range of flavour volatiles have shown the outlying position of CH grown in 2011 and the trend of high concentration in 2011 and low concentrations in 2010 and 2012 (Figure 4.13A-C), a range of anthocyanins were present at high concentrations in 2011 and low concentrations in 2010 and 2012, with the expectation of CH (Figure 4.13D-F). It was suggested that the second PC has an impact on flavour volatiles and anthocyanins, depending on their location.

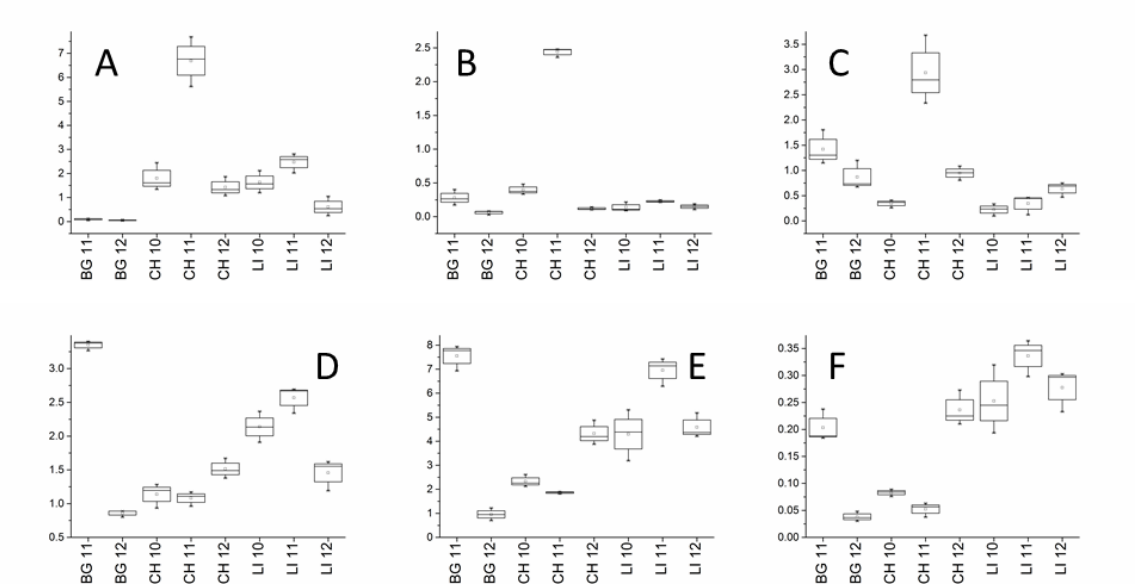


Figure 4.13. Box and whisker plots of selected phytochemicals in blueberry cultivars grown in different years (10, 11, 12 indicated the growing years 2010, 2011 and 2012) to confirm the location in PC2. A, Lim; B, 6-MHO; C, Euc; D, pet-ara; E, mal-pen; F, peo-pen. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Seasonal variation

Different seasonal conditions are likely to have an impact on the phytochemical content of blueberry fruit. Table 4.2 illustrates the average values for a range of phytochemical parameters in the eight cultivars, which were analysed in all three years. The differences among the means were compared between blueberry juices and acidified methanolic extracts and were compared between different growing years using one-way ANOVA; the significance of these effects was performed using Fisher's protected least significance. The Glc and Fru content were significantly higher ($p < 0.05$) in extracts than in juice in the harvesting years 2010 and 2012. Conversely to the studies of Sandell et al. (2009) in black currant, sugar content was higher in juice. Organic acids have shown trends to higher content in blueberry juices; with significant differences ($p < 0.05$) of CA in the harvesting year 2010 and significant differences ($p < 0.05$) of MA in the harvesting year 2010 and 2011. In agreement to the studies of Sandell et al. (2009) in black currant, acid contents in juice were higher than in press residue. The

antioxidant properties were significantly ($p < 0.05$) higher in acidified methanolic extracts than in juices. The different content between juice and extracts occurred due to the removal of the skin during fruit pressing, the skin containing the major content of antioxidant compounds (Cho et al., 2004). Further studies in black currant confirmed the higher levels of polyphenols in press residue compared to the juice (Sandell et al., 2009).

Sugars and MA were significantly influenced by growing season; the blueberry extracts grown in 2012 had a significantly higher content ($p < 0.05$) than those grown in 2010 or 2011 quantified in acidified methanolic extracts. Conversely, the CA content in blueberry extracts grown in 2012 was significantly lower ($p < 0.05$) than those grown in 2010 and 2011. Additionally T-AO and T-Anth were significantly influenced by growing season; the blueberry extracts grown in 2011 had significantly higher concentrations ($p < 0.05$) than those grown in 2010 or 2012. Similarly T-Anth content was significantly higher ($p < 0.05$) in blueberry juices grown in 2010. The T-Phen content in blueberry juice varied significantly by growing season ($p < 0.05$) with the highest content 2011, followed by 2012 and 2010. Similar results were observed in blueberry extracts.

Table 4.2. Concentrations of selected phytochemicals in the eight blueberry cultivars (AU, BE, BL, CH DA, LI, OZ, SP), which were available in the harvesting years 2010, 2011 and 2012 were analysed in juice and acidified methanolic extracts. The table indicates the mean scores ± standard deviation (n = 3). The list of the phytochemicals including the abbreviations is shown in Appendix D. Values in bold are significant ($p < 0.05$).

	2010	2011	2012
Glc[1]			
juice	2.32 ± 0.78^{a}	4.14 ± 0.90^{b}	5.12 ± 0.75^{c}
extract	3.54 ± 0.78^{a}	3.94 ± 0.66^{a}	6.22 ± 1.23^{b}
	p = 0.000	p = 0.105	**p = 0.000**
Fru[1]			
juice	3.32 ± 1.21^{a}	4.97 ± 0.95^{b}	5.13 ± 0.68^{b}
extract	4.00 ± 0.89^{a}	4.46 ± 0.62^{a}	6.48 ± 1.15^{b}
	p = 0.044	p = 0.453	**p = 0.001**
CA[1]			
juice	0.82 ± 0.27^{a}	0.81 ± 0.28^{a}	0.53 ± 0.23^{b}
extract	0.63 ± 0.18^{a}	0.67 ± 0.30^{a}	0.45 ± 0.24^{b}
	p = 0.005	p = 0.104	p = 0.240
MA[1]			
juice	0.038 ± 0.012^{a}	0.033 ± 0.009^{a}	0.036 ± 0.014^{a}
extract	0.017 ± 0.008^{a}	0.019 ± 0.006^{a}	0.030 ± 0.020^{b}
	p = 0.000	**p = 0.000**	p = 0.232
T-AO[2]			
juice	1128.2 ± 721.2^{a}	1154.8 ± 313.7^{a}	996.1 ± 218.5^{a}
extract	2805.8 ± 627.3^{a}	4362.8 ± 1672.5^{b}	3299.4 ± 595.6^{a}
	p = 0.000	**p = 0.000**	**p = 0.000**
T-Anth[2]			
juice	38.77 ± 20.59^{a}	11.76 ± 7.58^{b}	8.11 ± 4.12^{b}
extract	299.19 ± 44.10^{a}	366.34 ± 138.30^{b}	300.80 ± 71.84^{a}
	p = 0.000	**p = 0.000**	**p = 0.000**
T-Phen[2]			
juice	111.47 ± 33.11^{a}	455.16 ± 176.16^{b}	343.44 ± 39.72^{c}
extract	496.22 ± 153.67^{a}	1305.91 ± 395.11^{b}	1233.30 ± 307.10^{b}
	p = 0.000	**p = 0.000**	**p = 0.000**

[1] concentrations in g/100 ml
[2] concentrations in mg/100 ml.
[a,b,c] different letter superscripts within row indicate significant differences at $p < 0.05$.

Meteorological records recorded at the Hutton site were examined to test for relationships between prevailing conditions and fruit antioxidant content. However, there were no obvious correlations between temperature, rainfall or sun exposure during fruit development and fruit antioxidant properties. The hottest, driest with the most sun exposure was the summer 2010; the coldest, wettest, with the least sun exposure was the summer 2012 and the summer 2011 was in between (Table 4.3). The antioxidant properties did not relate to the total sun exposure. Recent studies reported a significantly higher ($p < 0.05$) T-AO capacity, including T-Anth and T-Phen contents after UV-B and UV-C exposure in blueberries and raspberries (Eichholz et al., 2011, Wang et al., 2009, Bañados, 2006). It was suggested that the fruit developmental stage during harvesting influenced the T-AO capacity, T-Anth and T-Phen contents.

Table 4.3. Meteorological Data from June to August of the three harvesting years at the James Hutton Institute, Invergowrie, Scotland.

	Harvesting years		
	2010	2011	2012
Mean monthly daytime temperature [°C]	15.0	13.9	13.9
Mean monthly rain [mm]	2.7	3.7	4.6
Total sun hours	562.4	449.0	349.4

On the contrary, the impact of weather conditions on sugar and organic acid content did appear to be significant. The hottest summer with the most sun exposure (2010) produced fruit with the lowest Glc and Fru contents and highest CA content, while the summer of 2012 with least sun exposure produced the highest Glc and Fru contents and lowest organic acid content (Table 4.2). These data are in accordance with research in strawberries indicating that higher temperature decreased the sugar content (Bañados, 2006). Conversely, in tomato high temperatures resulted in high sugar content (Rosales et al., 2007, Kassim et al., 2009) suggesting that influences of temperature on fruit phytochemistry are species dependent. The MA content was not significantly different ($p > 0.05$) between the years. Lobit et al. (2006) has shown a negative relation of high

temperature and MA content in peaches. Environmental conditions influence the activity of invertase and sucrose synthase.

Further environmental conditions such as water availability has an influence on metabolite accumulation in fruit, in accordance to several studies in grapes where high water availability increased the sugar content and total soluble acids (Matthews and Anderson, 1988, Santesteban and Royo, 2006). Furthermore, Glc and Fru contents were increased and CA was decreased with high water availability. In contrast several studies on fruit indicated that a high water deficit increased the total soluble solids and titratable acid (Spreer et al., 2009, Navarro et al., 2010, Pérez-Pérez et al., 2009).

The environmental conditions e. g. temperature, sun exposure and water availability influenced the accumulation of metabolites in blueberries. The weather conditions were suggested to influence the metabolite transport and accumulation in blueberries (Retamales et al., 2008, Kalt et al., 2003, Glass et al., 2005b)

The T-Anth content in blueberry cultivars has shown no relation to the weather conditions ($p > 0.05$). The T-AO capacity was non-significant different ($p > 0.05$) between the harvesting years. Rosales et al. (2007) showed that higher temperature and solar radiation facilitated the production of antioxidants due to the accumulation of hexoses in fruits.

Influence of other external factors

Furthermore, the growing location and practices are likely to influence fruit phytochemistry. A number of cultivars were grown at multiple sites under different agronomic conditions (Table 4.4). This allowed an analysis of the impact of additional external factors such as plant age, growing regime and influences of microclimate on fruit phytochemistry.

Table 4.4. Growing conditions of the same blueberry cultivars grown at different locations. For identification of the blueberry cultivars codes refer to Table 2.2. JHI refers to James Hutton Institute; Muirhead and Blairgowrie were commercial farms.

Cultivars	Growing location	Planted year	Growing condition
BL	JHI	1969	Open field
	Muirhead	2006	Open field
BR	Blairgowrie	1997	Open field
	Muirhead	2006	Open field
CH	Blairgowrie	2005	Polytunnel
	Muirhead	2006	Open field
SP	Blairgowrie	2005, 2008	Polytunnel
	Muirhead	2006	Open field

PCA of fruit phytochemical data revealed significant impacts of growing location (Figure 4.14A). The first two PCs accounted for 45 % of the total sample variance. 29 % of total variance was explained by the first PC with strong positive scores for BL grown in Muirhead. Lipids and antioxidant properties contributed to positive scores with C_6 flavour volatiles contributing negative scores (Figure 4.14B). 16 % of variance was explained by the second PC, with strong positive scores for BL grown at the James Hutton Institute and BR grown in Muirhead. Anthocyanins contributed positive scores, antioxidant properties and flavour volatiles provided a negative contribution. SP, BL, CH grown in Muirhead were separated from the SP and CH grown in Blairgowrie with PC1 contributing to this separation. BL grown at the James Hutton Institute and BR grown in Muirhead and Blairgowrie were generally separated from CH, SP, BL grown in Muirhead with PC2 contributing to this separation.

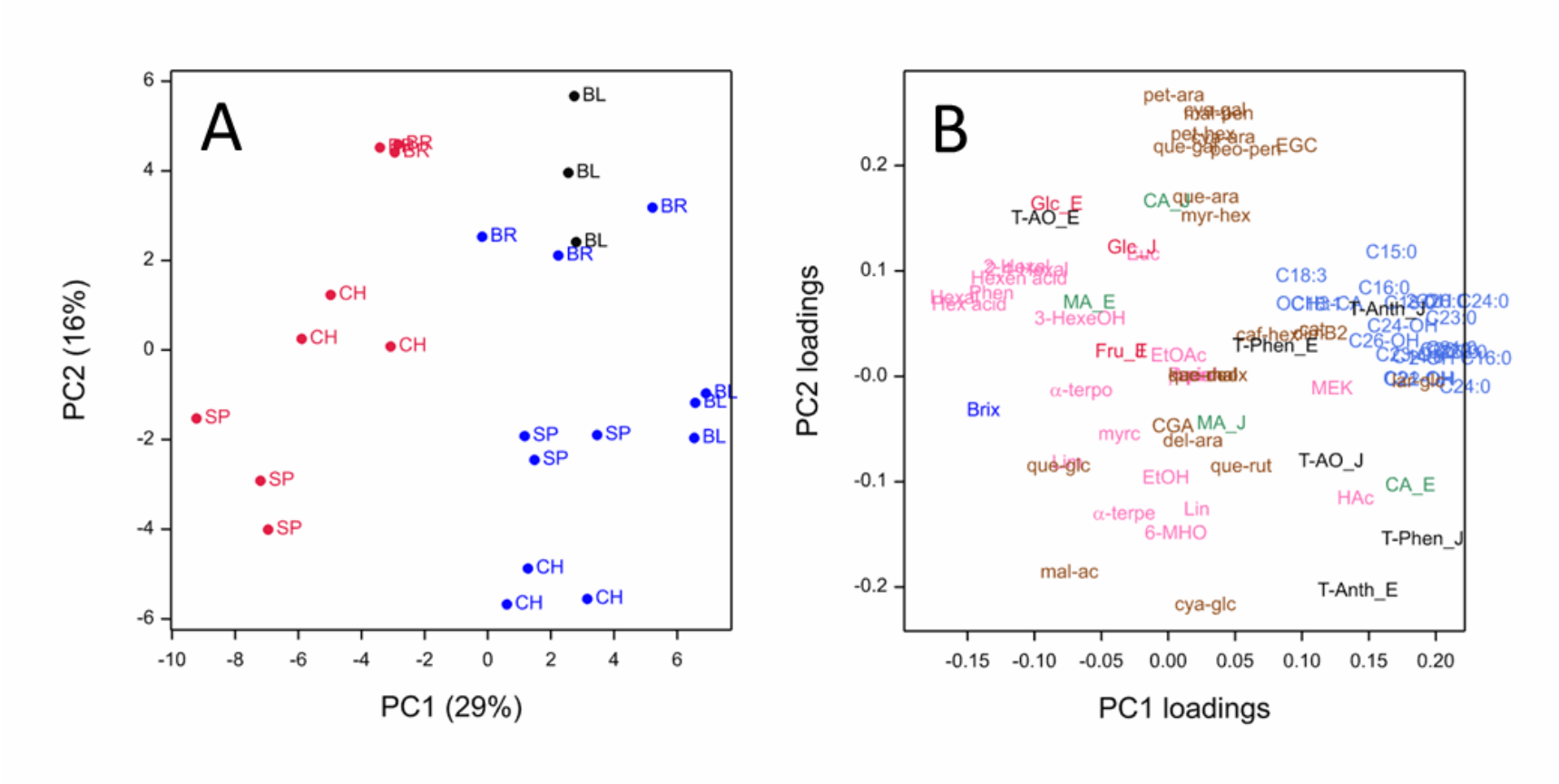


Figure 4.14. Principal component analysis (PCA) of metabolites (flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer) from blueberry cultivars in grown at different places. Cultivars grown at the commercial farm in Muirhead refers to blue colour, cultivars grown at the commercial farm in Blairgowrie refers to red colour and cultivars grown at the James Hutton Institute refers to black colour. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ volatiles, ■ lipids, ■ individual polyphenols and anthocyanins, ■ antioxidant components, ■ sugars, ■ organic acids, ■ brix). The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

The cultivars SP, CH and BR, which were grown at the commercial farm in Blairgowrie had high levels of C_6 flavour volatiles, while SP, CH, BL and BR grown in Muirhead and BL grown at the James Hutton Institute, Dundee were expected to exhibit high levels of lipids and antioxidant properties. The box and whisker plots confirmed the high levels of C_6 flavour volatiles in SP and CH grown in Blairgowrie and the low levels of antioxidants (Figure 4.15A-B,D-F), while levels of T-Anth content were high in CH and SP grown in Blairgowrie suggesting other compounds were contributing to the position of these cultivars (Figure 4.15C). Furthermore, age differences of plants and growing conditions such as under tunnels or in open field conditions could have an impact (Table 4.4). Cultivars grown in Blairgowrie were grown under polytunnels, except BR, while the remaining cultivars were grown open field.

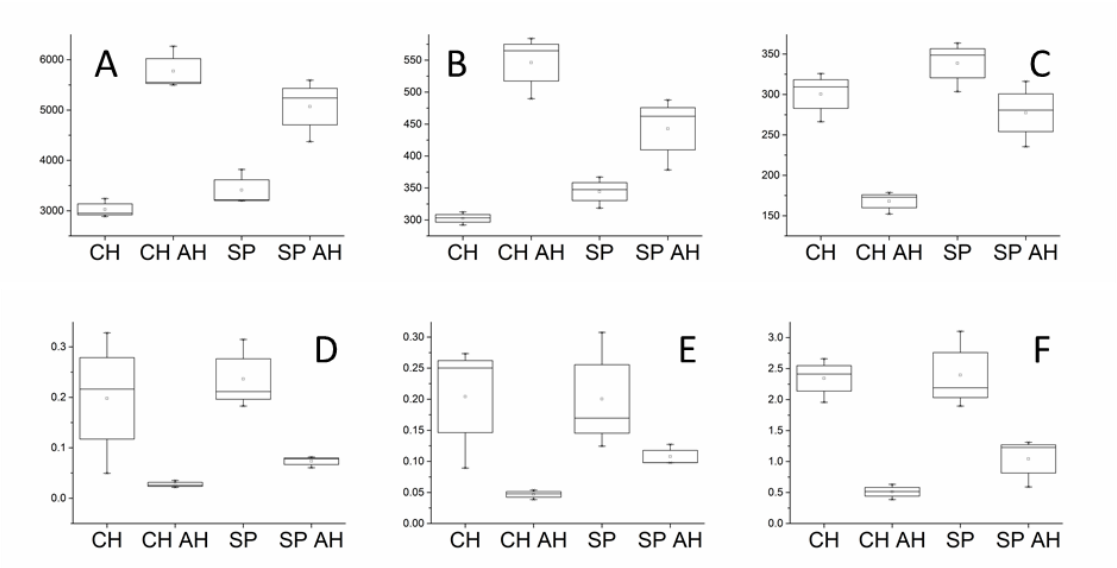


Figure 4.15. Box and whisker plots of CH and SP grown at different places. Cultivars grown at the commercial farm in Muirhead refers to AH, the remaining cultivars were grown at the commercial farm in Blairgowrie. A, T-AO_E; B, T-Phen_J; C, T-Anth_E; D, Hex acid; E, Hexen acid; F, Hexal. The data are given as mean, n = 3. The list of the phytochemicals including the abbreviations is shown in Appendix D. CH refers to Chandler, SP refers to Spartan.

Previous work has demonstrated a link between the absorbance of UV light by polytunnel sheeting and reduced fruit antioxidant capacity (Eichholz et al., 2011). The differences among the means were compared between different growing places using

one-way ANOVA, the significance of these effects was performed using Fisher's protected least significance.

In this study CH and SP grown at two different commercial farms exhibited no significant difference ($p > 0.05$) in the T-Phen and T-Anth contents (Table 4.5). The blueberry juices of CH and SP exhibited no significant difference in the T-AO capacity, while the acidified methanolic blueberry extracts were significantly different ($p < 0.05$). The acidified methanolic extracts of CH and SP grown under polytunnel at the commercial farm in Blairgowrie were significant lower ($p < 0.05$) in T-AO capacity in agreement to Tsormpatsidis (2008) (Tsormpatsidis et al., 2008). The UV radiation facilitates the production of flavonoids and related antioxidant compounds in plants (Tevini and Teramura, 1989, Rozema et al., 1997).

Table 4.5. Antioxidant properties of CH and SP grown in 2012 at two different commercial farms were analysed in juice and acidified methanolic extracts. The table indicates the mean scores ± standard deviation (n = 3) and quantified in mg/100 ml. The list of the phytochemicals including the abbreviations is shown in Appendix D. CH refers to Chandler, SP refers to Spartan. Values in bold are significant ($p < 0.05$).

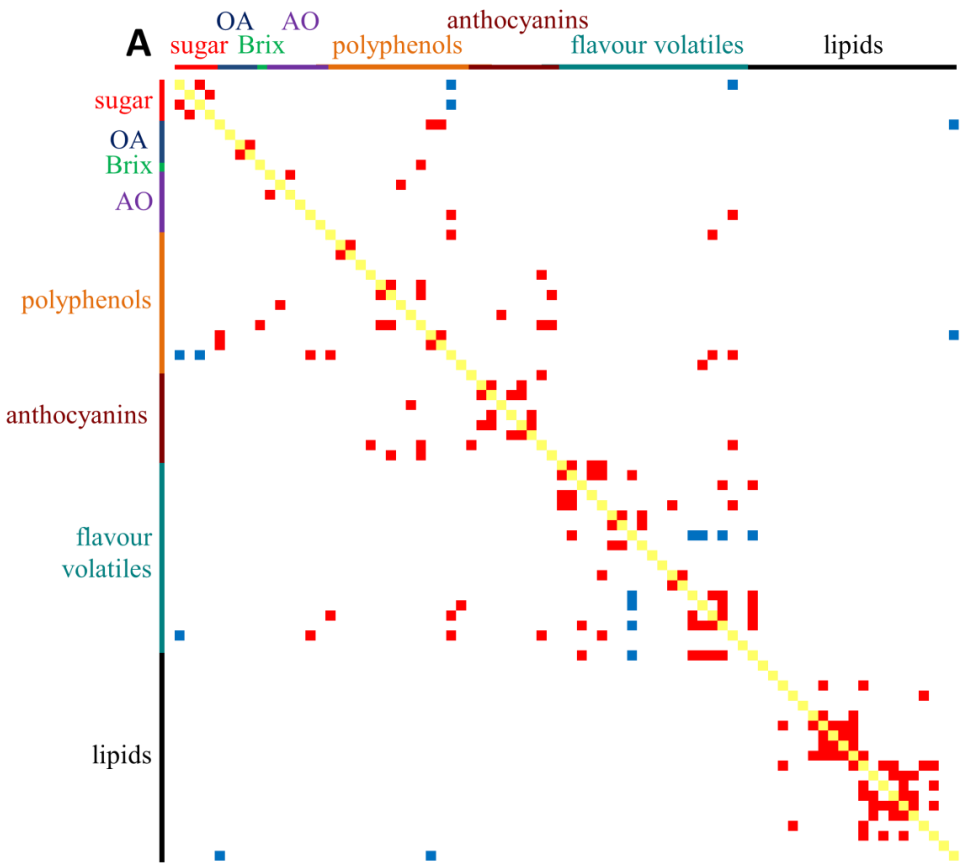
	Chandler		Spartan	
	Blairgowrie	Muirhead	Blairgowrie	Muirhead
T-AO[1]				
Juice	955.60 ± 50.22	875.98 ± 78.31	850.67 ± 87.56	1028.10 ± 65.62
	p = 0.235		p = 0.067	
Extract	3027.70± 188.38	5774.56 ± 428.66	3410.81 ± 354.79	5069.91 ± 627.58
	p = 0.010		**p = 0.028**	
T-Anth[1]				
Juice	5.92 ± 1.47	6.74 ± 3.08	7.07 ± 3.49	24.12 ± 6.21
	p = 0.719		**p = 0.025**	
Extract	300.50 ± 30.69	167.93 ± 13.90	338.58 ± 31.22	277.42 ± 40.47
	p = 0.021		p = 0.130	
T-Phen[1]				
Juice	302.57 ± 10.16	344.37 ± 24.40	546.20 ± 49.76	442.73 ± 57.28
	p = 0.014		p = 0.112	
Extract	1226.58 ± 31.17	1147.15 ± 59.12	1107.40 ± 76.02	1217.61 ± 98.62
	p = 0.132		p = 0.213	

4.2.3 Correlation analysis

Correlation analysis was undertaken to expose potential linkages between a broad spectrum of phytochemicals. All of the analysed phytochemicals of blueberry cultivars in three different growing years were studied and analysed with HPLC, UV-spectrometer, LC-MS^2 and GC-MS. The mean values across the whole sample sets of each metabolite were plotted against every other compound within the cultivars. Correlations were estimated in each harvesting year separately and were considered significant if they displayed a Pearson's correlation coefficient higher than 0.7 and or less than -0.7.

4.2.3.1 Correlations overview

Phytochemical correlations are presented in Figure 4.16; cultivars grown in 2010 exhibited the highest number of correlations with fewer observed in 2011 and 2012. The correlations by phytochemical class were similar in 2011 and 2012; only 2010 exhibited negative correlations. The main specific correlations were within lipids, volatiles and anthocyanins and they are focusing on individual compound correlations in the following figures, such as Figure 4.17.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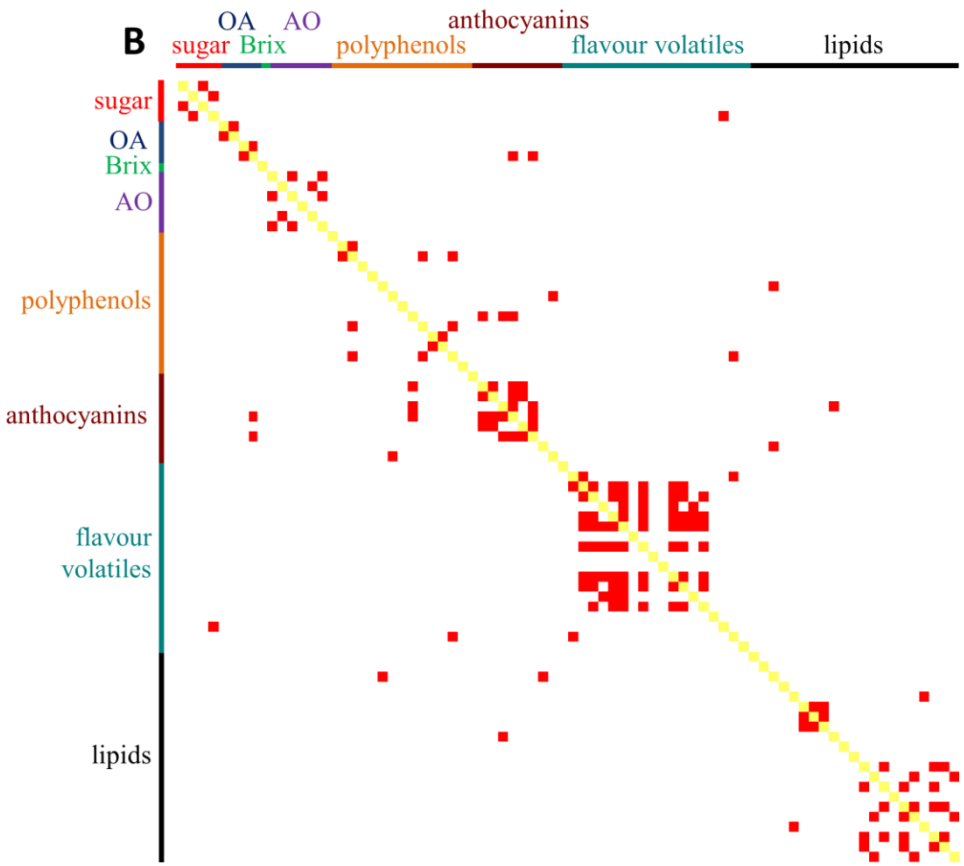
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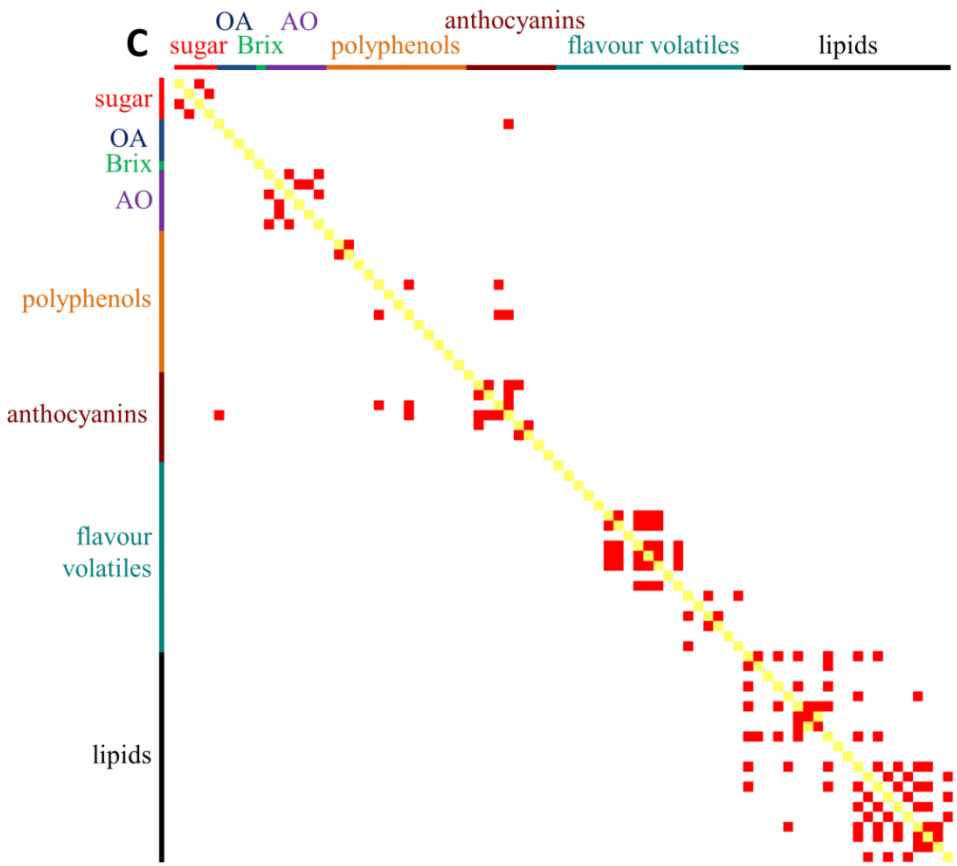


Figure 4.16. Correlations of phytochemicals in blueberry cultivars grown in different years. (A) refers to the harvesting year 2010; (B) refers to the harvesting year 2011 and (C) refers to the harvesting year 2012. Pearson's correlation coefficients $r > 0.7$ is indicated by ■, $r < -0.7$ is indicated by ■ and correlations between the same components is indicated by ■. The phytochemicals were analysed with flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-spectrophotometer in juice and acidified methanolic extracts in blueberry cultivars. The list of the phytochemicals including the abbreviations is shown in Appendix D.

4.2.3.2 Correlations between lipids

Lipids were analysed by GC-MS after derivatisation. In general cultivars grown in 2011 and 2012 are exhibited similar correlations (Figure 4.17). Two correlation clusters were observed; correlations of shorter chain fatty acids (C14:0 to C21:0) and longer chain fatty acids (C22:0 to C26:0).

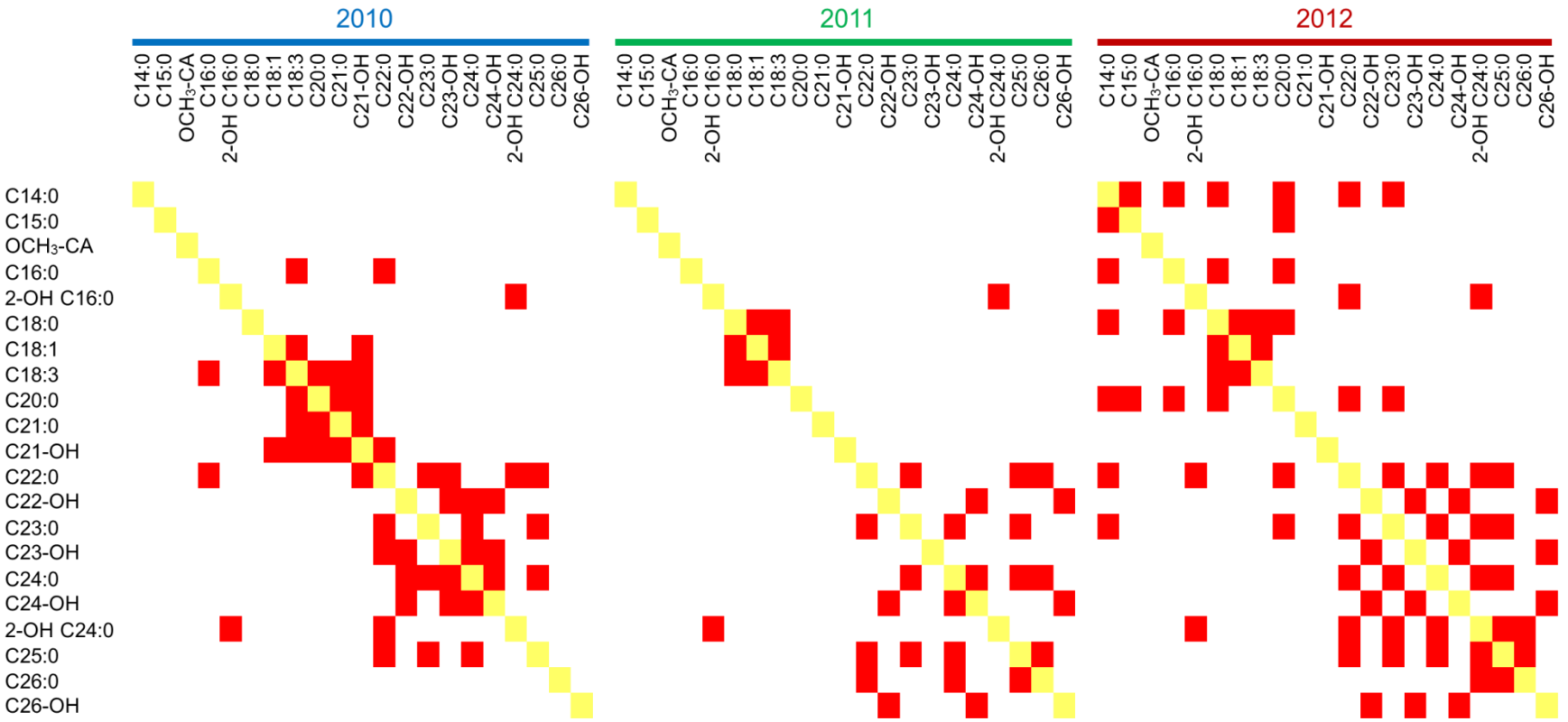


Figure 4.17. Correlations of lipids in blueberry cultivars in three different harvesting years. (A) refers to the harvesting year 2010; (B) refers to the harvesting year 2011 and (C) refers to the harvesting year 2012. Pearson's correlation coefficients r > 0.7 is indicated by ■, r < -0.7 is indicated by ■ and correlations between the same components is indicated by ■. The list of the phytochemicals including the abbreviations is shown in Appendix D. 45.

The shorter chain fatty acids had the most correlations in the growing years 2012, a little less in 2010 and considerably less in 2011. The few correlations of shorter chain fatty acids in 2011 were similar to 2012, while 2010 exhibited different correlations. C18:1 was correlated to C18:3 in all three years, while C18:0 correlated to C18:1 and C18:0 to C18:3 in 2011 and 2012. The 18 carbons long acyl chain may form double bounds in the plastid by acyl-ACP desaturase to C18:1 and C18:3 (Shanklin and Cahoon, 1998, Araújo et al., 2012).

The highest numbers of correlations were shown between the longer chain fatty acids with similar correlations in 2011 and 2012. The longer chain fatty acids and the corresponding alcohols are formed in the epidermal cell in a different elongation system than shorter chain fatty acids (Figure 4.18). Different elongation systems are related with different linked pathways that probably do not using the same precursors. This acyl elongation system ED2 detected in maize elongated C22:0 to the longer chain fatty acids (Shepherd and Wynne Griffiths, 2006). Yeast genes (*ELO*) are involved in the elongation of fatty acids; *ELO 1* is involved in the elongation of C14:0 to C16:0, *ELO 2* is involved in the elongation from C16:0 to C24:0, *ELO3* is mainly involved in the elongation from C24:0 to C26:0 (Oh et al., 1997). Additionally, correlations of even and odd long chain fatty acids were observed. Their biosynthesis pathways are similar, even chain fatty acids were formed by using acetyl-CoA, odd chain fatty acids were formed by using an alternative primer (Shepherd and Wynne Griffiths, 2006).

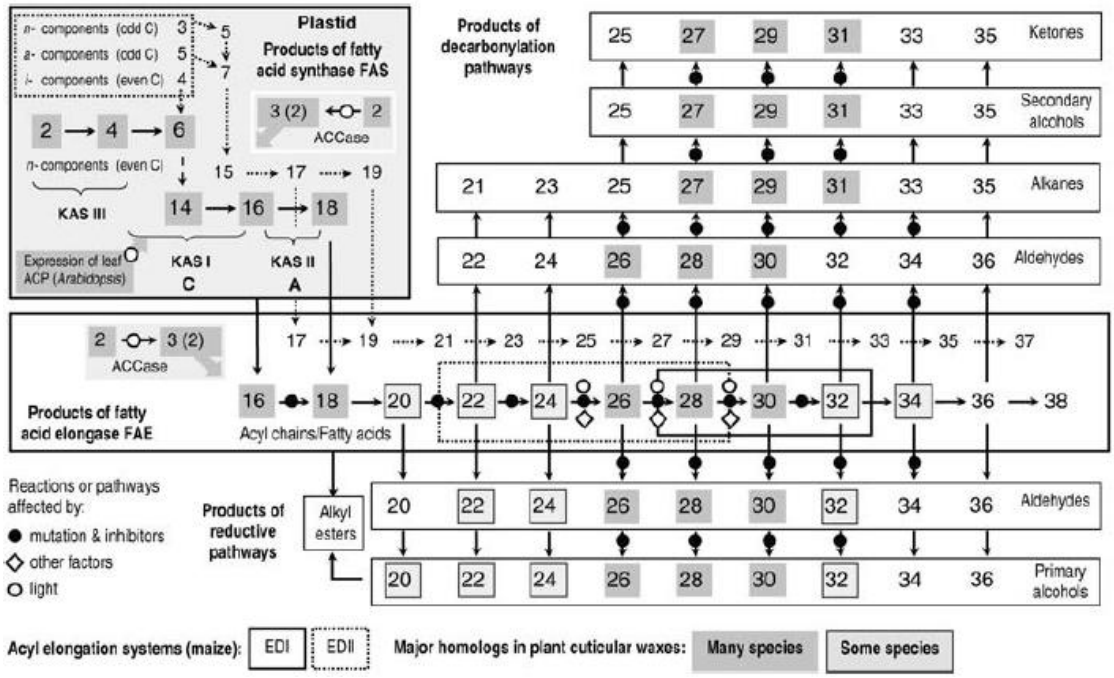


Figure 4.18. Scheme of the acyl chain elongation in plastid, and following reactions of acyl intermediates into the decarbonylative and reductive products. Numbers in the square describe the length of the acyl chains (Shepherd and Wynne Griffiths, 2006).

Further correlations were shown between alcohols. The secondary alcohols 2-OH C16:0 and 2-OH C24:0 were correlated in all three harvesting years (Shepherd and Wynne Griffiths, 2006). The decarbonylation pathway forms secondary alcohols while the reductive pathways form primary alcohols. Therefore a correlation of secondary alcohols with different chain length is more likely than correlations between primary and secondary alcohol (Figure 4.19).

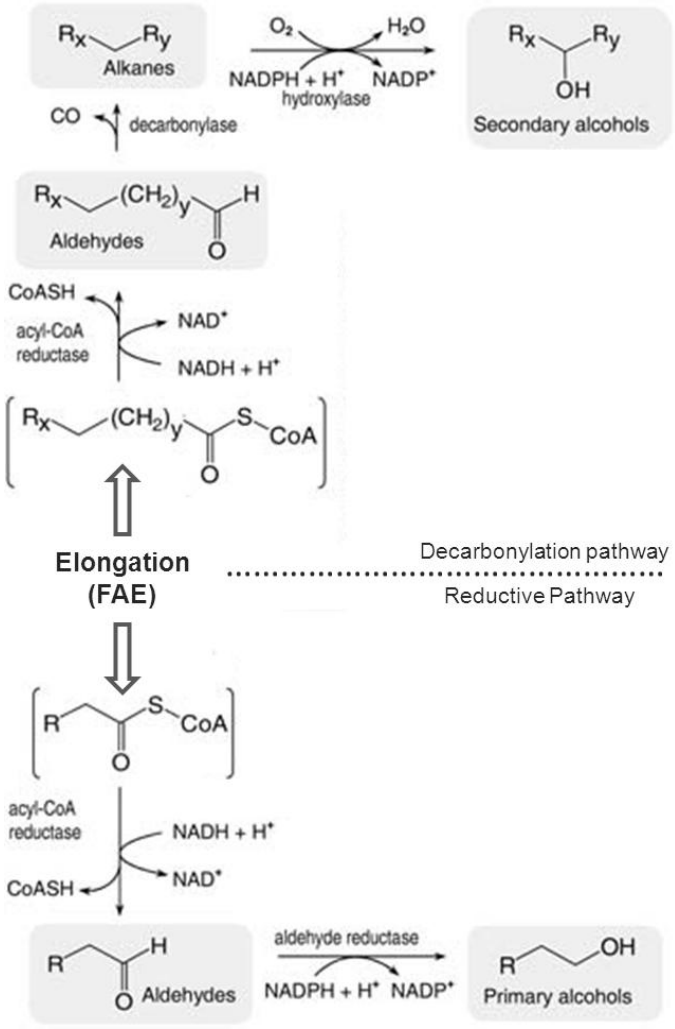


Figure 4.19. The decarbonylation pathway produces secondary alcohols and the reductive pathway produces primary alcohols. The substrates were produced by the elongation of fatty acid elongases (FAE), adapted from (Shepherd and Wynne Griffiths, 2006).

4.2.3.3 Correlations between flavour volatiles

Flavour volatiles were analysed by SPME-GC-MS in the three separate harvest years. The highest number of correlations between volatiles were observed in 2011, followed by 2010 and 2012; the only negative correlations observed were in the harvesting year 2010 (Figure 4.20). The majority of flavour volatiles detected were fatty acid derivatives along with a number of terpenoids and single compounds derived from the shikimate pathway or carotenoids (Table 4.6) (Tomás-Barberán and Robins, 1997).

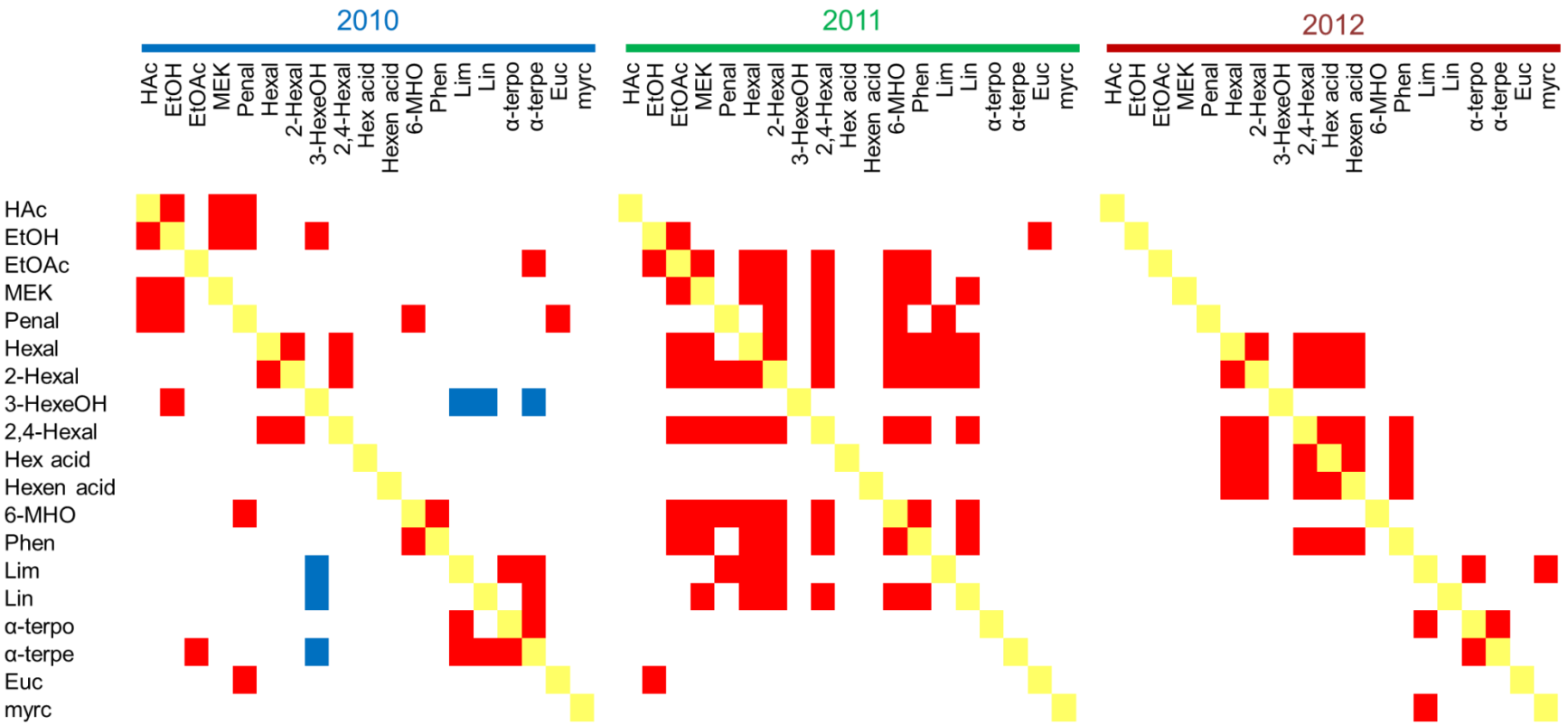


Figure 4.20. Correlations of flavour volatiles in blueberry cultivars in three growing years. (A) refers to the harvesting year 2010; (B) refers to the harvesting year 2011 and (C) refers to the harvesting year 2012. Pearson's correlation coefficients r > 0.7 is indicated by ■, r < -0.7 is indicated by ■ and correlations between the same components is indicated by ■. The list of the phytochemicals including the abbreviations is shown in Appendix D.

Table 4.6. The corresponding precursor of identified volatiles in blueberry cultivars according to Tomás-Barberán and Robins (1997). The identified were estimated with SPME-GC-MS to analyse blueberry cultivars.

Fatty acids	Terpenoids	Shikimate derivates	Carotenoids
HAc	Lim	Phen	6-MHO
EtOH	Lin		
EtOAc	α-terpo		
MEK	α-terpe		
Penal	Euc		
Hexal	myrc		
2-Hexal			
3-HexeOH			
2,4-Hexal			
Hex acid			
Hexen acid			

C_6 flavour volatiles had the most correlations in 2011, followed by 2010 with similar correlations in the three years. Baldwin et al. (1991) reported an increase in concentrations of C_6 flavour volatiles and 6-MHO in tomato during ripening. The increase in the latter was correlated with the accumulation of its carotenoid precursor lycopene (Buttery et al., 1971). 6-MHO was also previously correlated to Lin which shares a common intermediate (Stevens, 1970). Few correlations were observed between different terpenoids in the harvesting years 2010 and 2012. The common precursor of all of these compounds was geranial diphosphate (GPP) (Figure 4.21) (Croteau, 1987, Rajaonarivony et al., 1992) and the finding that GPP derivatives were strongly correlated suggests common regulation of the biosynthetic pathways. 3-HexeOH only exhibited correlations in the growing year 2010 with negative correlations to the terpenoids Lim, Lin, α-terpe. Several studies have reported that during ripening the concentration of terpenes increased in different fruits, while C_6 aldehydes decreased (Baldwin et al., 1991, Horvat and Chapman, 1990, Lalel et al., 2003, Lewinsohn et al., 2001). A range of flavour volatiles derived from fatty acid metabolism were positively correlated to Lin in 2011, in accordance to the studies of Horvat (1990) and Baldwin (1991).

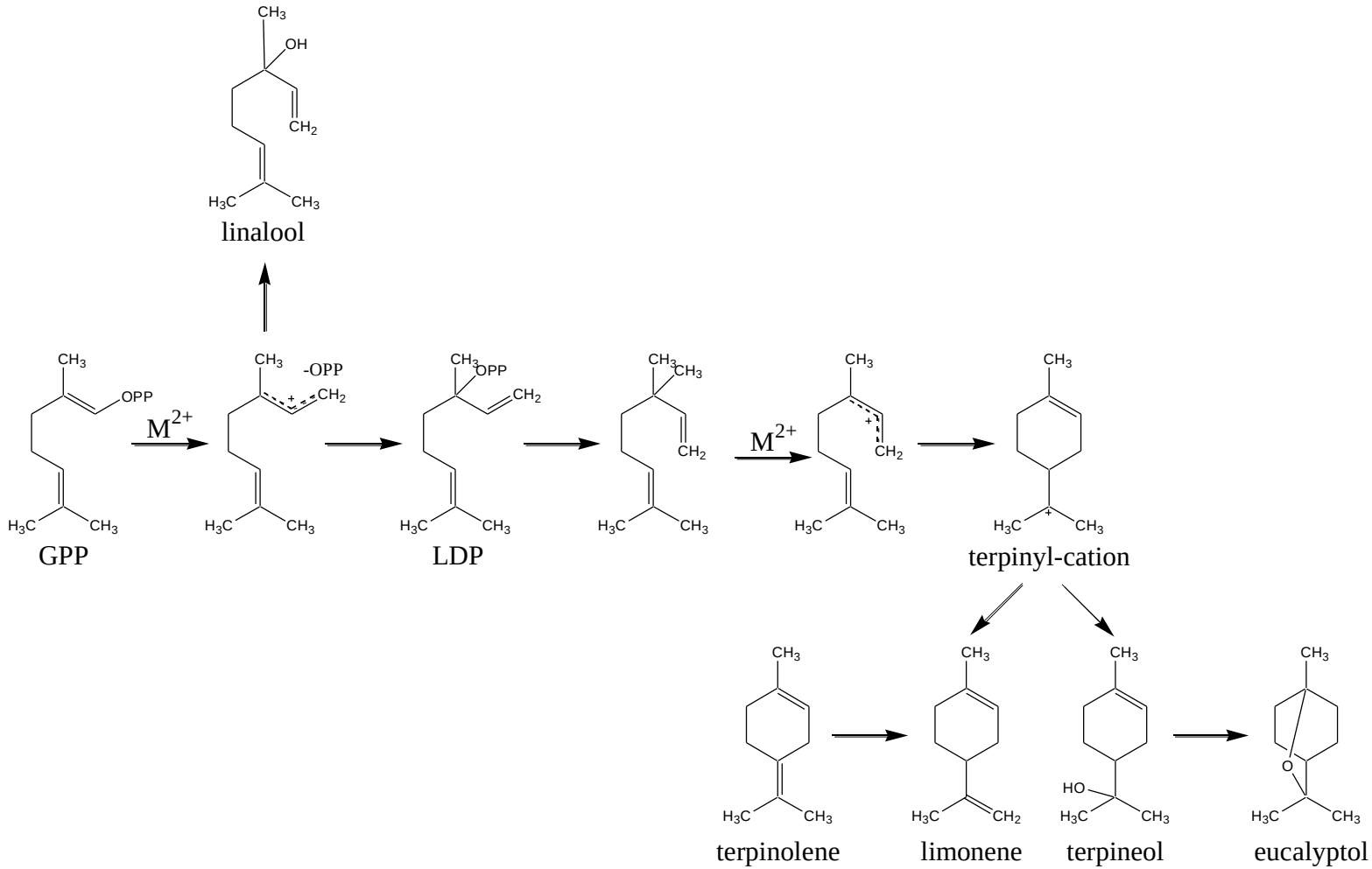


Figure 4.21. Biosynthesis of monoterpenes that were identified in blueberry cultivars. Geranyl pyrophosphate (GPP); LDP, linalyl pyrophosphate.

4.2.3.4 Correlations between anthocyanins

The anthocyanins were analysed by LC-MS^2. In general cultivars grown in 2011 exhibited the most correlations, followed by 2010 and 2012 (Figure 4.22). Consistent correlations within the three different harvesting years were cya-ara to cya-gal and pet-ara and cya-gal to peo-pen. Further pet-ara was correlated to pet-hex in 2011 and 2012, mal-pen to pet-ara in 2010 and 2011, pet-ara to cya-gal and pet-hex in 2011 and 2012.

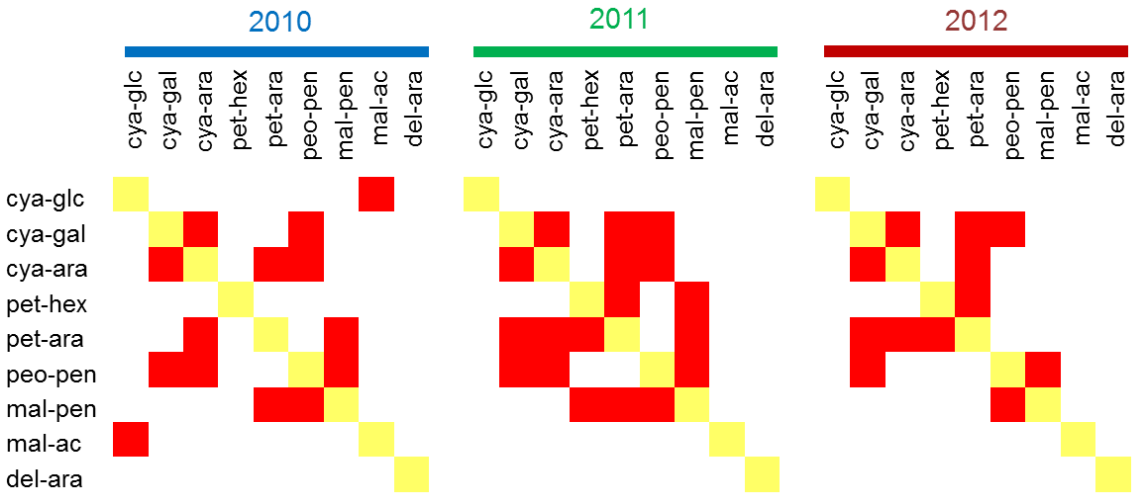


Figure 4.22. Correlations of anthocyanins in blueberry cultivars in the growing years 2010, 2011 and 2012. Pearson's correlation coefficients r > 0.7 is indicated by ■, r < -0.7 is indicated by ■ and correlations between the same components is indicated by ■. The list of the phytochemicals including the abbreviations is shown in Appendix D.

Cya-gal was more highly correlated than cya-glc with higher response ratios (Table 4.7). Studies of Vorsa and Polashock (2005) in cranberries have shown that the tetraploid V. *macrcarpon* had higher levels of anthocyanins galactoside (≈ 60 %), while the diploid V. *oxycoccus* had high levels of glucoside (≈ 70 %). The ratio of galactoside and glucoside in cyanidin differed considerably between the two species. Glucosides form via catalysis by UDP-glucose:flavonoid-3-O-glucosyltransferase anthocyanidin-3-O-glucosides (Holton and Cornish, 1995), while UDP-glucose:flavonoid-3-O-glucosyltransferase did not conjugate UDP-galactose (Ford et al., 1998). It could be hypothesized that tetraploid level is set by an allele that forms predominatly galactosides; while diploid level is set by an allele encoding an enzyme that conjugates

predominantely glucosides. Similar to cranberries, the predominant tetraploid level in highbush blueberry may rather produce cya-gal than cya-glc.

Table 4.7. The averaged response ratio ± standard deviation (n = 3) of cya-gal, cya-glc and cya-ara in the blueberry cultivars in three different growing years. The list of the phytochemicals including the abbreviations is shown in Appendix D.

cya-gal	2010	2011	2012
cya-gal	1.03 ± 0.45	0.90 ± 0.54	0.60 ± 0.33
cya-glc	0.19 ± 0.06	0.63 ± 0.29	0.42 ± 0.19
cya-ara	0.69 ± 0.27	0.17 ± 0.05	0.12 ± 0.03

Further cya-ara was correlated to pet-ara in the harvesting years 2011 and 2012. The illustrated anthocyanins pathway (Figure 4.23) showed the formation of cya-ara and pet-ara. The nearest common precursor to both of these compounds is naringenin and it has previously been proposed that the correlations between downstream anthocyanins can be explained in terms of naringenin formation being rate limiting rather than enzyme activities beyond this point. However it is also possible that naringenin turnover limits its availability for subsequent conversions or indeed that downstream enzyme activity could limit the rate of formation of anthocyanins. Further studies of the anthocyanin pathway would be required to resolve this question. Mal-pen correlated to peo-pen and pet-ara in all three harvesting years and mal-pen was correlated to pet-ara in 2011 and 2012. The petunidin and malvidin derivatives are formed by the action of O-methyl transferases on delphinidin derivatives. The close correlation between intermediates with different glycosyl decoration could be indicative of the formation of free delphinidin, petunidin and malvidin pools prior to glycosylation.

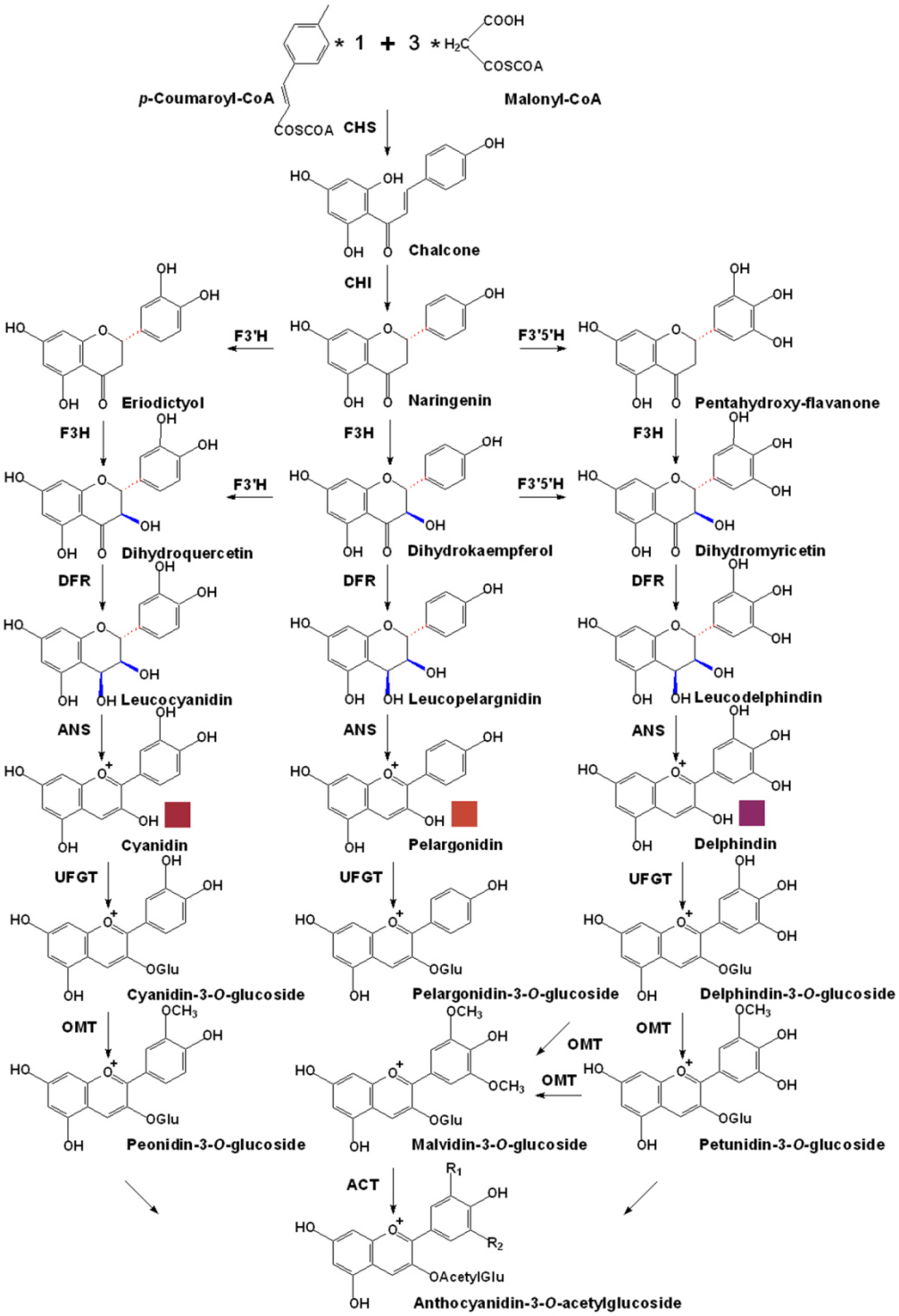


Figure 4.23. The flavonoid pathway to anthocyanins in blueberry cultivars. CHS, Chalcone synthase; CHI chalcone isomerase; F3'H, flavonoid 3'hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; UFGT, flavonoid glucosyltransferase; OMT, o-methyltransferase; ACT, anthocyanin acyltransferase (He et al., 2010)

4.2.3.5 Correlations between polyphenols

Individual polyphenols were analysed by LC-MS^2. Only a few correlations were found with cultivars grown in 2011 exhibiting the highest number of correlations; little less in 2010 and considerably less in 2012 (Figure 4.24).

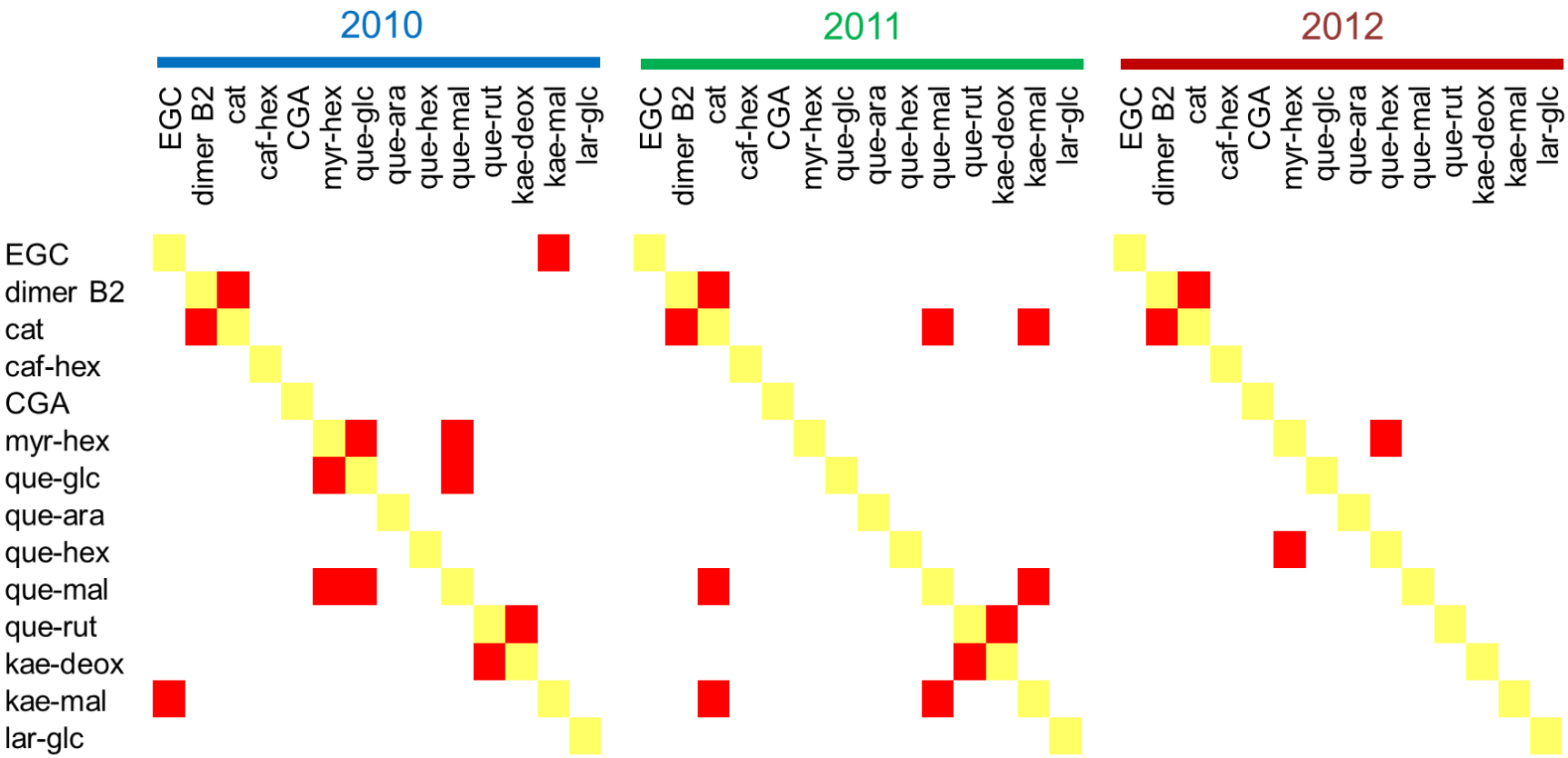


Figure 4.24. Correlations of polyphenols in blueberry cultivars grown in 2010, 2011 and 2012. Pearson's correlation coefficients r > 0.7 is indicated by ■, r < -is indicated by ■ and correlations between the same components is indicated by ■. The list of the phytochemicals including the abbreviations is shown in Appendix D.

The polyphenols were synthesised via the flavonoid pathway in the cytosol (Figure 4.25). A consistent correlation within the three different harvesting years was cat to dimer B2. The common precursor leucoanthocyanidin interacted with leucoanthocyanidin reductase (LAR) to produce cat or interacted with anthocyanidin synthase (ANS/LDOX) to produce anthocyanidin, the precursor to dimer B2 (Dixon et al., 2002). Myricetin correlated to quercetin in 2010 and 2012, furthermore quercetin correlated to kaempferol in 2010 and 2011. Naringenin interacted with F3'H (flavonoid 3'hyrdroxylase) to produce dihydrokaempferol which can interact with F3'H to produce dihydroquercetin, the precursor to quercetin or interact with F3'5'H (flavonoid 3'5'hydroxylase) to produce dihydromyricetin, the precursor to myricetin (Sparvoli et al., 1994, Jaakola et al., 2002, Deluc et al., 2006).

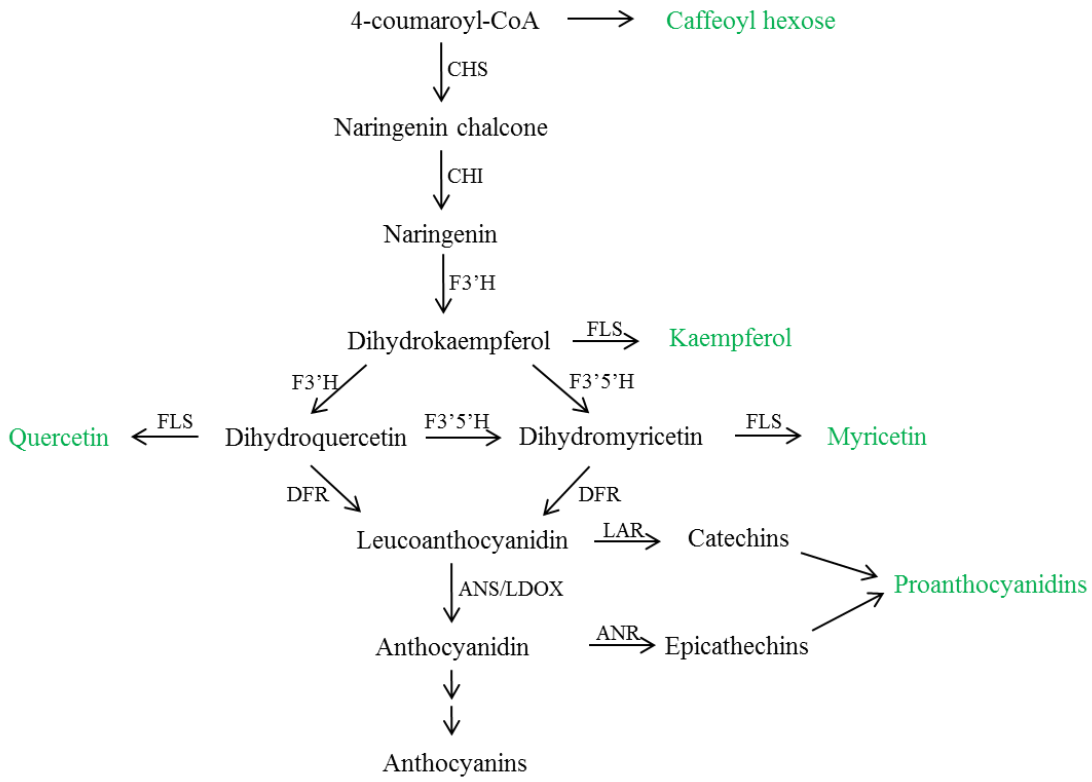


Figure 4.25. Scheme of the flavonoid pathway in blueberries (ANR, anthocyanidin reductase; ANS-LDOX, anthocyanidin synthase; CHI, chalcone isomerase; CHS, chalcone synthase; DFR, dihydroflavonol 4-reductase; F3'H, flavonoid 3'hydroxylase, F3'5'H, flavonoid 3'5'hydroxylase; FLS, flavonol synthase; LAR, leucoanthocanidin reductase; LDOX, leucoanthocyanidin dioxygenase), adapted from (Deluc et al., 2006, Jaakola et al., 2002).

4.2.3.6 Correlations between antioxidant properties

The T-AO capacity, T-Phen and T-Anth contents were analysed by UV-photometer. In general cultivars grown in 2012 exhibited the highest number of correlations; little less in 2011 and considerably less in 2010 (Figure 4.26). The analysed blueberry juice exhibited correlations between T-Anth and T-Phen content within the harvesting years. Previous studies confirmed the relationship between T-Phen and T-Anth (Kalt et al., 2001, Prior et al., 1998).

Kalt et al. (2001) and Prior et al. (1998) quantified the T-Phen content with the Folin-Ciocalteu assay, but a range of other compounds beside phenols were significantly reactive with the Folin-Ciocalteu reagent. The Folin-Ciocalteu assay should rather be used to quantify the T-AO capacity than the T-Phen content. It could be only used for a rough approximation of T-Phen content because polyphenols belong to the largest group of antioxidants in most plants (Everette et al., 2010).

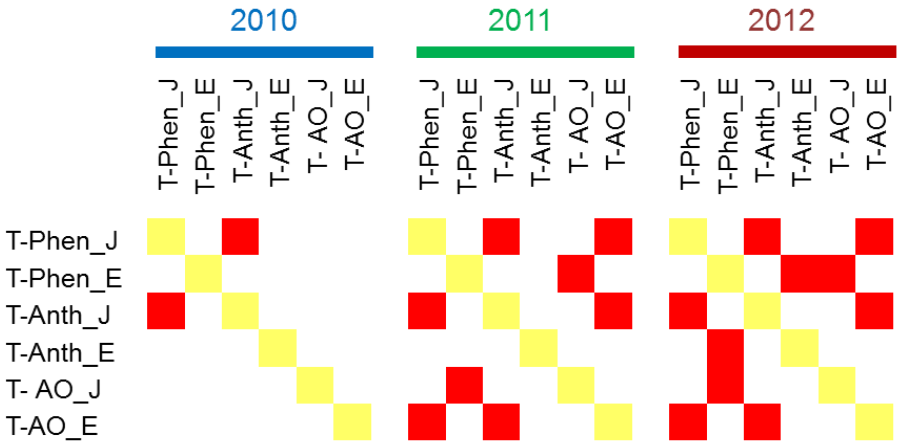


Figure 4.26. Correlations of antioxidants in blueberry cultivars grown in 2010, 2011 and 2012. Pearson's correlation coefficients r > 0.7 is indicated by ■, r < -0.7 is indicated by ■ and correlations between the same components is indicated by ■. The list of the phytochemicals including the abbreviations is shown in Appendix D.

4.2.3.7 Correlations between different compound classes

The analysis of correlations between different classes was undertaken to obtain information regarding the relationship of phytochemicals. The correlations of phytochemical classes were similar in 2011 and 2012, only 2010 has shown negative correlations. The main correlations between polyphenols and anthocyanins were further discussed. Further correlations between different compound classes were random.

Correlations between polyphenols and anthocyanins

Anthocyanins, flavonols, flavan-3-ols and hydroxycinnamic acid derivates showed the highest number of correlations in 2010, little less in 2011 and considerably less in 2012. Polyphenols and anthocyanins are formed by the same precursors (Figure 4.27). 4-coumaroyl CoA resulted from the phenylpropanoid metabolism is a substrate for the flavonoid pathway as well as a substrate for hydroxycinnamic acid derivates. The flavonoid metabolism forms anthocyanins, flavonols, and flavan-3-ols. Different correlations between polyphenols and anthocyanins within the three harvesting years were shown. The environmental conditions influenced the polyphenolic biosynthesis by initiation, inhibition and turnover rate of enzymes (Macheix et al., 1990).

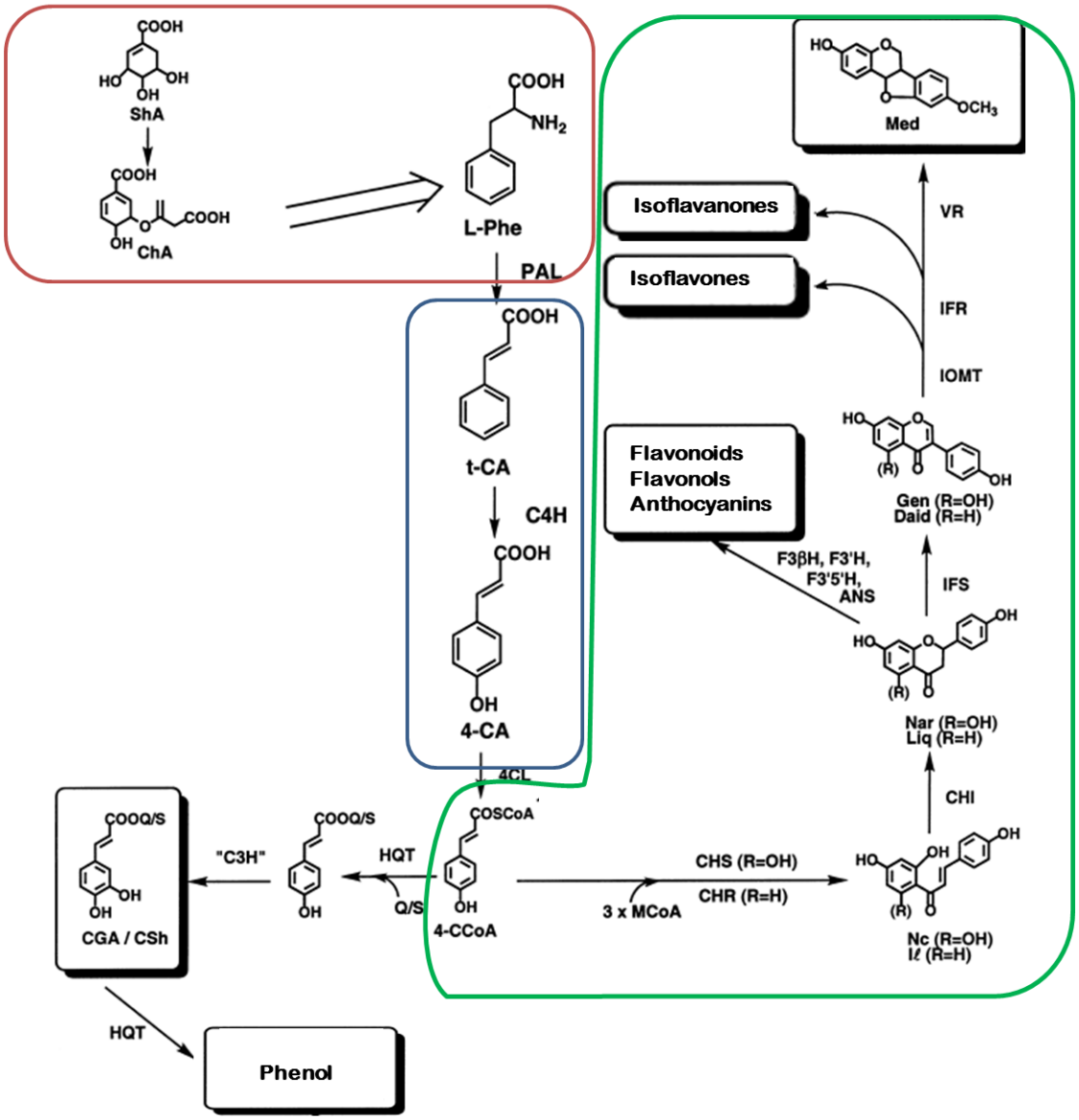


Figure 4.27. Biosynthetic pathways to flavonoids (ChA, chorismic acid; ShA, shikimic acid; L-Phe, L-phenylalanine; PAL, L-phenylalanine ammonia-lyase; t-CA, trans-cinnamic acid; C4H, cinnamate 4-hydroxylate; 4CL, 4-courmarate CoA ligase; 4-CCoA, 4-coumaroyl CoA; HQT, hydroxycinnamoyl-CoA Quinate hydroxycinnamoyl transferate; C3H, coumarate CoA ligase; CGA chlorogenic acid; CSh, 4-coumaroyl shikimate; Med, medicarpin; VR, vestitone; IFR, isofavone reductase; IOMT, isoflavone O-methyltransferase; Gen, genistein; Daid, daidzein; IFS, isoflavone synthase; F3βH, flavanone 3-β-hydroxylase; F3'H, Flavonoid 3'hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; ANS anthocyanidin synthase; Nar, naringenin; Liq, liquiritigenin; CHI, chalcone isomerase; Nc, naringenin chalcone; Il, isoliquiritigenin; MCoA, malonyl CoA; ■ shikimate, ■ phenylpropanoid and ■ flavonoid pathway), adapted from Dixon et al. (2002).

4.3 Conclusions

Genetic and environmental differences were analysed. In general blueberries grown in 2010 were distinct from those grown in 2012, indicating that the environment has a substantial influence in the same cultivars across the seasons, while limited genetic influences were observed in phytochemical content of individual cultivars grown in the same location.

Evidence is presented that seasonal environmental differences affected metabolite accumulation in the same cultivar across growing years with higher temperatures and longer sun exposure resulting in a higher content of CA, while in wetter, colder seasons higher contents of Glucose and Fructose were observed. In future studies cultivars should be grown across the UK under the same agronomic conditions in order to obtain information regarding how blueberries perform at different UK sites. Additional cultivars should be grown under polytunnels and open field conditions to determine the impact on the key quality traits.

Chapter 5

Modelling of relationships between compositional and sensory data

5.1 Introduction

At present less than 3 % of UK consumed blueberries are grown indigenously. One factor hindering development of the industry is the availability of UK adapted germplasm producing fruit meeting the sensory expectations of consumers. Both appearance and flavour contribute to fruit quality. Aroma is a sum of flavour volatiles that are perceived by receptors of the olfactory system (retronasal), while taste is a sum of sensory components that stimulate the gustatory receptors on the tongue (Meilgaard et al., 1999). Breeders are currently focussed on appearance (size, colour, shape), but flavour is also an important fruit quality character for consumers (Bruhn et al., 1991). A market review regarding apples reported that for the consumer, fruit quality is more important than the price (Market Review, 1996). Correlations between phytochemical and sensory data of different blueberry cultivars within different harvesting years were examined. While human perception is an important tool for the evaluation of fruit quality, sensory analysis requires large quantities of fresh fruits, trained assessors and considerable time making direct breeding for quality traits impractical. An alternative approach is to link sensory traits to fruit phytochemical content and then to breed for specific levels of easily quantified fruit chemical components.

Correlation analysis between the phytochemical and sensory data of blueberry cultivars in three different harvesting years were examined in order to determine

which phytochemicals predicted which sensory attribute. A model was developed to link the analysed compounds (sugars, organic acids, flavour volatiles, lipids, antioxidant properties, polyphenols and anthocyanins) to 17 different sensory attributes, which were scored by a trained panel. Direct correlation analysis (Pearson's correlation) plotted every metabolite in the individual blueberry cultivars against every sensory attribute within the cultivars. The benefits are that the method is easy to apply and interpret for continuous non-normal data, independent of the amount of data (Chok, 2008). On the contrary, this statistical technique can only measure linear relationships between phytochemical and sensory data. In addition, outliers can result in the identification of false correlations (Cohen, 1988) and the technique is not applicable for normally distributed data. In order to overcome these limitations, a second statistical method was applied to the data. PLS was applied to find interaction between sensory and phytochemical data. PLS is further described chapter 3 under the section 3.5.

The aim of the work presented in this chapter was to understand the underlying phytochemical drivers of the blueberry sensory experience. By a combination of this knowledge with an appreciation of the sensory expectations of UK consumers and the genetic determinants of fruit phytochemistry it is intended to provide a tool to breed elite UK adapted germplasm. The relationship between sensory and phytochemical fruit characteristics were analysed from eight blueberry cultivars harvested in 2010, twelve in 2011 and fourteen in 2012.

5.2 Results and Discussion of univariate analysis

The relationship between compositional and sensory data within the cultivars was determined. Correlation analysis was undertaken to expose potential linkages between phytochemical and sensory data. The content of each phytochemical in individual cultivars was plotted against the corresponding sensory data score. Correlations were estimated in each harvesting year separately and were considered significant if they displayed a Pearson's correlation coefficient higher than 0.6 and or less than -0.6.

5.2.1 Correlations overview

The correlation analysis is presented in Figure 5.1; cultivars grown in 2010 exhibited the highest number of correlations with fewer observed in 2011 and 2012. The correlations between the years were not consistent. Sensory profiling (Chapter 3) revealed that sourness and sweetness were important contributors to the overall sensory experience. Therefore specific analyses were conducted to examine the relationship between sugars and acids with sweetness and sourness. Further analyses revealed that flavour volatiles also played a role in determining flavour intensity.

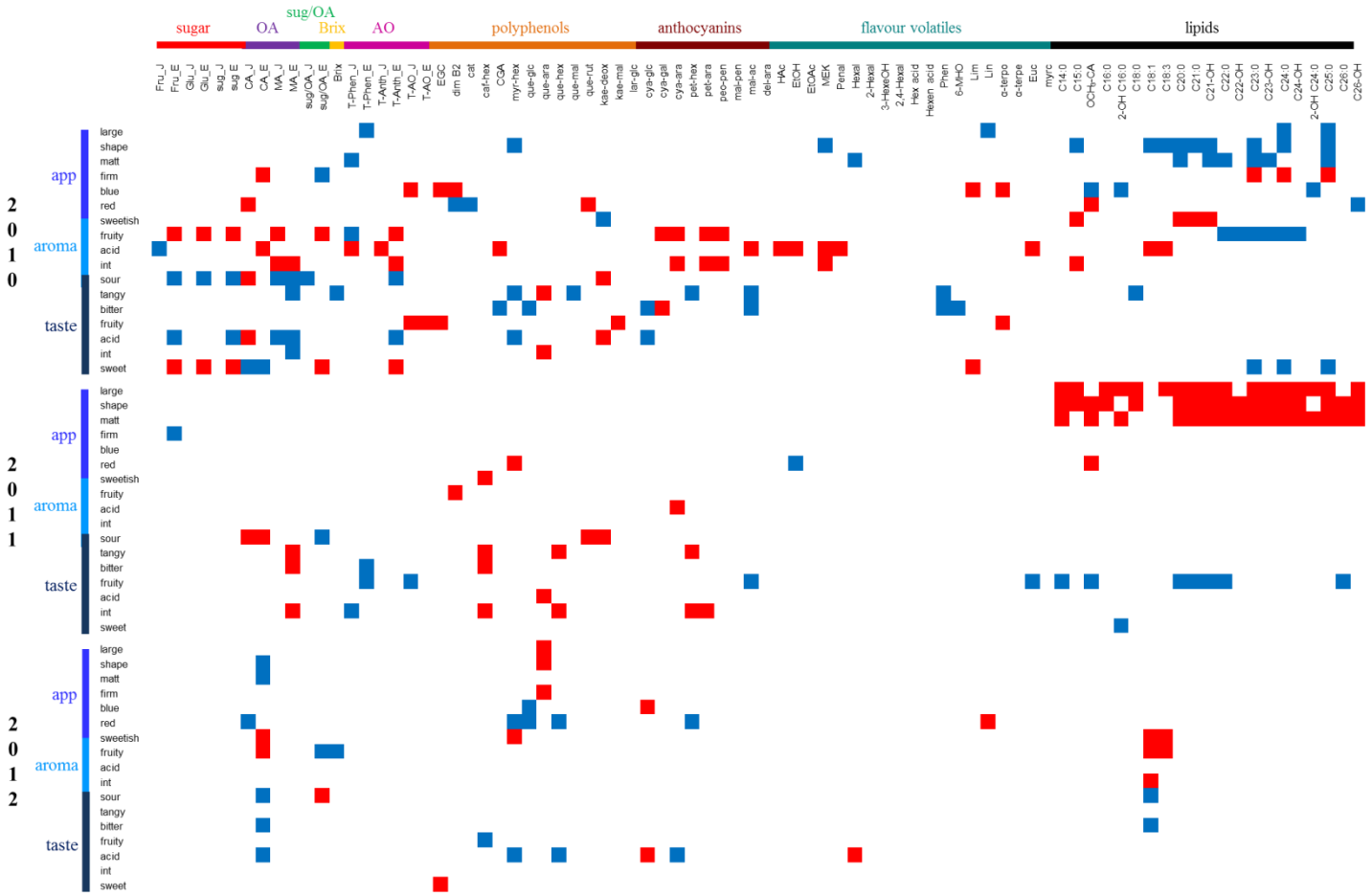


Figure 5.1. Correlations between phytochemical and sensory data in blueberry cultivars in the growing years 2010, 2011 and 2012; each square display r (Pearson's correlation coefficient r of a pair of sensory and phytochemical data), and the value of r > 0.6 is characterised by ■, the value of r < -0.6 is characterized by ■. The list of phytochemicals is shown in Appendix D; int, intensity.

5.2.2 Univariate analysis: Correlations to sugars and organic acids

Sweet, fruity, intensity, sour, acid, tangy and bitter contribute to sensory taste quality. These attributes were scored significantly different ($p < 0.05$) among the cultivars in all three harvesting years, expect fruity in 2010 (Table 3.9). The correlation analysis between sugars and organic acids to selected sensory attributes is shown in Table 5.1.

Correlations of cultivars grown in 2010

Sweetness was highly correlated to sugars (only in acidified methanolic blueberry extract) with higher correlations to Fru than Glc (Table 5.1) in agreement with other studies that indicate that Fru is perceived as the sweeter of the two sugars (Bañados, 2006). The sug/OA ratio exhibited the highest Pearson's correlation coefficient to sweetness. Previous studies in different fruits reported that sug/OA ratio is a better indicator for sweetness than sugar content alone (Tang et al., 2001, Poll, 1981, Tiitinen et al., 2005). Sweetness was highly negatively correlated to CA in agreement with studies in sea buckthorn that indicated negative correlations between acid content and sweetness (Tiitinen et al., 2005). Interestingly, fruitiness exhibited similar trends to sugars and CA as sweetness suggesting a link between the perceptions of these two flavours. Sugars (only in acidified methanolic extracts), sug/OA and MA were negatively correlated to sourness and acidity. On the contrary, sourness and acidity were similarly positively correlated to CA. These data are in agreement with previous studies in different fruits that reported a correlation of organic acids to sourness (Sandell et al., 2009, Tiitinen et al., 2005), and titratable acids were described as a good indicator for sourness and acidity (Harker et al., 2002, Baldwin et al., 1998). In agreement to Baldwin et al. (1998) in tomato, taste descriptors have shown strong correlations to sugars and acids. Acid, tangy and bitter taste descriptors have shown a positive correlation to CA (only in acidified methanolic blueberry extract). Tangy and intensive taste descriptors have shown similar correlations to sourness and acidity.

Table 5.1. Pearson's correlation coefficients between taste attributes and chemical parameters of blueberry cultivars grown in 2010. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The phytochemicals were analysed in juice and acidified methanolic extracts in blueberry cultivars and sensory attributes were scored on a nine point scale assessed in blueberry juice. The list of phytochemicals is shown in Appendix D; int, intensity.

	Fru		Glc		CA		MA		sug/OA		Brix
	Juice	Extract	Juice	Extract	Juice	Extract	Juice	Extract	Juice	Extract	
sweet	0.172	0.863	-0.139	0.723	-0.768	-0.863	0.335	0.219	0.539	0.849	-0.079
fruity	-0.228	0.537	-0.520	**0.402**	**-0.442**	**-0.391**	-0.075	-0.136	0.080	0.246	**0.334**
int	**-0.301**	-0.073	-0.226	-0.113	**0.421**	0.037	**-0.419**	-0.641	**-0.367**	-0.122	-0.153
sour	-0.174	-0.684	-0.025	-0.620	0.869	0.526	-0.663	-0.734	-0.609	-0.531	**-0.417**
acid	-0.017	-0.639	-0.094	-0.559	0.776	**0.435**	-0.708	-0.801	-0.510	**-0.470**	**-0.392**
tangy	-0.237	-0.145	**-0.307**	-0.155	0.605	0.054	-0.598	-0.849	-0.520	-0.033	-0.634
bitter	0.080	-0.086	0.113	0.193	**0.390**	0.313	0.136	-0.092	-0.230	-0.106	-0.266

Correlations of cultivars grown in 2011

In consideration of thawed assessed blueberries in cultivars grown in 2010 and fresh fruits grown in 2011, it can be seen that the correlations were similar (Table 5.2). Similar to the harvesting year 2010, sweetness was positively related to Fru, sug/oa and sourness showed positively correlations to CA. Tanginess and bitterness were correlated to MA in contrast to cultivars grown in 2010.

Correlations of cultivars grown in 2012

In contrast to the harvesting years 2010 and 2011, sourness and acidity were negatively correlated to CA for cultivars grown in 2012 and positively related to sug/OA (Table 5.3). This was an unexpected result that is difficult to reconcile with previous studies in different fruits (Sandell et al., 2009, Tiitinen et al., 2005). However, it may be that an unknown factor or combinations of factors are responsible for this result. Brix was a reliable predictor for sourness. Previous studies reported that Brix was a reliable predictor for sweetness in fruits (Harker et al., 2002, Kallio et al., 2000), while studies in blueberries could not confirm this correlation (Saftner et al., 2008). This finding highlights the potential problems of using Brix readings as a proxy for total sugars, a practice that is common in the horticulture industry (Harker et al., 2002, Kallio et al., 2000).

In general cultivars grown in 2010 and 2011 exhibited similar correlations with respect to compounds associated with sweetness and acidity. These data illustrates the significance of sugars and organic acids in defining these sensory traits in both juice extracted from thawed blueberries and in fresh fruits. On the contrary, cultivars grown in 2012 did not exhibit the same phytochemical-sensory correlations as those grown in the preceding years illustrating the impact of growing environment and suggesting that components other than sugars and acids contributed to the sensory traits of sweetness and acidity.

Table 5.2. Pearson's correlation coefficients between taste attributes and chemical parameters of blueberry cultivars grown in 2011. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The phytochemicals were analysed in juice and acidified methanolic extracts in blueberry cultivars and sensory attributes were scored on a nine point scale assessed in entire blueberries. The list of phytochemicals is shown in Appendix D; int, intensity.

	Fru		Glc		CA		MA		sug/OA		Brix
	Juice	Extract	Juice	Extract	Juice	Extract	Juice	Extract	Juice	Extract	
sweet	**0.454**	**0.491**	0.293	0.251	-0.550	-0.553	**0.438**	0.025	0.506	0.523	0.209
fruity	0.217	0.160	**0.411**	**0.385**	**0.324**	**0.386**	0.255	0.051	-0.038	-0.112	0.072
int	0.089	0.032	0.099	0.079	-0.012	0.053	0.538	0.718	0.031	0.046	0.143
sour	**-0.331**	**-0.327**	-0.018	-0.001	0.650	0.775	-0.053	0.187	-0.581	-0.606	-0.146
acid	-0.093	-0.055	0.217	0.186	0.276	0.297	-0.013	0.192	-0.254	-0.208	0.237
tangy	-0.296	**-0.312**	-0.296	-0.262	**0.349**	**0.337**	**0.414**	0.717	-0.296	-0.273	-0.201
bitter	-0.165	**-0.303**	0.029	-0.097	**0.449**	0.513	**0.493**	0.651	**-0.429**	**-0.498**	**-0.349**

Table 5.3. Pearson's correlation coefficients between taste attributes and chemical parameters of blueberry cultivars grown in 2012. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The phytochemicals were analysed in juice and acidified methanolic extracts in blueberry cultivars and sensory attributes were scored on a nine point scale assessed in entire blueberries. The list of phytochemicals is shown in Appendix D; int, intensity

	Fru		Glc		CA		MA		sug/OA		Brix
	Juice	Extract	Juice	Extract	Juice	Extract	Juice	Extract	Juice	Extract	
sweet	0.228	-0.130	**0.405**	0.021	**0.325**	-0.079	**-0.372**	-0.136	-0.042	0.178	-0.087
fruity	-0.067	-0.238	-0.021	-0.076	0.266	**0.339**	**-0.355**	-0.184	-0.200	-0.123	-0.129
int	**0.415**	0.142	0.239	0.070	**-0.377**	-0.602	0.178	0.185	**0.339**	0.511	0.202
sour	**0.490**	**0.309**	**0.309**	0.229	**-0.470**	-0.732	0.091	0.259	**0.480**	0.617	0.509
acid	0.284	0.217	0.073	0.077	-0.591	-0.645	**0.434**	**0.404**	**0.478**	**0.334**	**0.355**
tangy	**0.300**	0.122	0.209	0.156	-0.050	-0.253	-0.221	-0.005	0.155	**0.414**	0.198
bitter	**0.487**	**0.302**	**0.338**	0.240	**-0.314**	-0.649	0.067	0.208	**0.353**	0.599	**0.497**

5.2.3 Univariate analysis: Correlations to flavour volatiles

Taste and aroma character contribute to sensory quality flavour. The correlation analysis between selected sensory attributes and flavour volatiles are discussed in detail here.

Cultivars grown in 2010 exhibited the highest number of correlations between volatile components and sensory attributes (Table 5.4). Shorter chain flavour volatiles (HAc, EtOH, EtOAc and MEK) were positively related to intensive and acid aroma while the bitter taste descriptor was weakly positively correlated to the terpenoids Lin and α-terpe. The fruity taste attribute was positively related to terpenoids, especially Lim, α-terpo and Euc. Further α-terpo was positively related to sweet taste and was also negatively related to intensive aroma. The majority of flavour volatiles were negatively related with sweetish aroma in the harvesting year 2011 (Table 5.5). According to other studies Lim provides a fruity, orange and lemon odour (Kaack et al., 2006). Fruity taste was positively related to shorter chain flavour volatiles (EtOH, EtOAc, MEK and Penal) in cultivars grown in 2012 (Table 5.6). Further EtOAc, MEK were negatively related to acid taste. These data illustrate the difficulties that trained assessors have in perception of odour compared with the more basic flavours such as sweet, sour, salty and bitter confirming previously published results (Martin et al., 2006, Darnell and Williamson, 1997).

Table 5.4. Pearson's correlation coefficients between flavour descriptors and flavour volatiles of thawed blueberry cultivars grown in 2010. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The sensory attributes were scored on a nine point scale assessed in blueberry juices. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma				Taste						
	sweetish	fruity	int	acid	sweet	fruity	int	sour	acid	tangy	bitter
HAc	0.184	-0.238	0.581	0.949	-0.553	**-0.435**	**-0.339**	0.083	-0.194	**-0.335**	-0.115
EtOH	0.250	-0.290	0.582	0.809	**-0.423**	**-0.438**	**-0.449**	0.006	-0.291	**-0.383**	**-0.307**
EtOAc	0.109	**0.328**	**0.322**	0.518	-0.180	0.104	0.211	0.008	-0.057	0.028	**0.426**
MEK	0.547	0.086	0.690	0.784	-0.242	-0.050	-0.223	-0.231	-0.525	**-0.448**	-0.070
Penal	0.028	-0.547	0.114	0.898	**-0.392**	-0.131	**-0.410**	0.060	-0.048	-0.221	**-0.382**
Hexal	**0.380**	**-0.419**	-0.149	-0.175	-0.245	0.087	0.150	0.193	0.031	-0.009	**-0.327**
2-Hexal	0.221	-0.254	-0.163	**-0.356**	0.038	0.234	0.274	0.148	0.021	0.178	**-0.371**
3-HexeOH	0.003	-0.534	0.274	0.512	**-0.465**	**-0.410**	-0.234	0.240	-0.026	-0.155	**-0.466**
2,4-hexal	0.160	-0.149	-0.196	-0.560	0.170	0.233	0.208	-0.031	-0.094	0.001	**-0.353**
6-MHO	-0.158	**-0.491**	0.049	0.509	0.060	-0.054	-0.544	-0.175	-0.222	-0.256	-0.707
Phen	0.048	**-0.392**	0.222	0.281	-0.007	-0.084	**-0.495**	**-0.409**	-0.560	-0.720	-0.729
Lim	-0.150	**0.446**	**-0.398**	**-0.542**	0.610	0.522	0.207	-0.270	0.045	0.229	0.286
Lin	**0.345**	0.266	**-0.334**	-0.300	0.078	**0.474**	**0.385**	0.002	0.155	0.207	**0.484**
α-terpo	**-0.412**	0.025	-0.550	-0.154	**0.410**	0.746	0.401	-0.082	0.169	**0.352**	-0.072
α-terpe	-0.133	**0.381**	-0.187	-0.090	0.189	**0.430**	**0.385**	-0.081	0.094	0.194	**0.428**
Euc	-0.022	-0.016	0.187	0.630	0.232	0.510	-0.036	**-0.326**	**-0.387**	-0.133	**-0.382**

Table 5.5. Pearson's correlation coefficients between flavour descriptors and flavour volatiles of fresh blueberry cultivars grown in 2011. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The sensory attributes were scored on a nine point scale assessed in entire blueberries for taste and blueberry juices for aroma. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma				Taste						
	sweetish	fruity	int	acid	sweet	fruity	int	sour	acid	tangy	bitter
HAc	-0.269	-0.144	**0.303**	**0.465**	0.108	0.266	-0.170	0.102	0.590	-0.254	0.134
EtOH	**-0.483**	**-0.317**	**-0.389**	-0.015	0.034	-0.127	-0.506	0.067	-0.031	**-0.394**	-0.201
EtOAc	**-0.384**	-0.204	-0.273	0.024	-0.064	-0.208	-0.293	0.110	-0.032	-0.045	-0.203
MEK	**-0.314**	-0.236	-0.259	0.011	-0.104	0.031	-0.113	0.153	0.123	0.111	-0.019
Penal	**-0.376**	-0.061	-0.247	0.261	-0.150	-0.144	-0.147	**0.351**	0.265	-0.025	-0.103
Hexal	**-0.473**	-0.082	-0.073	0.197	0.200	-0.091	-0.264	-0.090	0.097	-0.177	-0.280
2-Hexal	**-0.473**	-0.138	-0.079	0.233	0.051	-0.144	-0.293	0.040	0.165	-0.155	-0.277
3-HexeOH	-0.235	0.097	0.296	0.242	0.260	**-0.312**	0.069	**-0.326**	-0.087	-0.022	-0.504
2,4-hexal	**-0.311**	-0.110	0.005	0.144	-0.087	-0.097	**-0.358**	0.115	0.070	-0.090	-0.267
6-MHO	-0.500	0.052	-0.220	**0.319**	0.048	-0.128	-0.194	0.133	**0.331**	-0.259	-0.203
Phen	**-0.372**	0.075	-0.293	0.042	-0.264	-0.608	**-0.397**	0.104	-0.108	**-0.312**	**-0.320**
Lim	-0.184	**0.335**	-0.035	-0.016	**0.327**	-0.008	**-0.372**	**-0.337**	-0.010	**-0.469**	**-0.395**
Lin	-0.287	-0.145	-0.065	-0.064	0.079	-0.081	**-0.300**	-0.046	-0.060	-0.067	-0.238
α-terpo	**-0.461**	-0.247	-0.063	0.174	0.011	-0.061	-0.255	0.029	0.202	-0.126	**-0.328**
α-terpe	-0.183	0.066	0.138	0.172	0.070	0.196	-0.003	0.028	**0.349**	**-0.339**	-0.091
Euc	**-0.341**	-0.170	-0.183	0.013	-0.038	-0.224	-0.117	0.107	-0.001	0.086	-0.155

Table 5.6. Pearson's correlation coefficients between flavour descriptors and flavour volatiles of fresh blueberry cultivars grown in 2012. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; values in bold indicate for r > 0.3 and r < -0.3. The sensory attributes were scored on a nine point scale assessed in entire blueberries for taste and blueberry juices for aroma. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma				Taste						
	sweetish	fruity	int	acid	sweet	fruity	int	sour	acid	tangy	bitter
HAc	-0.022	-0.173	0.121	0.282	-0.136	0.087	0.039	0.062	-0.019	0.155	-0.007
EtOH	0.108	-0.047	0.239	0.266	-0.021	**0.415**	-0.113	-0.079	-0.211	0.156	-0.135
EtOAc	**0.444**	0.249	0.093	**-0.366**	0.282	**0.389**	-0.200	**-0.330**	**-0.402**	0.099	**-0.332**
MEK	0.292	0.080	**0.374**	**0.429**	0.282	**0.462**	-0.004	-0.158	**-0.495**	**0.361**	-0.041
Penal	0.050	-0.059	-0.031	0.128	0.139	**0.365**	0.185	0.135	-0.107	**0.403**	0.229
Hexal	**-0.400**	-0.198	**-0.427**	-0.062	-0.225	**-0.374**	0.043	**0.331**	0.630	-0.264	0.134
2-Hexal	-0.190	0.008	**-0.366**	-0.087	-0.057	-0.199	-0.143	0.103	**0.404**	**-0.341**	-0.030
3-HexeOH	-0.050	0.039	-0.070	0.151	0.057	-0.063	**-0.315**	-0.086	0.091	**-0.321**	-0.219
2,4-Hexal	-0.127	-0.012	-0.221	0.130	-0.127	-0.033	-0.201	0.030	0.212	-0.259	-0.088
Hex acid	-0.036	0.160	0.035	0.174	**-0.373**	-0.028	-0.187	-0.057	0.284	**-0.354**	-0.204
Hexen acid	0.092	0.279	-0.058	0.010	-0.003	0.058	**-0.380**	-0.217	0.075	**-0.423**	-0.290
6-MHO	-0.156	-0.237	0.002	**0.458**	-0.110	0.116	-0.010	0.171	0.136	0.117	0.080
Phen	0.149	**0.302**	0.121	0.261	-0.216	0.174	**-0.305**	-0.222	0.028	**-0.312**	**-0.314**
Lim	**-0.332**	-0.261	**-0.350**	0.112	-0.237	-0.139	0.124	0.344	0.447	0.035	**0.354**
Lin	**-0.478**	**-0.462**	**-0.442**	-0.122	**-0.363**	-0.548	0.087	0.380	0.563	-0.183	0.284
α-terpo	-0.289	-0.236	**-0.381**	0.034	-0.081	-0.022	0.244	0.380	0.355	0.190	**0.435**
α-terpe	**-0.364**	-0.294	**-0.321**	0.069	**-0.359**	-0.285	0.138	0.371	0.532	-0.031	**0.346**
Euc	-0.064	0.036	-0.251	-0.075	0.058	-0.082	0.054	0.089	0.151	-0.020	0.100
myrc	-0.226	-0.191	-0.162	0.170	**-0.387**	-0.299	-0.119	0.147	**0.389**	-0.221	0.119

Fatty acids are the precursors of C_6 flavour volatiles; 2-Hexal and 3-HexeOH contributing to the typical blueberry flavour in highbush blueberries (Horvat and Senter, 2006, Hirvi and Honkonen, 1983). The few correlations between C_6 flavour volatiles and flavour descriptors resulted due to the poor flavour intensity in highbush blueberry cultivars (Table 5.4, Table 5.5 and Table 5.6). Precursors for the detected C_6 flavour volatiles Hexal, 2-Hexal, 3-HexeOH and 2,4-Hexal are C18:2 and acid (C18:3) (Figure 5.2) (Klee, 2010).

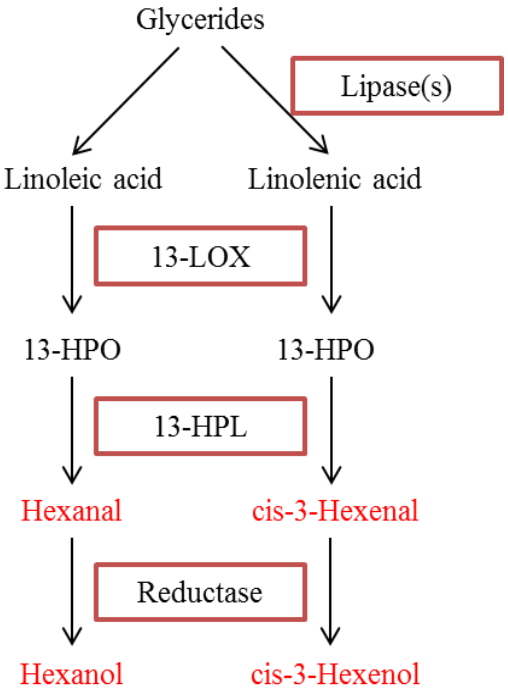


Figure 5.2. C_6 flavour volatiles synthesised from fatty acids. 13-LOX, 13-lipoxygenase; 13-HPO, 13-hydroperoxide; 13-HPL, 13-hydroxyperoxide lyase (Klee, 2010).

These fatty acids are synthesised from C18:0 via C18:1 by the action of lipid desaturases. In the present study only C18:0, C18:1 and C18:3 were identified in the analysed highbush blueberry cultivars. A significant number of strong correlations were observed between the lipid precursors of these volatiles and the flavour descriptors (Table 5.7, Table 5.8 and Table 5.9). In 2010 and 2012, all three C18 fatty acids identified in blueberry fruit exhibited positive relations to sweetish aroma and negative correlations with sour and acid taste albeit with different strengths of correlation in the different years. Similar to the harvesting year 2010, intensive aroma was related to C18:1 and C18:3 in cultivars grown in 2012, while cultivars

grown in 2011 have shown negative relations between intensive aroma and theses fatty acids. Fatty acids can't be used as predictors of sensory attributes due to their weak relation between lipids and flavour volatiles, further described in Chapter 4.

Table 5.7. Pearson's correlation coefficients between sensory attributes and lipids of thawed blueberry cultivars grown in 2010. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma				taste						
	sweetish	fruity	int	acid	sweet	fruity	int	sour	acid	tangy	bitter
C18:0	**0.303**	**-0.343**	0.156	0.195	-0.105	0.072	-0.287	**-0.357**	-0.525	-0.686	-0.558
C18:1	0.554	**-0.384**	**0.352**	0.647	**-0.462**	-0.145	**-0.342**	-0.096	**-0.359**	-0.542	**-0.311**
C18:3	0.579	**-0.339**	**0.406**	0.634	**-0.378**	-0.145	**-0.381**	-0.128	**-0.402**	-0.517	**-0.347**

Table 5.8. Pearson's correlation coefficients between sensory attributes and lipids of fresh blueberry cultivars grown in 2011. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma				Taste						
	sweetish	fruity	int	acid	sweet	fruity	int	sour	acid	tangy	bitter
C18:0	-0.184	-0.250	**-0.400**	-0.254	-0.247	-0.261	-0.218	-0.270	0.021	-0.238	-0.198
C18:1	-0.248	**-0.395**	-0.521	-0.257	-0.084	0.016	-0.267	-0.276	-0.035	**-0.333**	-0.178
C18:3	**-0.351**	**-0.328**	-0.506	-0.189	-0.151	-0.229	**-0.342**	-0.297	-0.076	**-0.413**	**-0.305**

Table 5.9. Pearson's correlation coefficients between sensory attributes and lipids of blueberry cultivars grown in 2012. Pearson's correlation coefficients r > 0.5 is indicated by ■, r < -0.5 is indicated by ■; value in bold indicate for r > 0.3 and r < -0.3. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma				Taste						
	sweetish	fruity	int	acid	sweet	fruity	int	sour	acid	tangy	bitter
C18:0	0.561	0.588	**0.397**	-0.027	-0.010	-0.057	-0.334	-0.535	**-0.333**	-0.288	**-0.445**
C18:1	0.800	0.716	0.666	0.122	-0.117	0.105	-0.536	-0.764	-0.545	**-0.316**	-0.677
C18:3	0.716	0.632	0.593	0.095	0.090	0.151	-0.305	-0.602	-0.523	-0.089	**-0.479**

5.2.4 Univariate analyis: Correlations to anthocyanins

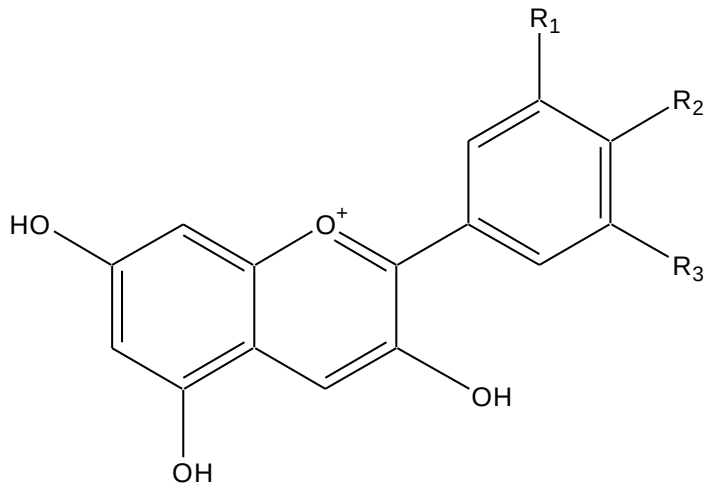
Colour is an important parameter for sensory quality (Saftner et al., 2008). The type and quantity of anthocyanins have a major impact on the blueberry colour. Only a few correlations of individual anthocyanins to red and blue colour were observed (Table 5.10). Relations of T-Anth content to red and blue colour were infrequent and inconsistent. In general, there were only small variations of the intensity in redness and blueness within the cultivars; red and blue were scored as not significantly different ($p > 0.05$) among the cultivars in all three harvesting years (Table 3.9).

The correlation analysis between selected sensory attributes and individual anthocyanins is shown in Table 5.10. In general the harvesting years 2010 and 2011 exhibit similar relationships in consideration of provided thawed blueberries in 2010 and fresh blueberries in 2011. Mal-pen was negatively related to blue color in cultivars grown in 2010 and 2011; further pet-ara was surprisingly positively correlated to red color.

Table 5.10. Pearson's correlation coefficients of anthocyanins to red and blue color in the three harvesting years. Pearson's correlation coefficients $r > 0.5$ is indicated by ■ and $r < -0.5$ is indicated by ■; value in bold indicate $r > 0.3$ and $r < -0.3$. The intensity of red and blue color were scored on a nine point scale assessed on entire thawed blueberries in 2010 and on entire fresh blueberries in 2011 and 2012. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensive taste.

	2010		2011		2012	
	blue	red	blue	red	blue	red
cya-glc	0.098	-0.164	-0.081	-0.287	0.660	0.510
cya-gal	-0.521	**0.482**	-0.038	**0.400**	-0.245	-0.292
cya-ara	**-0.432**	**0.472**	-0.115	**0.454**	-0.243	-0.261
del-ara	-0.161	0.015	**0.471**	-0.168	-0.220	-0.570
pet-hex	0.117	-0.234	-0.168	**0.406**	-0.426	-0.674
pet-ara	-0.540	0.552	**-0.388**	0.573	**-0.387**	-0.505
peo-pet	**-0.327**	**0.321**	-0.292	0.281	0.044	**0.352**
mal-pen	-0.528	**0.425**	**-0.378**	0.267	-0.122	0.104
mal-ac	0.284	-0.163	-0.440	**0.376**	-0.170	-0.045
T-Anth_J	0.036	0.043	0.260	**-0.350**	-0.508	-0.109
T-Anth _E	-0.021	-0.020	0.222	-0.298	-0.102	**-0.336**

The redness level rises with increasing methylation of hydroxyl groups and the blueness level rises with increasing numbers of free hydroxyl groups in the aglycone anthocyanidin (Figure 5.3) (He et al., 2010).



Anthocyanidin	R_1	R_2	R_3
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Malvidin	OCH_3	OH	OCH_3
Pelargonidin	H	OH	H
Peonidin	OCH_3	OH	H
Petundin	OCH_3	OH	OH

Figure 5.3. Structure of the most frequent anthocyanins in fruits (Lohachoompol et al., 2008).

Malvidin is described as the reddest of the individual anthocyanins, while delphinidin is described as the bluest anthocyanin in fruit, followed by cyanidin. Cultivars grown in 2012 have shown unexpected correlations; petunidin derivates and delphinidin were negatively correlated to red colour. Surprisingly cya-gal and cya-ara were positively correlated to red and negatively correlated to blue in the harvesting years 2010 and 2011. The correlations are likely to be influenced by cellular pH with anthocyanins forming the blue quinonoid form above pH 6, while at lower pH they tend to form the red flavylium form (Figure 5.4) (McGhie and Walton, 2007, Holcroft and Kader, 1999, Brouillard et al., 1997).

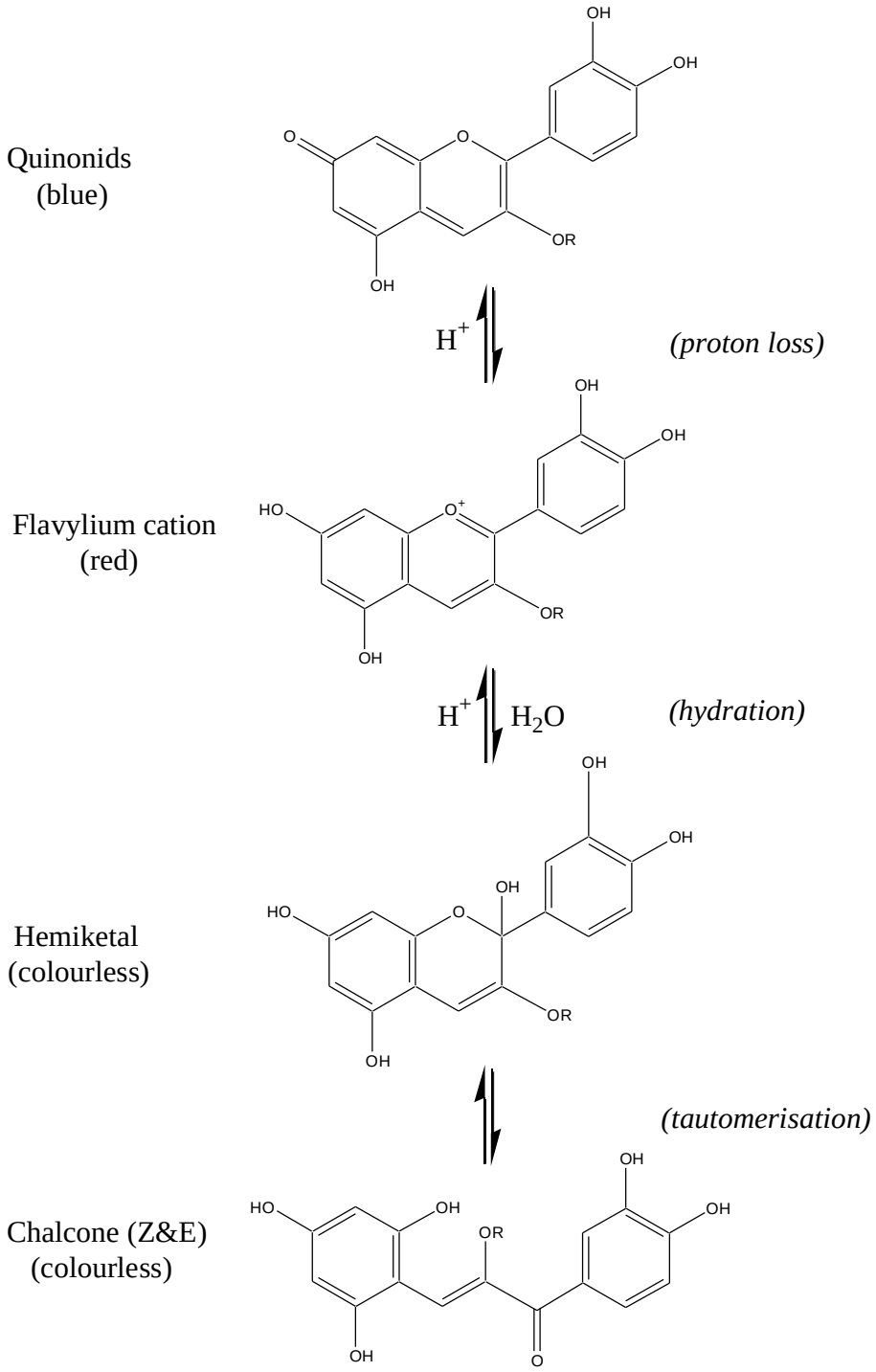


Figure 5.4. The change of pH transforms the structure of anthocyanins (McGhie and Walton, 2007).

The pK_a values for the change to the blue form from the flavylium cation range from 5.3 to 6.0. It depends on the anthocyanin. The pK_a were calculated according to the concentration of the quinoid form (HA), flavyllium form (A^-) and the concentration of hydrogen ions (H^+) (Equation 5.1).

$$pK_a = -\log \frac{c(H^+) \cdot c(A^-)}{c(HA)}$$ **Equation 5.1**

These data are perhaps in contrast to previous work that suggested that other factors like the type and amount of individual anthocyanins, temperature and light exposure had a larger impact on fruit colour than pH (Routray and Orsat, 2011, Sapers et al., 1984), because the range of pH in highbush blueberry cultivars is fairly small (Sapers et al., 1984). The lower correlations of cya-glc to the sensory descriptor may result due to their lower content in tetraploid fruits, further described under 4.2.3.4.

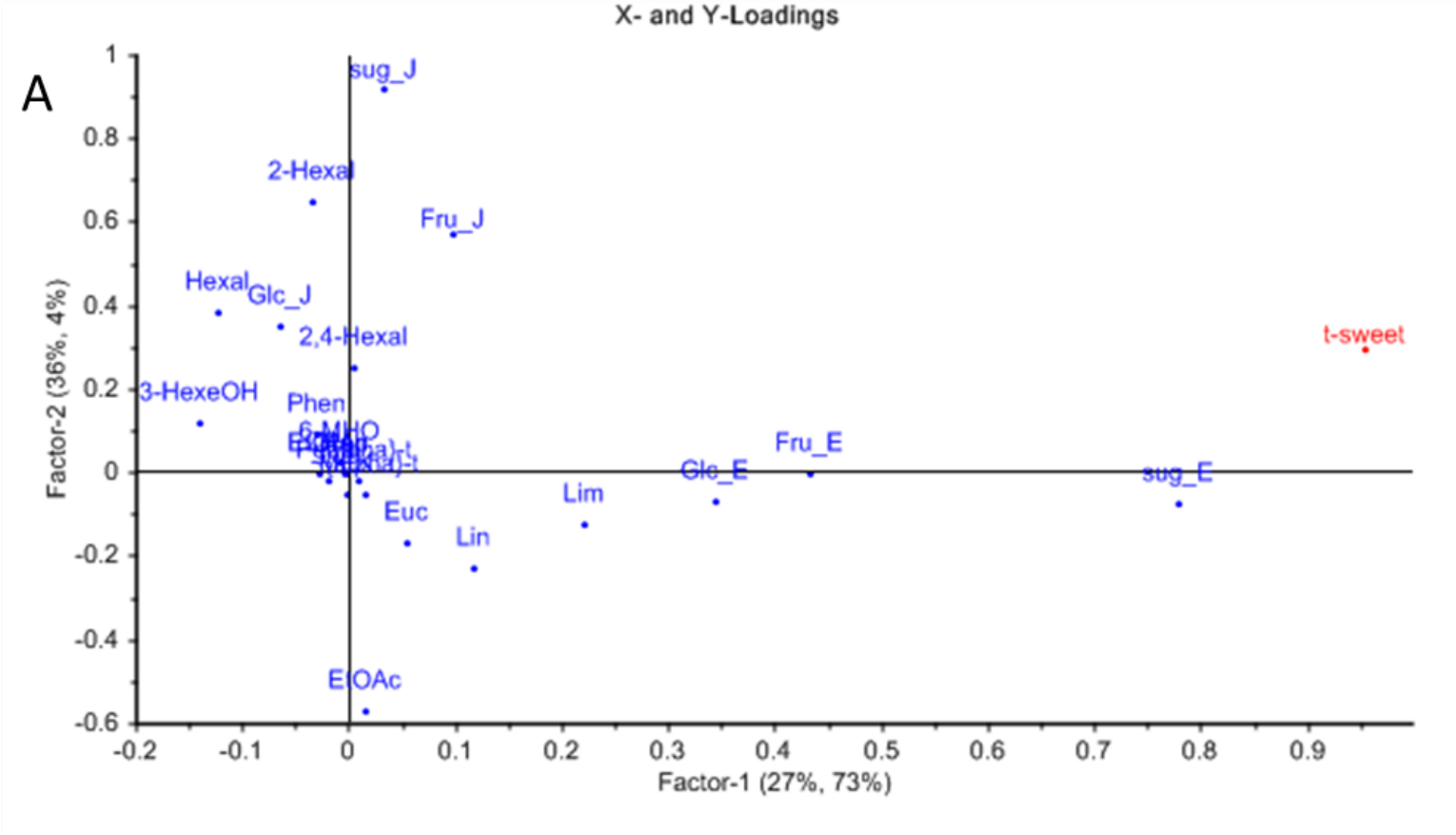
5.3 Results and Dission of multivariate analysis

5.3.1 PLS modelling

PLS was applied to identify interaction between sensory and phytochemical data. Phytochemical data were chosen as the x-variables (predictors) while the sensory data were chosen as the y-variables (responses).

The advantages of using PLS is that it produces robust interactions of independent variables taking into account the multiplicity of factors that may contribute to a given sensory perception further described under 3.5. Figure 5.5, Figure 5.6 and Figure 5.7 illustrate the interaction of sweetness to sugars, acids and flavour volatiles content in blueberry varieties grown in different harvesting years. The PLS-1 plot was applied to analyse the sensory attributes separately. However, a statistical analysis revealed that the model cannot be trusted for several reasons; the wide divergence between predicted (blue) and measured (red) plots and the not applicable R^2 in the harvested years 2011 and 2012 and the low R^2 ($R^2 = 0.049$) in 2010.

As a totality of data failed to produce robust PLS models, the technique was applied with subsets of the data. Table 5.11 illustrates the x- and y-variables investigated and provides the value of the resulting R^2. The low R^2 values obtained indicated the lack of robustness of the models generated. One reason for the limited robustness may be the low variance of sugars and CA in the analysed blueberry cultivars which was significantly lower than previously reported in raspberry (Table 5.12) (Zait, 2010).


X- and Y-Loadings
A
sug_J
2-Hexal
Fru_J
Hexal
Glc_J
2,4-Hexal
t-sweet
3-HexeOH
Phen
Fru_E
Glc_E
sug_E
Lim
Euc
Lin
EtOAc
Factor-2 (36%, 4%)
Factor-1 (27%, 73%)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9

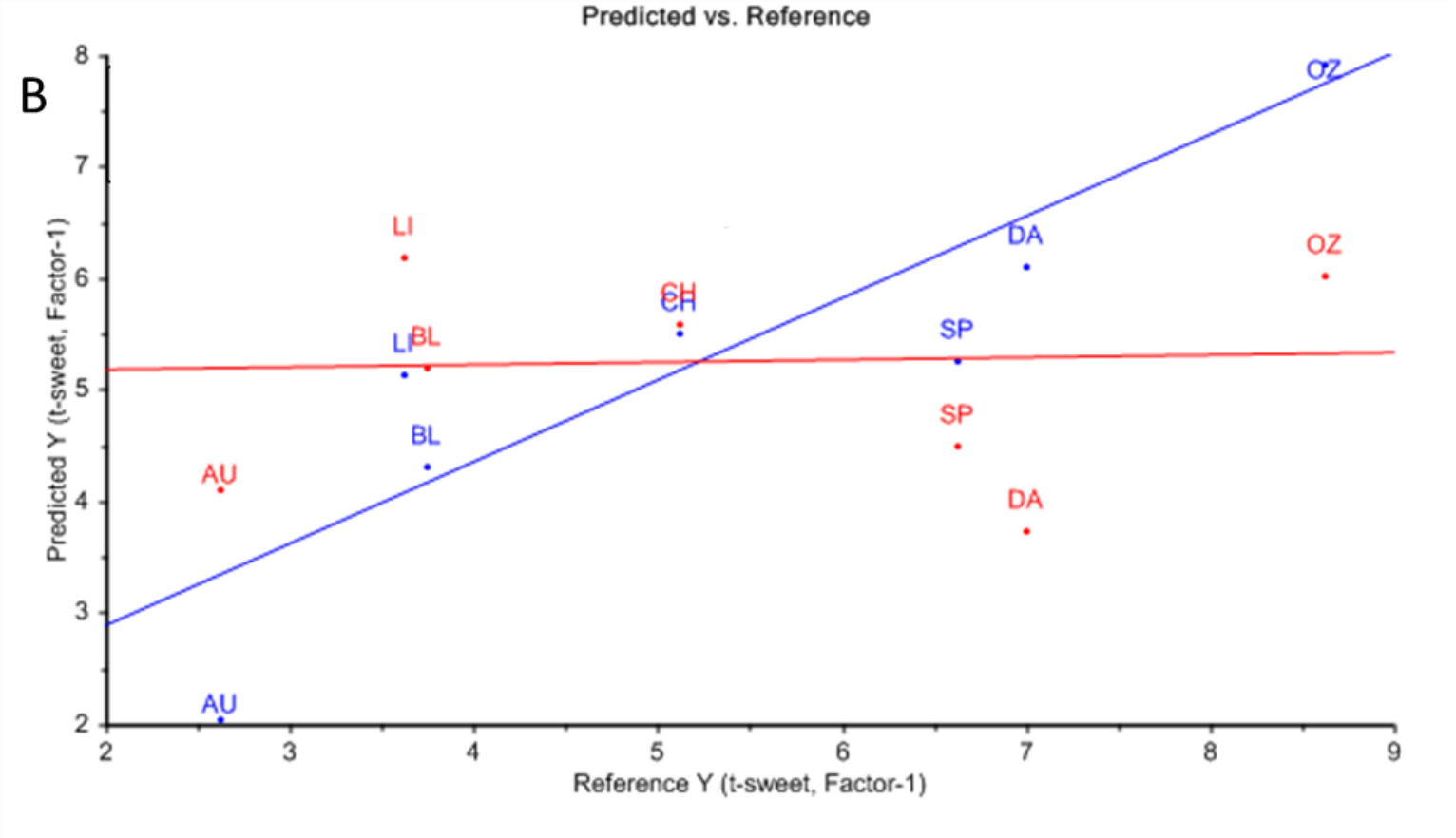
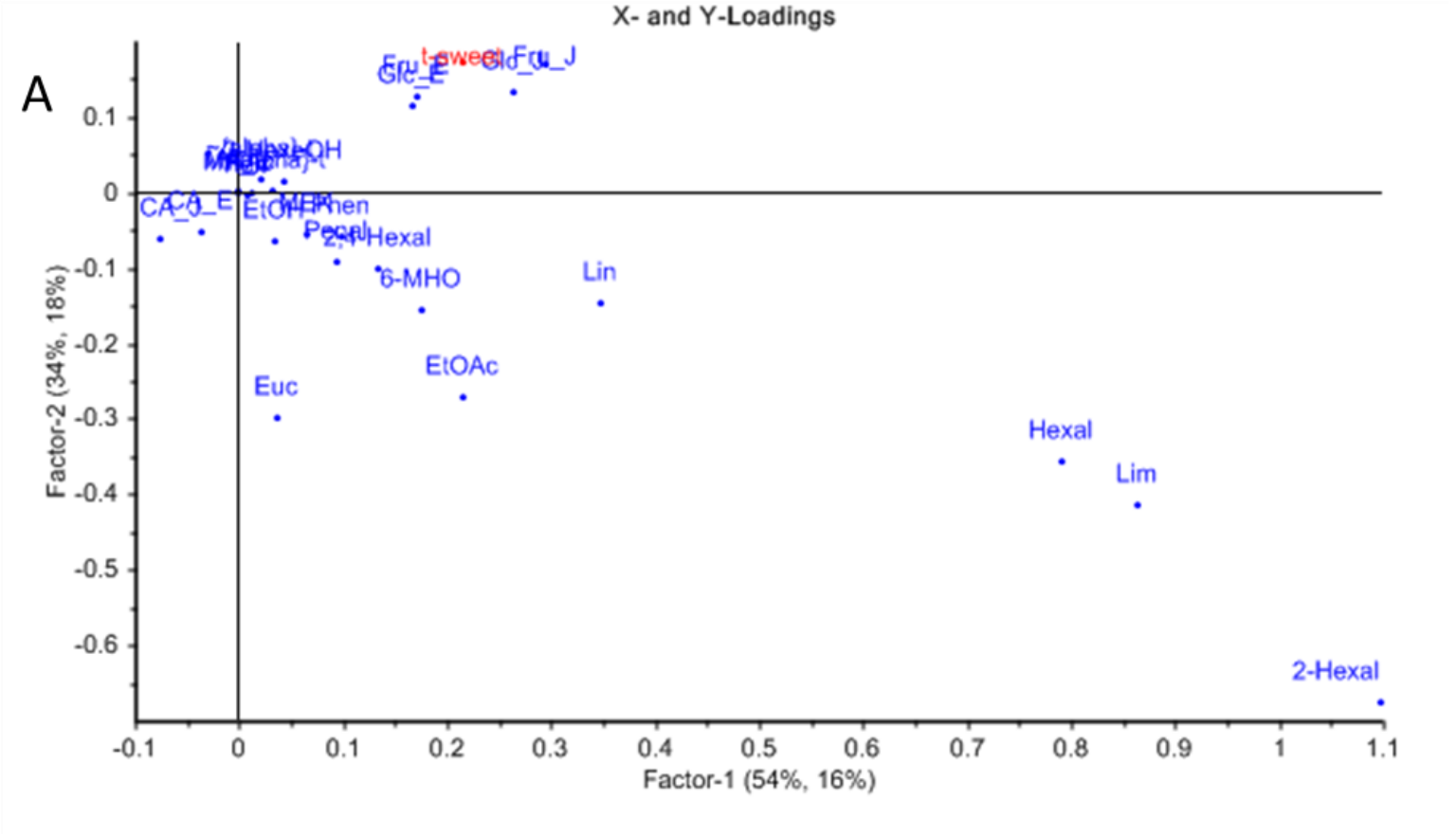


Figure 5.5. Result of PLS-1 showing the relationship between the analysed phytochemicals (sugars, organic acids and flavour volatiles) and sensory sweet taste scored on a nine-point scale of eight thawed blueberry cultivars grown in 2010. (A) x- and y-loading plot fom PLS; blue indicate phytochemical data and red indicates sweetness (t-sweet) scoring, (B) predicted (blue) vs measured (red) plot from PLS-1. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.


X- and Y-Loadings
A
Factor-2 (34%, 18%)
Factor-1 (54%, 16%)
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Hexal
6-MHO
Lin
EtOAc
Euc
Hexal
Lim
2-Hexal

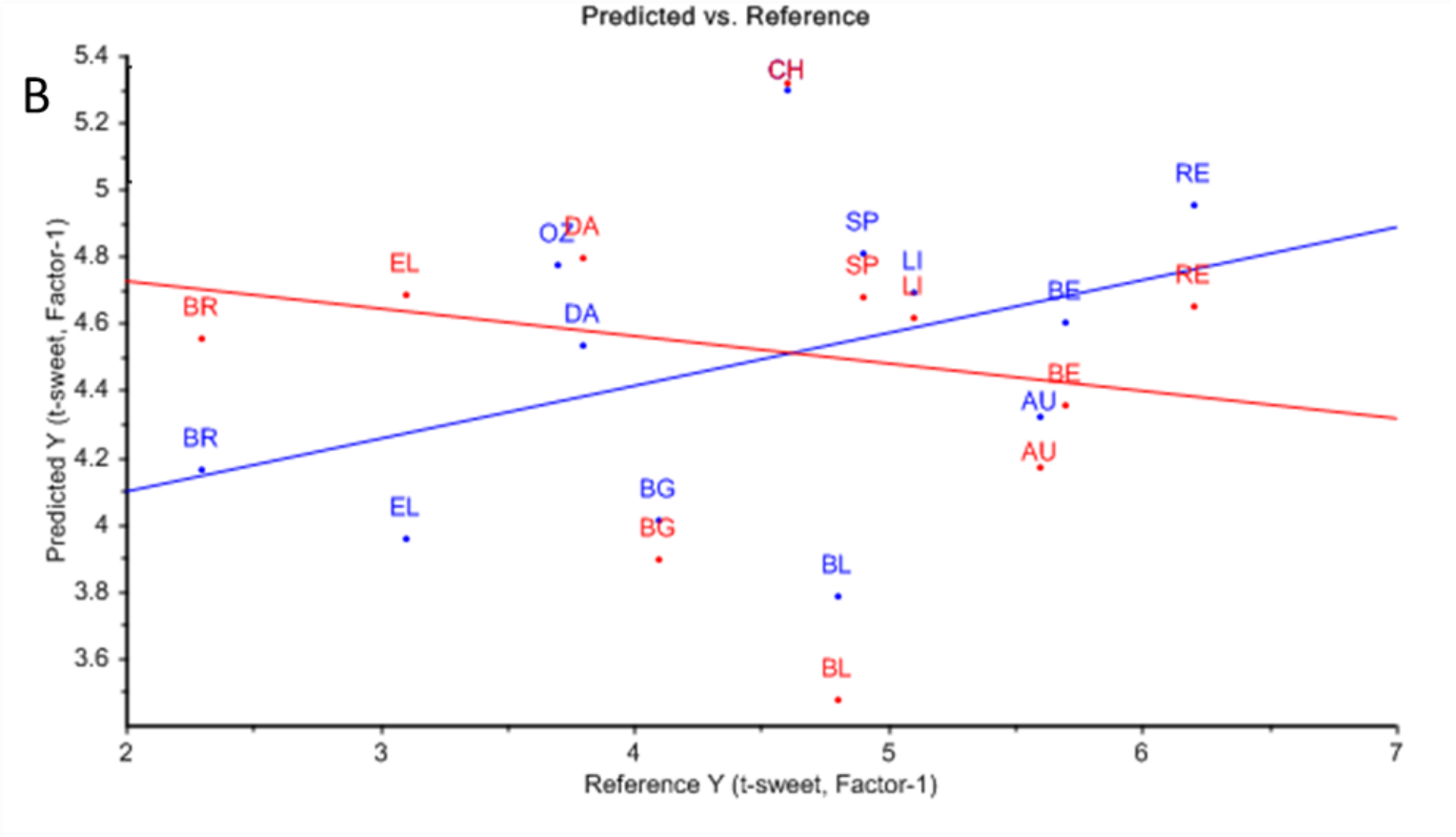
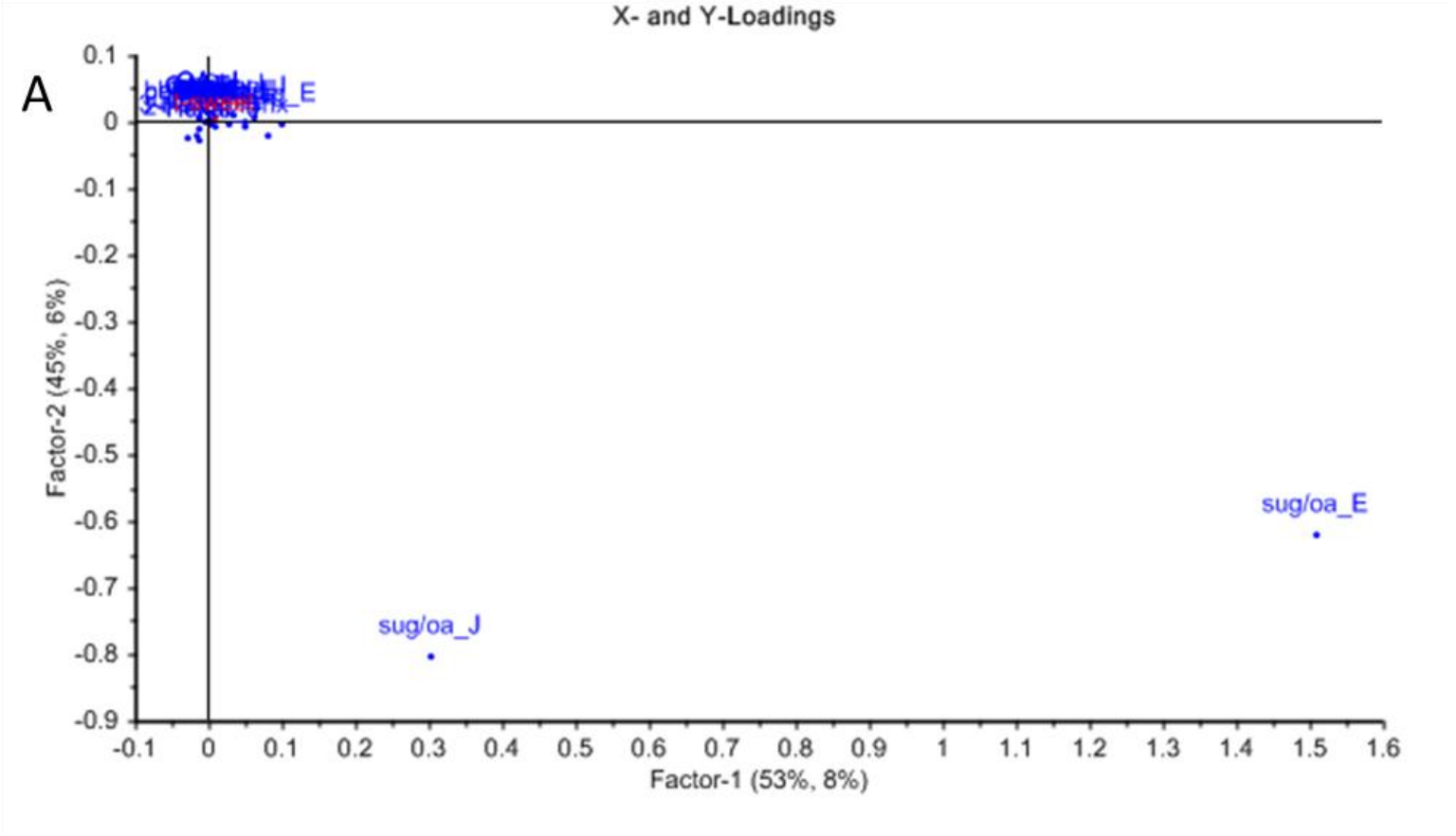


Figure 5.6. Result of PLS-1 showing the relationship between the analysed phytochemicals (sugars, organic acids and flavour volatiles) and sensory sweet taste scored on a nine-point scale of twelve fresh blueberry cultivars grown in 2011. (A) x- and y-loading plot fom PLS; blue indicate phytochemical data and red indicates sweetness (t-sweet) scoring, (B) predicted (blue) vs measured (red) plot from PLS-1. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.


X- and Y-Loadings
A
sug/oa_E
sug/oa_J
Factor-2 (45%, 6%)
Factor-1 (53%, 8%)
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6

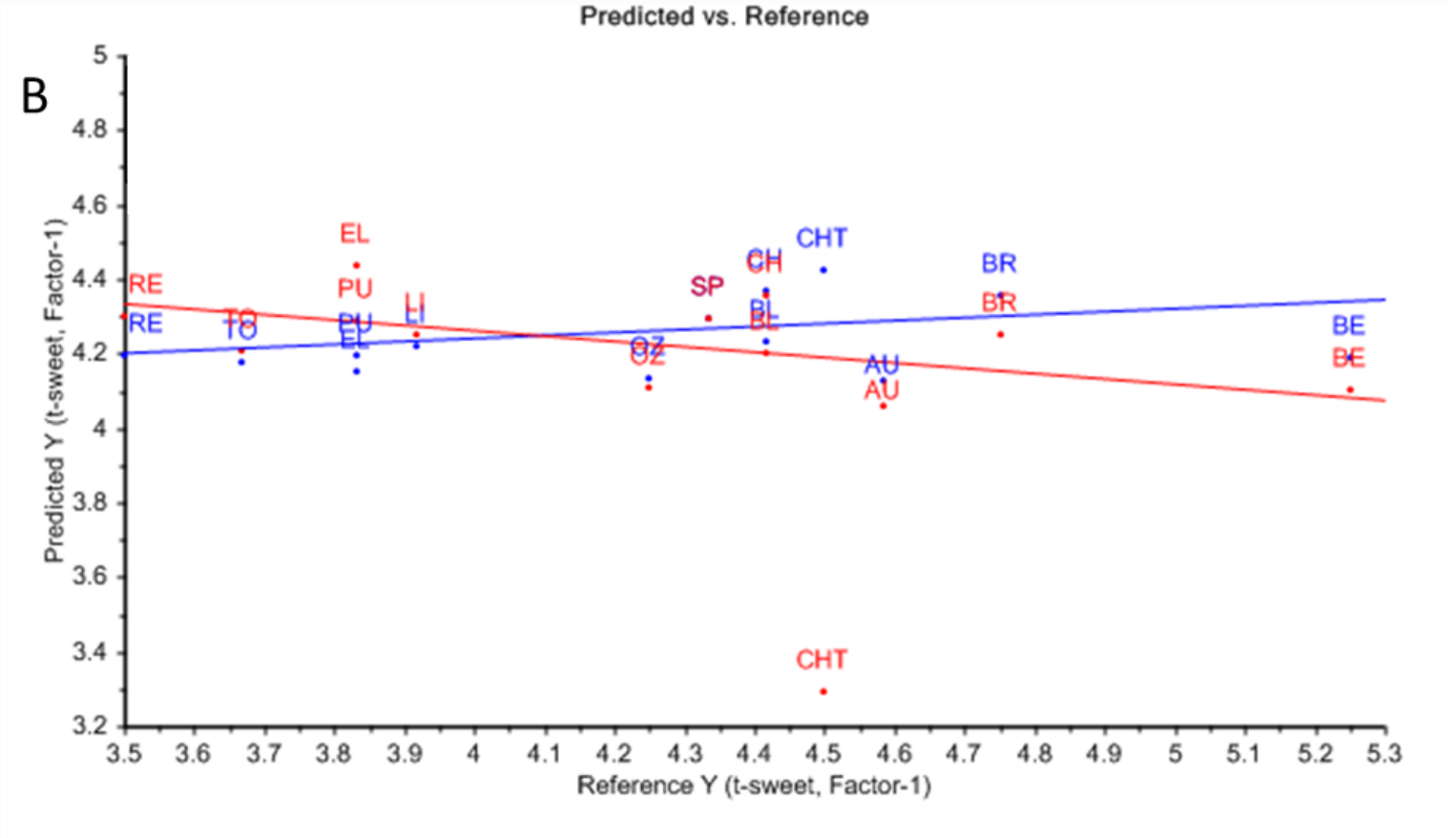


Figure 5.7. Result of PLS-1 showing the relationship between the analysed phytochemicals (sugars, organic acids and flavour volatiles) and sweetness on a nine-point scale of fourteen fresh blueberry cultivars grown in 2012. (A) x- and y-loading plot fom PLS; blue indicate phytochemical data and red indicates sweetness (t-sweet) scoring, (B) predicted (blue) vs measured (red) plot from PLS-1. The list of the phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Table 5.11. Validation about the robustness of the model. The applied PLS used for the x-variables phytochemicals and for the y-variables selected sensory attributes descriptor scored on a nine-point scale for eight thawed blueberry cultivars grown in 2010, twelve fresh in 2011 and fourteen fresh in 2012. The regression coefficient factors (R^2) provided the robustness of the model. NA, not applicable.

y-variable	x-variable	R^2		
		2010	2011	2012
All attributes	All phytochemicals	NA	NA	NA
All aroma attributes	All phytochemicals	NA	NA	NA
All taste attributes	All phytochemicals	NA	NA	NA
Sweetness	All phytochemicals	0.110	0.056	NA
Sourness	All phytochemicals	0.025	NA	NA
Sweetness	Sugars, organic acids, flavour volatiles	0.161	NA	NA
Sourness	Sugars, organic acids, flavour volatiles	0.216	NA	NA
All taste attributes	Sugars, organic acids, flavour volatiles	NA	NA	0.409
All aroma attributes	Sugars, organic acids, flavour volatiles	NA	0.179	0.400
Intensive taste	Sugars, organic acids, flavour volatiles	NA	NA	0.408
Sweetness	Sugars, organic acids, Brix	NA	NA	NA
Sourness	Sugars, organic acids, Brix	NA	NA	0.412
Aroma	Flavour volatiles	NA	0.179	NA
Intensive aroma	Flavour volatiles	NA	NA	NA
Intensive taste	Flavour volatiles	NA	NA	NA
Red	Anthocyanins	NA	NA	0.516
Blue	Anthocyanins	NA	NA	NA

Table 5.12. The lowest and highest average of the three replicates of sugars and citric acid in acidified methanolic extracted raspberries and blueberries. The sugar and citric acid content in blueberry extracts were analysed in three different harvesting years.

	Fru [g/100ml]	Glc [g/100ml]	CA [g/100ml]
Raspberry	0.069 – 0.354	0.325 – 2.630	0.090 – 0.215
Blueberry			
2010	2.50 – 5.21	2.19 – 4.08	0.51 – 1.28
2011	3.31 – 5.68	2.62 – 4.86	0.30 – 1.08
2012	4.59 – 7.87	4.32 – 7.57	0.30 – 1.07

5.3.2 Relations between chemical and sensory parameters

Given the poor fit of the PLS models, PCA was used to identify trends relating phytochemical to sensory data. PCA has previously been used to evaluate the relationship between phytochemical and sensory data in sweet cherries (Esti et al., 2002) and apples (Mehinagic et al., 2003). PCA determines trends between sensory and phytochemical, while PLS does modelling and prediction to indicate the model robustness (Moertsell et al, 2001). PCA and PLS are further described in 3.5.2.3. Additional correlation analysis of sensory attributes in extreme positions in the loading plot with compositional data was performed.

Relations between chemical and sensory parameters of blueberry cultivars grown in 2010

The principal component product space of eight blueberry cultivars in the growing year 2010 is shown in Figure 5.8A. The first two PCs accounted for 50 % of total sample variance. 30 % of total variance was explained by the first PC, which highlighted the outlying nature of BL. Acid aroma and the metabolites lipids, flavour volatiles derived from fatty acids and a range of polyphenols contributed to positive scores with high levels for BL; a range of sensory attributes (shiny, fruity aroma, bitter and sweet taste) and the metabolites sugars and a range of anthocyanins and terpenoids contributing negative scores with high levels for OZ and LI (Figure 5.8B). 20 % of the variance was explained by the second PC indicating the outlying nature of AU and LI. A range of sensory attributes (blue, sweet and fruity taste) and the metabolites sugars, T-Anth content and T-AO capacity measured in acidified methanolic blueberry extract contributed to positive scores with high levels for SP, BE and low levels of AU and LI. A range of sensory attributes (red, sour, acid, tangy, bitter and intensive taste) and CA contributed negatives scores with high levels for AU and LI and low levels for SP and BE.

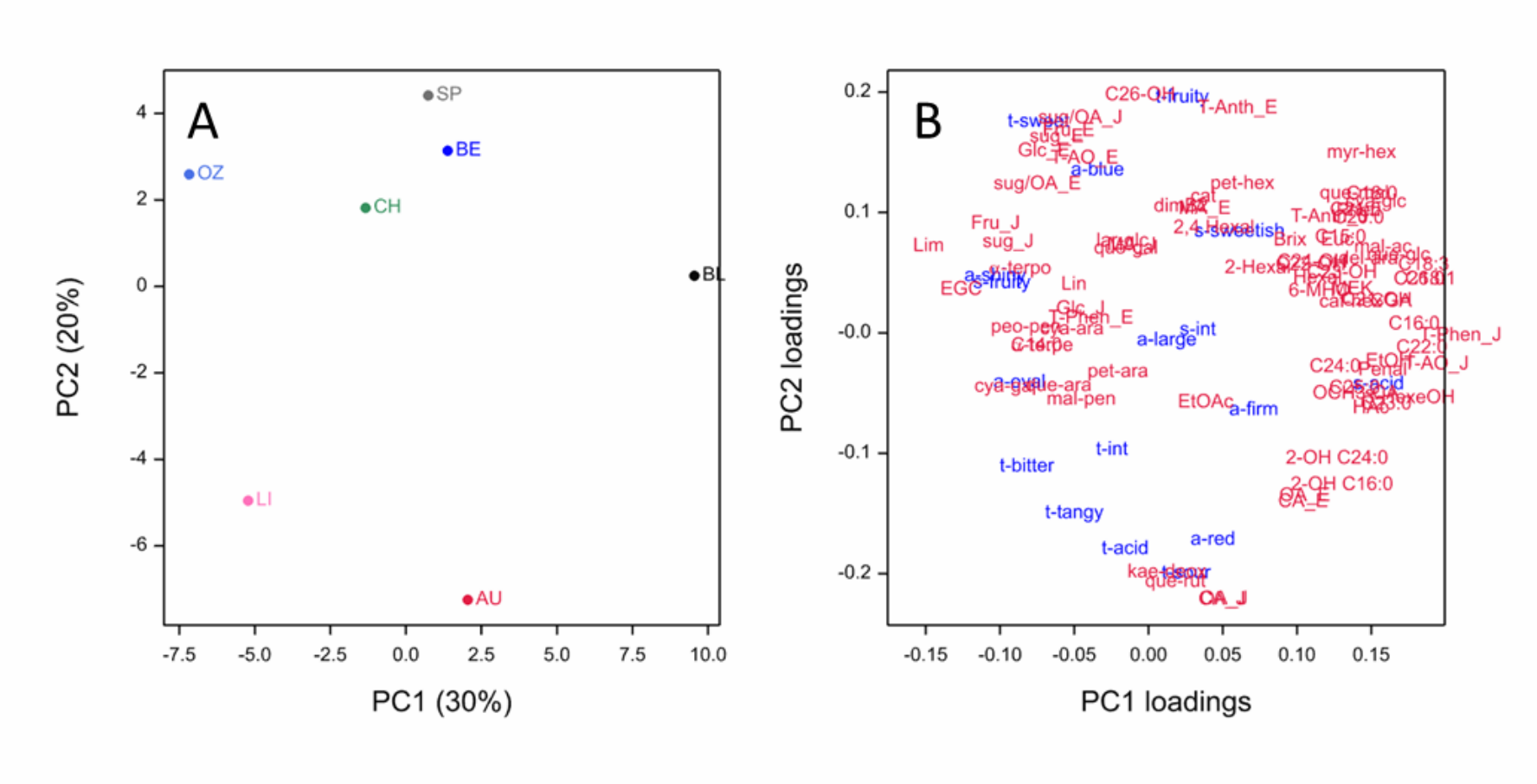


Figure 5.8. Principal component analysis (PCA) of compositional and sensory data from eight blueberry cultivars grown in 2010. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ compositional and ■ sensory data). The compositional data consist of flavour volatiles and lipids analysed with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer. The sensory descriptors were scored on a nine-point scale; a- refers to appearance attributes, s- to aroma attributes and t- to taste attributes. The list of phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Table 5.13 lists the Pearson's correlations coefficient between phytochemicals and sensory attributes with extreme positions in the loading plot in the first PC in blueberry cultivars grown in 2010. The sensory attributes acid and fruity aroma and sweet and bitter taste and a range of lipids, flavour volatiles and polyphenols were selected to find further correlations between metabolites and sensory attributes in blueberry cultivars. As previously mentioned in 5.2.3 sweet taste and fruity aroma descriptors had a negative correlation to fatty acids. The acid aroma descriptor was positively correlated to fatty acids and to flavour volatiles 3-HexeOH and 6-MHO. Further the bitter taste descriptor was negatively related to flavour volatiles derived from the fatty acids as discussed in the 5.2.3. Polyphenols were not discussed in the univariate analysis (5.2); however polyphenols were correlated to the acid aroma descriptor (Table 5.13). Drewnowski and Gomez-Carneros (2000) reported that phenolic compounds were considered as bitter.

Table 5.13. Pearson's correlation coefficients between sensory attributes and phytochemicals in extreme positions in the loading plot in PC1 in eight blueberry cultivars grown in 2010. Pearson's correlation coefficients r > 0.5 indicates by ■ and r < -0.5 indicated by ■; values in bold indicate for r > 0.3 and r < -0.3. The sensory attributes were scored on a nine point scale assessed on thawed blueberries. The list of phytochemicals including the abbreviations is shown in Appendix D.

	Aroma		Taste	
	acid	fruity	sweet	bitter
C22:0	**0.397**	-0.688	-0.531	-0.115
C26:0	**0.325**	-0.564	**-0.425**	**-0.467**
3-HexeOH	0.512	-0.534	**-0.465**	**-0.466**
Penal	0.898	-0.547	**-0.392**	**-0.382**
Lim	-0.542	**0.446**	0.610	0.286
α-terpo	-0.154	0.025	**0.410**	-0.072
CGA	0.862	**-0.402**	-0.660	-0.611
que-glc	**0.406**	-0.513	-0.127	-0.729
sug/oa_E	**-0.474**	0.607	0.849	-0.106
Fru_E	-0.228	0.663	0.863	-0.086
Fru_J	-0.679	0.044	0.172	0.080

Table 5.14 lists the Pearson's correlations coefficient between phytochemicals and sensory attributes with extreme positions in the loading plot in the second PC in blueberry cultivars grown in 2010. The sensory attributes blue, red, sweet, fruity, sour, acid, tangy, bitter and intensive taste and the phytochemicals sugars, antioxidants and CA were selected to find further correlations between metabolites and sensory attributes in blueberry cultivars. As previously discussed in 5.2.2 sweetness was positively correlated to Fru (only in acidified methanolic blueberry extract) and to sug/OA and negatively correlated to CA. Further sourness was negatively related to Fru (only in acidified methanolic blueberry extract) and to sug/OA and positively related to CA. Further sweetness and fruitiness was negatively related to CA and sour and acid taste exhibited similar relations to sugars and CA.

Table 5.14. Pearson's correlation coefficients between sensory attributes and analysed phytochemicals in extreme positions in the loading plot in PC2 in eight blueberry cultivars grown in 2010. Pearson's correlation coefficients r > 0.5 indicates by ■ and r < -0.5 indicates by ■; values in bold indicate for r > 0.3 and r < -0.3. The sensory attributes were scored on a nine point scale assessed on thawed blueberries. The list of phytochemicals including the abbreviations is shown in Appendix D.

	Aroma	Taste						
	sweetish	sweet	fruity	sour	acid	tangy	bitter	int
sug/OA_J	0.148	0.539	0.080	-0.609	-0.510	-0.520	-0.230	**-0.367**
sug/OA_E	0.034	0.849	0.246	-0.531	**-0.470**	-0.033	-0.106	-0.122
Fru_J	0.135	0.172	-0.228	-0.174	-0.017	-0.237	-0.080	**-0.301**
Fru_E	0.248	0.863	0.537	-0.684	-0.639	-0.145	-0.086	-0.073
CA_J	**-0.310**	-0.768	**-0.442**	0.869	0.776	0.605	**0.390**	**-0.421**
CA_E	0.073	-0.863	**-0.391**	0.526	**0.435**	0.054	**0.313**	0.037

Relations between chemical and sensory parameters of blueberry cultivars grown in 2011

The principal component product space of twelve blueberry cultivars in the growing year 2011 is shown in Figure 5.9A. The first two principal components accounted for 42 % of total sample variance. 26 % of total variance was explained by the first principal component (PC), with a strong positive score for BG and a strong negative score for CH. A range of sensory attributes (shiny, sweetish aroma, tangy, bitter and intensive taste) and the metabolites lipids, a range of anthocyanins and MA contributed positive scores and oval, large and the flavour volatiles provided a negative contribution (Figure 5.9B). 16 % of the variance was explained by the second PC with strong positive scores for EL, OZ, AU and BR and a strong negative score for SP. Sour and bitter taste, firm and CA contributed positive scores and a range of sensory attributes (red, sweet and intensive taste and fruity aroma) and sugars and Brix provided a negative contribution.

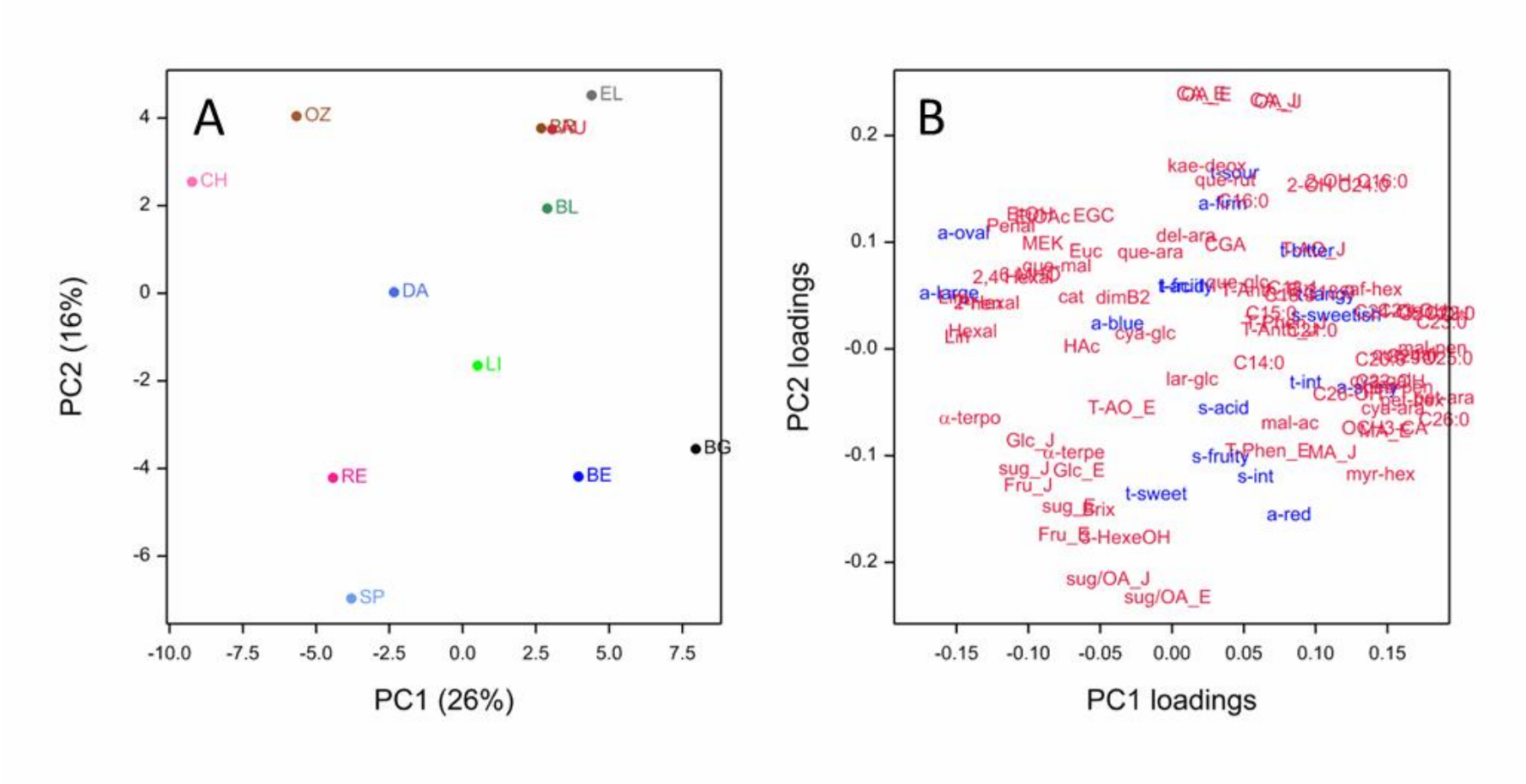


Figure 5.9. Principal component analysis (PCA) of compositional and sensory data from twelve blueberry cultivars grown in 2011. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ compositional and ■ sensory data). The compositional data consist of flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS-MS, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer. The sensory descriptors were scored on a nine-point scale; a- refers to appearance attributes, s- to aroma attributes and t- to taste attributes. The list of phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Table 5.15 lists the Pearson's correlations coefficient between phytochemicals and sensory attributes with extreme positions in the loading plot in the first PC in blueberry cultivars grown in 2011. The sensory attributes tangy, bitter and intensive taste and sweetish aroma and a range of lipids, anthocyanins, flavour volatiles and MA were selected to find further correlations between metabolites and sensory attributes in blueberry cultivars. As previously discussed under 5.2.3 Lim was negatively related to tangy, bitter and intensive taste. According to other studies Lim provides a fruity, orange and lemon odour (Kaack et al., 2006). Additional to the previously described correlations between sensory attributes and phytochemcials under 5.2, C_6 flavour volatiles and 6-MHO were negatively related to sweetish aroma. In agreement to other studies C_6 flavour volatiles provides a fresh cut grass odour (Kaack et al., 2006). Additional red colour was positively related to cya-gal (r = 0.400) and pet-ara (r = 0.573). In correspondence to the structure cyanidin contribute to blue colour as already described in 5.2.4) (He et al., 2010).

Table 5.15. Pearson's correlation coefficients between sensory attributes and analysed phytochemicals in extreme positions in the loading plot in PC1 in twelve blueberry cultivars grown in 2011. Pearson's correlation coefficients r > 0.5 indicates by ■ and r < -0.5 indicates by ■; values in bold indicate for r > 0.3 and r < -0.3. The sensory attributes were scored on a nine point scale assessed on fresh blueberries. The list of phytochemicals including the abbreviations is shown in Appendix D.

	Aroma	Taste		
	sweetish	tangy	bitter	int
C26:0	-0.063	-0.107	-0.210	-0.022
CIN	**0.351**	**0.394**	0.118	0.387
MA_J	**0.432**	**0.414**	**0.493**	0.538
MA_E	0.579	0.717	0.651	0.718
Hexal	**-0.473**	-0.177	-0.280	-0.264
2-Hexal	**-0.473**	-0.155	-0.277	-0.293
6-MHO	-0.500	-0.259	-0.203	-0.194
Lim	-0.184	**-0.469**	**-0.395**	**-0.372**

Table 5.16 lists the Pearson's correlations coefficient between phytochemicals and sensory attributes with extreme positions in the loading plot in the second PC in blueberry cultivars grown in 2011. The sensory attributes sour, bitter and sweet taste, fruity and intensive aroma and CA, sugars and brix were selected to find further correlations between metabolites and sensory attributes in blueberry cultivars grown in 2011. As expected, sweetness was positively correlated to Fru and sug/OA and negatively correlated to CA. Sourness exhibited inverse correlations being negatively related to Fru and sug/OA and positively correlated to CA and negative correlation to Fru (only in acidified methanolic extracts) as discussed in 5.2.2. Sour and bitter taste descriptors exhibited similar relations to sugars and CA. Additional to the univeriate analysis in 5.2.2 bitter taste was negatively related to sug/OA. A positive correlation between CA and firmness was additional observed (r = 0.431 in blueberry juice; r = 0.519 in blueberry extracts), it is likely that such correlations are not causative. Firmness contributes to sensory quality. Mehinagic et al. (2003) reported in apples a relationship of firmness to sweetness and sourness. Fruit softening occurs due to modifications in cell wall polymers (Cantu et al., 2008, Hancock et al., 1987).

Table 5.16. Pearson's correlation coefficients between sensory attributes and analysed phytochemicals in extreme positions in the loading plot in PC2 in twelve blueberry cultivars grown in 2011. Pearson's correlation coefficients r > 0.5 indicates by ■ and r < -0.5 indicates by ■; values in bold indicate for r > 0.3 and r < -0.3. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma		Taste		
	fruity	int	sour	bitter	sweet
Fru_J	**0.323**	-0.064	**-0.331**	-0.165	**0.454**
Fru_E	0.083	0.263	**-0.327**	**-0.303**	**0.491**
Brix	-0.170	0.069	-0.146	**-0.349**	0.209
CA_J	-0.527	**-0.365**	0.650	**0.449**	-0.550
CA_E	-0.296	**-0.390**	0.775	0.513	-0.553
sug/oa_J	**0.350**	0.199	-0.581	**-0.429**	0.506
sug/oa_E	0.149	0.294	-0.606	**-0.498**	0.523

Relations between chemical and sensory parameters of blueberry cultivars grown in 2012

The principal component product space of fourteen blueberry cultivars in the growing year 2012 is shown in Figure 5.10A. The first two principal components accounted for 39 % of total sample variance. 22 % of total variance was explained by the first principal component (PC), with positive scores for AU, EL and BL and negative scores for CH, DA and SP. The aroma attributes sweetish, fruity and intensity and the metabolites lipids and CA contributed positive scores and a range of sensory attributes (sour, acid, bitter, intensive taste, oval and firm), brix, sugars and terpenoids provided a negative contribution (Figure 5.10B). 17 % of the variance was explained by the second PC with high scores for PU and CHT and a very low score for SP. Red and blue colour, acid taste, C_6 flavour volatiles and a range of lipids contributed to positive scores and fruity taste and a range of anthocyanins provided a negative contribution.

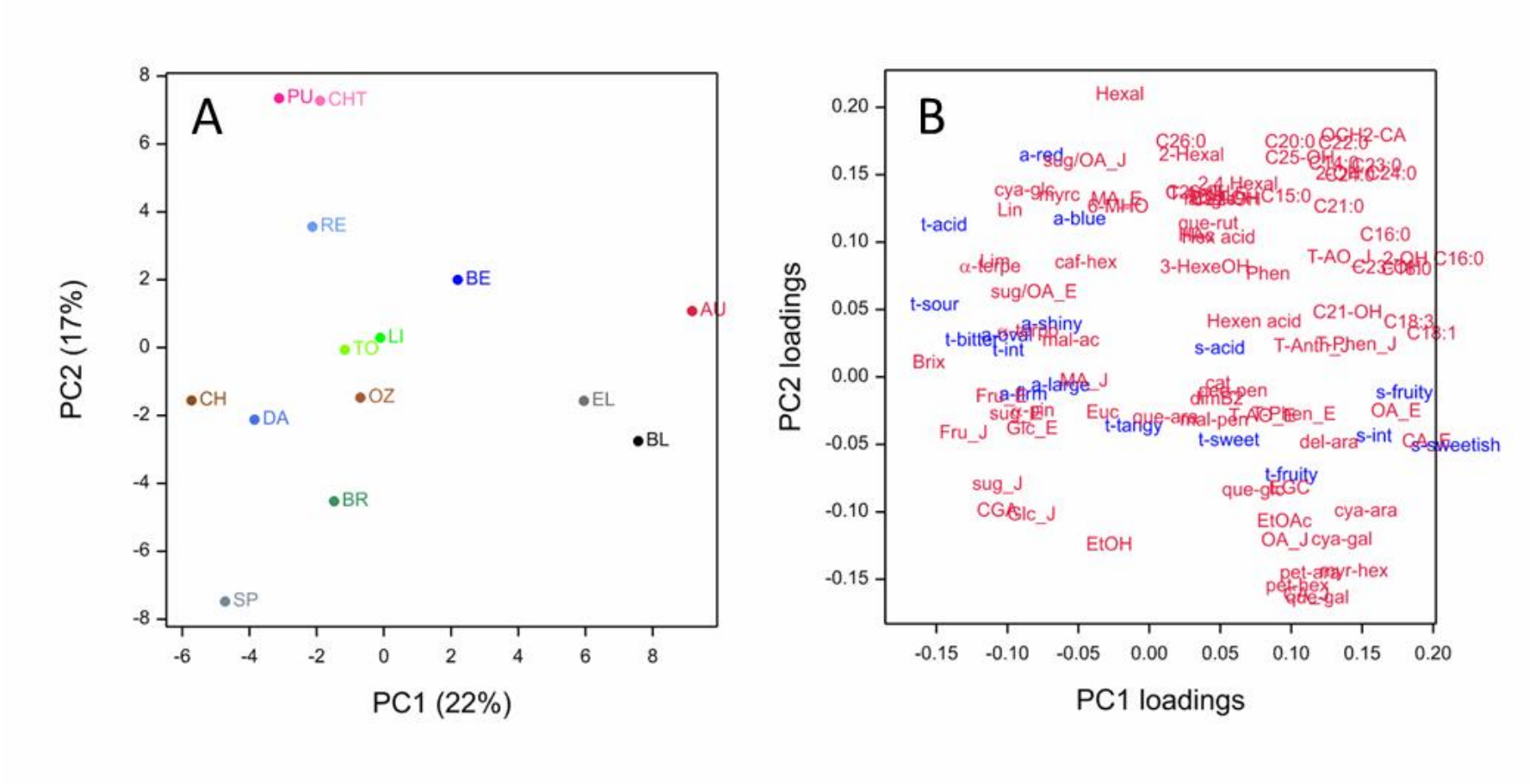


Figure 5.10. Principal component analysis (PCA) of compositional and sensory data from fourteen blueberry cultivars grown in 2012. PCA scores plot (A) and the corresponding PCA loading plot (B, ■ compositional and ■ sensory data). The compositional data consist of flavour volatiles and lipids measured with from GC-MS, individual polyphenols and anthocyanins with LC-MS^2, sugars and organic acids with HPLC, brix with refractometer and antioxidant compounds with UV-photometer. The sensory descriptors were scored on a nine-point scale; a- refers to appearance attributes, s- to aroma attributes and t- to taste attributes. The list of phytochemicals including the abbreviations is shown in Appendix D. For identification of the blueberry cultivars codes refer to Table 2.2.

Table 5.17 lists the Pearson's correlations coefficient (r) between phytochemicals and sensory attributes with extreme positions in the loading plot in the first PC in blueberry cultivars grown in 2012. The sensory attributes sour, acid, bitter and intensive taste, sweetish, fruity and intensive aroma tangy and a range of lipids, CA, sugars, brix and terpenoids were selected to find further correlations between metabolites and sensory attributes in blueberry cultivars. The intensive and sweetish aroma descriptors were positively correlated to lipids and CA (only in acidified methanolic extract) and the sour and acid taste descriptors were negative related to CA as described in 5.2.2. Additional the fruity aroma descriptor was positively related to lipids. Sweetish, fruit and intensive aroma descriptor were negatively related to Brix and sug/OA.

Table 5.17. Pearson's correlation coefficients between sensory attributes and analysed phytochemicals in extreme positions in the loading plot in PC1 in fourteen blueberry cultivars grown in 2012. Pearson's correlation coefficients r > 0.5 indicates by ■ and r < -0.5 indicates by ■; values in bold indicate for r > 0.3 and r < -0.3. The list of phytochemicals including the abbreviations is shown in Appendix D. Int, intensity.

	Aroma			Taste			
	sweetish	fruity	int	sour	acid	bitter	int
C18:1	0.800	0.716	0.666	-0.764	-0.545	-0.677	-0.536
C18:3	0.716	0.632	0.593	-0.602	-0.523	**-0.479**	**-0.305**
CA_J	**0.470**	0.246	0.089	**-0.470**	-0.591	**-0.314**	**-0.377**
CA_E	0.730	0.622	0.547	-0.732	-0.645	-0.649	-0.602
Brix	-0.601	-0.692	-0.531	0.509	**0.355**	**0.497**	0.202
Sug/oa_E	-0.582	-0.731	-0.542	0.617	**0.334**	0.599	0.511
Fru_J	**-0.497**	**-0.451**	**-0.339**	0.490	0.284	**0.487**	**0.415**
Fru_E	**-0.383**	-0.502	**-0.421**	**0.309**	0.217	**0.302**	0.142
α-terpe	**-0.364**	-0.294	**-0.321**	**0.371**	**0.532**	**0.346**	0.138
Lim	**-0.332**	-0.261	**-0.350**	**0.344**	**0.447**	**0.354**	0.124

Table 5.18 lists the Pearson's correlations coefficient between phytochemicals and sensory attributes with extreme positions in the loading plot in the second PC in blueberry cultivars grown in 2012. The sensory attributes fruit and acid taste descriptors, anthocyanins, flavour volatiles derived from fatty acids and lipids were selected to find further correlations between metabolites and sensory attributes in blueberry cultivars. Additional to the discussions in 5.2.3 the C_6 flavour volatiles were positively related to acid taste attributes. The relationship between anthocyanins

and taste descriptors were not discussed in the univariate analysis (5.2). However pet-hex, cya-gal were negatively correlated to the acid descriptor. Koes et al. (1994) reported that anthocyanins contribute to astringend and bitterness.

Table 5.18. Pearson's correlation coefficients between sensory attributes and analysed phytochemicals in extreme positions in the loading plot in PC2 in fourteen blueberry cultivars grown in 2012. Pearson's correlation coefficients r > 0.5 indicates by ■ and r < -0.5 indicates by ■; values in bold indicate for r > 0.3 and r < -0.3. The list of phytochemicals including the abbreviations is shown in Appendix D.

	Taste	
	fruity	acid
pet-hex	0.202	-0.563
cya-gal	0.156	-0.571
Hexal	**-0.374**	0.630
2-Hexal	-0.199	**0.404**
C16:0	-0.246	**0.343**
OCH_3 CA	0.083	-0.067

5.4 Conclusions

Modeling flavour is important to identify factors that contribute to flavour quality in order to optimise these important variables for breeding desirable blueberry cultivars. The applied PLS could not produce a robust model and is likely that a larger number of blueberry cultivars that exhibit greater sensory diversity would be required for a good model. PCA highlighted links between phytochemical and sensory data. There was little consistency found between the year to year variations highlighting the complexity of the underlying phytochemical drivers of the sensory experience and the limited capacity for even trained consumers to distinguish the cultivars presented in the current work. Nevertheless, the data presented here suggests that high sugar content increases the perception of fruitiness, while CA enhances the intensive taste perception in cultivars grown in 2010 and 2011. The environmental differences could occur due to seasonal effects like light, temperature and rainfall. In further sensory analysis experiments texture descriptors need to be added due to the suggested impact on sweetness. Other sensory characters like mouthfeel may have an impact on

flavour perception. It is more likely that the fresh, green flavour volatiles are associated to sourness and the fruity volatiles enhancing the perception of sweetness. Relations between terpenoids and fruity taste indicated possible interaction between sugars with terpenoids.

Chapter 6

Summary and Conclusions

6.1 Introduction

This project was focused on the genetic and environmental influences of the chemical composition in relation to sensory quality in a range of commercially available blueberry cultivars grown in UK. The aim was to provide breeders with knowledge of the appropriate sensory characteristics that appeal to the largest section of UK consumers and to provide an underlying knowledge of phytochemical-sensory relationships. Such information provides breeders with the building blocks to allow accelerated breeding of desirable cultivars by opening the potential to develop indirect markers for sensory character. The chemical characteristics of blueberries are well documented (Silva et al., 2005), but only few studies are available regarding the correlation between sensory and chemical profiles (Saftner et al., 2008). The sensory and phytochemical analyses were conducted on a range of commercially available highbush blueberry cultivars grown in Scotland. Northern highbush blueberry cultivars are preferred in the retail due to large fruit size, large high-yielding bushes, longer shelf life (Hancock et al., 2008). A range of sensory tests were performed, started with a questionnaire to get information about consumer fruit perceptions. FCP was a useful precursor to conventional sensory profiling providing information about the use of descriptors. DP described the relationship between cultivars in a multivariate product space. In addition, hedonic testing was conducted to determine consumer blueberry preferences. In order to persuade the consumer to buy high quality blueberries one must deliver fruit that meets consumer expectations for both sensory profile and potential health benefits. Phytochemicals that have

potential health promoting (polyphenols,) or sensory (sugar, organic acid, volatiles, anthocyanins) properties in a range of germplasm grown at a variety of UK locations were analysed. Modeling of the relationship between sensory and phytochemical data was conducted. Data collected on fruit phytochemistry integrated with sensory profiles to develop models of how fruit chemistry drives sensory character.

6.2 Sensory analysis

6.2.1 Questionnaire

The studies started with an online survey to obtain an overview regarding blueberry consumption and how this relates to socioeconomic group. Responses to the questionnaire were received from 421 consumers at three sites. Participants were self-selected. Blueberries were generally consumed for health benefits. The questionnaire demonstrated that older consumers and females, especially when they are marrieds were more likely to consume blueberries and be prepared to pay a premium for UK grown blueberries. Further the questionnaire found out that women, especially those that were marrieds preferrred blueberry compared to single women and would accept a higher price for premium UK grown blueberries, because they probably want to provide healthy food for their children. Therefore these data suggest that amongst current UK consumers promotion regarding the health properties of blueberries and information regarding their use (e.g. smoothie, yoghurt) could increase the consumption of blueberries. Further potential groups might be people with a higher income and sportsmen, because they are more interested in health food. In order to increase the consumption by these groups processed blueberries should be provided with long shelf-lives (e.g. bars), but without any cooling requirements. In general consumers had difficulties in describing their “ideal blueberries”, only 129 out of 421 consumers answered this question. Most of the answers were about sweetness, sourness and appearance, only few about aroma. On average, consumers had no a clear perception about their “ideal blueberries”. The ideal blueberry was: large, sweet, firm but juicy, blue and fruity in character.

Presumably the consumers don't know what cultivars they prefer. This might provide an opportunity to explain to consumers how premium blueberry cultivars should taste. Further UK blueberries could be promoted as a tastier and higher quality product compared to imported blueberries, despite the evidence that the consumer can't perceive any differences to imported blueberries. On average consumers were prepared to pay £10 - £15 per Kg. This asking price was below the market price. Additional it is important to meet consumers' expectations, if the consumers don't like it; they won't buy it again, regardless of the health issues. In future studies the questionnaire should be conducted across the UK, preferably in different supermarkets in order to get a broader view about potential blueberry consumers. However it is important provide the consumer with the same quality across the season. Beside the name of the cultivars additional hints on the package about the taste (e. g. sour, sweet, flowery) could give the consumer the opportunity to choose what cultivars they might like.

6.2.2 Free-choice profiling

FCP supplied important information of the interrelationship among the six blueberry cultivars and sensory characteristics (Jack and Piggott, 1991). Consumers who were prepared to spend additional time assessing blueberries were selected form the questionnaire. FCP was carried out with 25 assessors who were consumers of blueberries. The lexicon for six varietal frozen blueberries consisted of a minimum of 10 descriptors by individuals and a maximum of 29. The largest group (35 %) used 10 – 15 descriptors. The most frequently mentioned descriptors were related to appearance suggesting that this aspect of the fruit plays an important role in the decision to purchase.

In general flavour is weakly perceived in food; therefore the consumers had difficulties to describe aroma. It was suggested that only in few foodstuff aroma is important, e. g. coffee. Aroma might be less important in fruits and vegetables. In order to score the aroma in blueberry cultivars it was necessary to provide blueberry puree. Appearance and taste are more important for consumers than aroma. For a preferable taste the sug/OA ratio is important, e. g. a too low content of acid reduce

the flavour perception (Kader, 1991). The assessors struggled in aroma probably because of their unfamiliarity similar similar to previous studies (Silva et al., 2005). Further reasons for this low agreement were the limited aroma profile and the lack of aroma and the provided thawed blueberry cultivars presumably reduced the aroma perception. Additional germplasm needs to be brought in to develop cultivars with distinctive aroma profiles that might aid in the development of the UK market.

From frequency of usage and the product space a vocabulary for DP was developed with 17 descriptors; six for appearance, four for aroma, and seven for taste. The generated vocabulary was juicy vs. firm, size, red, blue, matt vs. shiny, round vs. oval by appearance, sweet, fruity, acid intensity by aroma and sour, tangy, bitter, fruity, acid, intensity by taste.

6.2.3 Descriptive Profiling

DP was carried out with eight panelists trained on 17 attributes in three different harvesting years. Beforehand the assessors were tested to ensure their capacity to rank fruit in terms of sweetness, sourness, and flavour intensity. The panel was recruited from the University of Strathclyde and varied in age, domestic status, ethnicity, receptor sensitivity, gender and profession. Scale usage was improved by DP training to reduce variation (Meilgaard et al., 1999). The assessors scored up to 14 cultivars over three harvesting years. Thawed cultivars were scored in the harvesting year 2010, while fresh cultivars were scored in the harvesting years 2011 and 2012. Fruit puree was provided for the aroma evaluation to enhance the aroma perception. In discrimination only 11 of 17 descriptors were significant ($p < 0.05$). Similar to previous studies discrimination in aroma and colour were not significant ($p > 0.05$) (Silva et al., 2005), possibly related to small colour differences and low aroma level. This covered with the discussion of the assessors after the sessions. Similar to FCP, the assessors had difficulties in aroma perceptions; therefore breeders should improve the aroma profile in highbush blueberries by bringing in additional germplasm with distinct aroma profile. Due to the small diversity of available genotypes growers could only grow a few cultivars, should preferably draw up a shortlist of early, middle and late cultivars in order to supply blueberries over a

long season. The promotion about the advantages of UK blueberries compared to imported fruits might be the key to increase the market share as described in the questionnaire section (6.2.1)

In further sensory tests additional descriptors like mouthfeel and texture should be added because they might have an impact on the perception of sweetness, sourness and flavour and more distinct cultivars should be provided. Further sensory tests of blueberry cultivars grown at different regions and under different conditions should be conducted in order to determine the environmental impact.

6.2.4 Consumer Preferences

The hedonic test was conducted in the harvesting year 2011 to range the consumer preferences of blueberry cultivars on a seven point scale from dislike extremely to like extremely. Before the consumer started with the hedonic test they got a brief introduction. In general the range of consumer preference scores of the nine provided blueberry cultivars was fairly low. Similar to the results of FCP and DP the consumers had difficulties in differentiating between cultivars due to the small diversity in commonly grown UK cultivars, but different socioeconomic groups demonstrated preference differences between cultivars. In order to meet consumer expectations a broader diversity of sensory characteristics in blueberry cultivars are required.

A relationship between consumer preferences and conventional profiling was conducted to determine sensory attributes that are important in blueberry cultivars for meeting consumer expectations. The applied PLS related the consumer preferences to sensory quality characteristics in order to integrate two data sets in a multidimensional map. PLS could not create a robust model due to small variations between the cultivars. In future research, blueberry cultivars with a larger diversity should be selected.

Alternatively to PLS, PCA was applied to determine at least trends and outliers. It should be focused on a number of cultivars aligned to different consumer needs, e. g. sweeter cultivars for younger consumers, more sour ones for females. Further the different genotypes should be used for the fresh market and the processing market. For instance softer fruits should be selected for juice manufacturing in order to ease

the yield of juice and smaller fruits should be selected due to their higher content of anthocyanins. In future research, blueberry cultivars with a larger diversity should be selected and additional consumer test should be conducted in different supermarkets across UK with a larger number of consumers. Customers of different supermarkets may prefer different blueberries.

6.3 Phytochemical analysis

During fruit development blueberry fruit go through changes in the phytochemical composition. Methods were developed for the analysis of fruit phytochemicals with impacts on health or sensory properties of the fruit (HPLC, LC-MS^2, GC-MS). These methods were used to quantify phytochemicals that have potential health promoting (polyphenols, anthocyanins) or sensory (sugar, organic acid, volatiles, anthocyanins, polyphenols, lipids) properties in a range of germplasm grown at three different seasons.

Genetic and environmental differences (growing location, weather conditions) of fruit metabolites were illustrated using PCA. Limited genetic variations were observed in phytochemical content of individual cultivars grown in the same location due to similar pedigrees of the germplasm. Environmental influences were manifested as different in the same cultivar across seasons and across locations. Evidence is presented that seasonal differences affected metabolite accumulation in the same cultivar across growing years. Higher temperatures and longer sun exposure contributed to a higher content of organic acids while in wetter, colder seasons contributed to a higher content of sugars. Light is an important parameter for photosynthesis and anthocyanin synthesis, while moisture is required for respiration and nutrition transport (Taiz and Zeiger, 1998). Different growing locations had an impact on different levels of flavour volatiles, lipids and antioxidant properties. Cultivars grown at the farm in Blairgowrie exhibited higher levels of flavour volatiles, while cultivars grown at the farm in Muirhead or at the James Hutton Institute exhibited higher levels of lipids and antioxidant properties. Several factors were different between the growing places like the age and ripeness of the cultivars

and the growing conditions; cultivars in Blairgowrie were grown under polytunnel in contrary to the remaining growing places. Such factors might have an impact on the phytochemical content. In agreement to previous studies blueberries grown under polytunnel have shown lower levels of antioxidant components compared to blueberries grown open field because polytunnel filtered the UVA and UVB (Eichholz et al., 2011). In order to gain a better understanding of the metabolic differences within location differences, the same blueberry cultivars should be analysed across UK, in order to understand the environmental impact on key quality traits. Physiological processes like sugar and acid accumulation and ripening are depending on external factors like growing location (Steyn et al., 2002, Etienne et al.). This study demonstrated that the environment has an impact on the phytochemical quality, but it is unknown what parameters influencing the phytochemicals. Therefore a larger range of cultivars should be grown open field und under polytunnel at the same growing locations in order to how the environment influences the cultivars. This would give the growers useful information with regard to the optimal conditions for highbush blueberry cultivars in UK and what parameters might be adjusted in order to get the required fruit quality.

6.4 Modelling the relation between sensory and phytochemical data

In order to persuade the consumer to buy high quality blueberries one must deliver fruit that meets consumer expectations for both sensory profile and potential health benefits. Correlation analysis between sensory and phytochemical data across three different harvesting years determined which metabolites contribute to which sensory quality character.

The key sensory attributes sweetness and sourness exhibited the highest correlations to sugars and acids in correspondence to previous studies in different food (Sandell et al., 2009, Tiitinen et al., 2005). Sweetness perception is presumably a combination of aroma and oral (gustation) information from detection of volatiles and non-volatiles

(Chandrashekar et al., 2006). In general cultivars grown in 2010 have shown the most correlations. CA was the best predictor for sourness, while Glc and Fru was the best predictor for sweetness. In agreement to previous studies, sug/oa is an important indicator for sweetness and sourness (Poll, 1981, Tang et al., 2001). Beside sourness, CA content contributed to acidity and tanginess. The panel could not differentiate between sourness, acidity and tanginess. Therefore further sensory tests could leave out these descriptors. Flavour is an important factor for fruit quality. Flavour intensity perceptions were weakly related with sugar and acid parameters. Similar to previous studies the analysed blueberry cultivars have shown low flavour intensities (Silva et al., 2005). The combination and intensity of flavour volatiles varied with the climatic conditions. Modeling of flavour quality to independent variables with PLS could not produce a robust model, because of the lack of the variety of blueberry cultivars. The applied PCA exhibited relations between phytochemical and sensory data with similar relations in 2010 and 2011. PCA exhibited that Fru has an impact on sweetness and fruitiness. Sugars increased the fruitiness perception, while CA increases the intensive flavour perception. For further studies a larger variety of blueberry cultivars is required to create a robust model. The fruit quality in blueberries depends on a range of variables. The flavour volatiles profile should be enhanced by breeding blueberry cultivars with a larger flavour profile.

6.5 Concluding remarks

This project was performed on commercial available highbush blueberries with limited variations between the different genotypes. Untrained and even a trained panels could not differentiate between cultivars. Especially they were struggled in aroma perception probably because these blueberries were weak in aroma. Further research needs to focus on improving the aroma profile in blueberries. The correlation analyses could only demonstrate that CA is a good indicator for sourness and Glc and Fru contents are good indicators for sweetness. There was no success in modelling relationships between sensory and phytochemical data due to the limited variations between cultivars. In the short term, further research should strongly

promote the healty properties of blueberries and suggest recipe could make the consumption of blueberries more diversified for current and potential consumers. Marketing research could explain consumers how premium blueberry shold taste and promote that UK blueberry are tastier and a higher quality product compared to imported blueberries. The label should contain additional hints about the main characteristic in the blueberry cultivrs ithat the consumer could choose what cultivars they prefer. Environmental studies on blueberry cultivars should be conducted in order to provide growers a tool to enhance the key sensory quality properties. In the longer term breeding of more distinct flavour cultivars are required to meet consumer preferences.

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Appendices

Appendix A Questionnaire

The University of Strathclyde is carrying out a survey about perceptions on blueberries and related fruits. Your opinion is extremely important; thank you for taking a moment to answer the following questions:

1. How would you describe yourself?

❑ *Optimistic* ❑ *Pessimistic*

2. Are you a smoker?

❑ *Yes* ❑ *No* ❑ *Don't know*

3. How do you rate your general health state?

❑ *Good* ❑ *Fair* ❑ *Poor*

4. How often do you exercise?

❑ *Every day* ❑ *Once a week* ❑ *Once a month*
❑ *More than week* ❑ *Fortnightly* ❑ *Occasionally*

5. How often do you consume the following products?

	Every day	*5 to 6 times a week*	*2 to 4 times a week*	*Once a week*	*Fort-nightly*	*Monthly*	*Less than once a month*
Blueberry	❑	❑	❑	❑	❑	❑	❑
Strawberry	❑	❑	❑	❑	❑	❑	❑
Raspberry	❑	❑	❑	❑	❑	❑	❑
Blackberry	❑	❑	❑	❑	❑	❑	❑
Black currant	❑	❑	❑	❑	❑	❑	❑
Apple	❑	❑	❑	❑	❑	❑	❑
Pear	❑	❑	❑	❑	❑	❑	❑
Banana	❑	❑	❑	❑	❑	❑	❑

6. **Do you like blueberries?**

❑ *Yes* ❑ *No* ❑ *Don't know*

7. **Do you agree or disagree with the statement "blueberry consumption provides health benefits"?**

❑ *Yes* ❑ *No* ❑ *Don't know*

8. **What would you be willing to pay for blueberries per 100g?**

❑ *£1,00 - £1,49* ❑ *£2,00 - £2,49* ❑ *£3,50 - £5,00*

❑ *£1,50 - £1,99* ❑ *£2,50 - £3,49* ❑ *I don't know*

9. **Please use this space to describe your "ideal blueberry" in terms of appearance, aroma and taste.**

_____

_____

_____

_____

_____

_____

10. **Why do you buy blueberries?**

_____

11. **Do you know where your blueberries come from?**

❑ *Yes* ❑ *No* ❑ *Doesn't matter*

12. **Would you be willing to pay more (a premium) for British grown blueberries?**

❑ *Yes* ❑ *No* ❑ *Don't know*

Thank you for your time and co-operation with this survey

We would like to know your demographic background and it would greatly assist us if you would fill in the personal details that follow.

If you would prefer not to provide this information please return the questionnaire now.
Otherwise please continue …

13. **Indicate how much you agree or disagree of the following statements.**

	Strongly agree	*Tend to agree*	*Neither agree nor disagree*	*Tend to disagree*	*Strongly disagree*
I am constantly sampling new and different fruits.	❑	❑	❑	❑	❑
I do not trust new fruits.	❑	❑	❑	❑	❑
If I am not familiar with a fruit, I won`t try it.	❑	❑	❑	❑	❑
I like fruits from different countries.	❑	❑	❑	❑	❑
I like to taste new fruits.	❑	❑	❑	❑	❑
I am very particular about the fruits I will eat.	❑	❑	❑	❑	❑

14. **If you are a non-smoker and live in the Strathclyde area would you like to participate in a voluntary sensory panel?**

❑ *Yes* ❑ *No*

If yes, please provide your email address

_____

15. **How old are you?**

❑ *Under 18* ❑ *45 - 60*

❑ *18 - 24* ❑ *Over 60*

❑ *25 - 44*

16. **Are you male or female?**

❑ *Male* ❑ *Female*

17. **What is your domestic status?**

❑ *Married/ co-habiting* ❑ *Single*

18. **Where do you live?**

The first section of your postcode
e.g. DD5 or G1 _____

19. **How would you describe your ethnicity?**

_____

Thank you for taking part in our survey.
We value your opinion.

Appendix B Examination

Name: _____

Date: _____

Please score the samples:

Appearance:

	Sample 1	Sample 2
Blue		
Red		
Round vs oval		
Matt vs shiny		
Firmness		
Size		

Please score the samples:

Aroma:

	Sample 1	Sample 2
Sweet		
Acid		
Fruity		
Intensity		

Please score the samples:

Taste:

	Sample 1	Sample 2
Sweet		
Sour		
Acid		
Bitter		
Intensity		
Fruity		

Appendix C Consumer preferences

1. **Are you female or male?**

- ❑ *Female*
- ❑ *Male*

2. **How old are you?**

- ❑ *Under 25*
- ❑ *26 - 34*
- ❑ *35 - 44*
- ❑ *45 - 55*
- ❑ *over 56*

3. **What is your occupation?**

- ❑ *Professional*
- ❑ *Office*
- ❑ *Self-employed*
- ❑ *Technical*
- ❑ *Student*
- ❑ *Sales*
- ❑ *Unemployed*

4. **How often do you exercise?**

- ❑ *Every day*
- ❑ *More than once a week*
- ❑ *Once a week*
- ❑ *Fortnightly*
- ❑ *Once a month*
- ❑ *Occasionally*

5. **Please sample the following samples according appearance.**

	Dislike ex-tremely			*Neither like nor dislike*			*Like ex-tremely*
Sample 897	❑	❑	❑	❑	❑	❑	❑
Sample 286	❑	❑	❑	❑	❑	❑	❑
Sample 508	❑	❑	❑	❑	❑	❑	❑
Sample 045	❑	❑	❑	❑	❑	❑	❑
Sample 453	❑	❑	❑	❑	❑	❑	❑
Sample 661	❑	❑	❑	❑	❑	❑	❑
Sample 114	❑	❑	❑	❑	❑	❑	❑
Sample 340	❑	❑	❑	❑	❑	❑	❑
Sample 227	❑	❑	❑	❑	❑	❑	❑

6. **Please sample the following samples according taste.**

	Dislike ex-tremely			*Neither like nor dislike*			*Like ex-tremely*
Sample 897	❑	❑	❑	❑	❑	❑	❑
Sample 286	❑	❑	❑	❑	❑	❑	❑
Sample 508	❑	❑	❑	❑	❑	❑	❑
Sample 045	❑	❑	❑	❑	❑	❑	❑
Sample 453	❑	❑	❑	❑	❑	❑	❑
Sample 661	❑	❑	❑	❑	❑	❑	❑
Sample 114	❑	❑	❑	❑	❑	❑	❑
Sample 340	❑	❑	❑	❑	❑	❑	❑
Sample 227	❑	❑	❑	❑	❑	❑	❑

7. **Please sample the following samples overall.**

	Dislike ex-tremely			*Neither like nor dislike*			*Like ex-tremely*
Sample 897	❑	❑	❑	❑	❑	❑	❑
Sample 286	❑	❑	❑	❑	❑	❑	❑
Sample 508	❑	❑	❑	❑	❑	❑	❑
Sample 045	❑	❑	❑	❑	❑	❑	❑
Sample 453	❑	❑	❑	❑	❑	❑	❑
Sample 661	❑	❑	❑	❑	❑	❑	❑
Sample 114	❑	❑	❑	❑	❑	❑	❑
Sample 340	❑	❑	❑	❑	❑	❑	❑
Sample 227	❑	❑	❑	❑	❑	❑	❑

Thank you for taking part.
We value your opinion.

Appendix D List of identified phytochemicals

Abbreviations	Phytochemicals
Sugars	
Fru	Fructose
Glc	Glucose
sug	Total sugar
Organic acid	
CA	Citric acid
MA	Malic acid
sug/OA	sugar to organic acid
Brix	Brix
Antioxidant components	
T-Phen	total polyphenol
T-Anth	total anthocyanin
T-AO	total antioxidant
Polyphenols	
dimer B2	(-)-epicatechin-(4β→8)-(-)-epicatechin
EGC	(epi)gallocatechin
caf-hex	caffeoyl hexose
cat	catechin
CGA	chlorogenic acid
kae-deox	kaempferol hexose deoxyhexoside
kae-mal	kaempferol hexoside-malonate
lar-glc	laricitrin-3-O-glucoside
myr-hex	myricetin hexoside
que-hex	quercetin hexoside
que-mal	quercetin hexoside-malonate
que-ara	quercetin-3-O-arabinoside
que-glc	quercetin-3-O-glucoside
que-rut	quercetin-3-O-rutinoside

Abbreviations	Phytochemicals
Anthocyanins	
cya-ara	cyanidin-3-O-arabinoside
cya-gal	cyanidin-3-O-galactoside
cya-glc	cyanidin-3-O-glucoside
del-ara	delphindin-3-O-arabinoside
mal-ac	malvidin-3-(6-acetyl)-hexoside
mal-pen	malvidin-3-O-pentoside
peo-pen	peonidin-pentose
pet-ara	petunidin-3-O-arabinoside
pet-hex	petunidin-3-O-hexoside
Flavour volatiles	
2,4-Hexal	(E,E)-2,4-hexadienal
2-Hexal	(E)-2-hexenal
3-HexeOH	(Z)-3-hexenol
6-MHO	6-methyl-5-hepten-2-one
EtOAc	ethyl acetate
EtOH	ethanol
Euc	eucalyptol
HAc	acetaldehyde
Hex acid	hexanoic acid
Hexal	hexanal
Hexen acid	(E)-2-hexenoic acid
Lim	D-limonene
Lin	linalool
MEK	2-butanone
myrc	myrcene
Penal	pentanal
Phen	phenol
α-terpe	α-terpineol
α-terpo	α-terpinolene

Abbreviations	Phytochemicals
Lipids	
C14:0	tetradecanoic acid
C15:0	pentadecanoic acid
OCH3-CA	3- or 4-methoxy, 3- or 4-hydroxy cinnamic acid
C16:0	hexadecanoic acid
2-OH C16:0	2-hydroxy-hexadecanoic acid
C18:0	octadecanoic acid
C18:1	octadenoic acid
C18:3	linolenic acid
C20:0	eicosanoic acid
C21:0	heneicosanoic acid
C21-OH	heneicosanol
C22:0	docosanoic acid
C22-OH	docosanol
C23:0	tricosanoic acid
C23-OH	tricosanol
C24:0	tetracosanoic acid
C24:OH	tetracosanol
2-OH C24:0	2-hydroxy tetracosanoic acid
C25:0	pentacosanoic acid
C26:0	hexacosanoic acid
C26-OH	hexacosanol

List of posters and presentations

Messner, C., Paterson A. and Hancock, R. (2011). *Highbush Blueberry Varietal Flavour Characteristics.* XIII Weurman Flavour Research Symposium, Zaragoza, Spain, 27th – 30th September 2011.

Messner, C., Paterson, A., McCallum, S., Graham, J., Hancock, R.D. (2012). *Genetic and Environmental Drivers of Fruit Composition in Relation to Sensory Quality in Blueberry*. Scottish Society for Crop Research Soft Fruit Meeting, Dundee, UK, 15th February 2012.

Messner, C., Paterson, A., McCallum, S., Graham, J., Hancock, R.D. (2012). *Genetic and Environmental Drivers of Fruit Composition in Relation to Sensory Quality in Blueberry*. SEB Annual Main Meeting, Salzburg, Austria, 29th June– 2nd July 2012.

Messner, C., Paterson, A., McCallum, S., Graham, J. & Hancock, R.D. (2012). *Genetic and Environmental Drivers of Fruit Composition in Relation to Sensory Quality in Blueberry*. 1st Agriscience Chemical Biology Postgraduate Symposium, London, UK, 1st – 2nd November 2012.

Messner, C., Paterson, A., McCallum, S., Graham, J. & Hancock, R.D. (2012). *Genetic and Environmental Drivers of Fruit Composition in Relation to Sensory Quality in Blueberry*. EuBerry International Berry School (IBS), Geisenheim, Germany, 5th – 8th April 2013.