Evaluating effects of climate variability on postharvest quality of strawberries

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DECLARATION

"I certify that this work has not been accepted in substance for any degree and is not concurrently being submitted for any degree other than that of Doctor of Philosophy being studied at the University of Greenwich. I also declare that this work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised the work of others".

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ABSTRACT

The strawberry is one of the most popular fruits worldwide cultivated in many regions of the globe contributing to human health and nutrition of consumers, as well as generating income for growers and retailers. The UK is one of the main importers of strawberries worldwide and one of the major strawberry exporters is Spain. The aim of this work was to evaluate the effects of environmental variability on several characteristics of strawberry postharvest quality. Acids, sugars, colour, firmness, anthocyanins, visual evaluation for detection of bruises and mould development were assessed. Work at the initial stages revealed a positive relationship between increased preharvest temperature and softening of fruits. Subsequent experiments took place in order to verify this finding and examine the physiological and biochemical mechanisms that contribute to this phenomenon. Contribution of pectate lyase (PEL) action to softening was assessed. No evidence was found to indicate that PEL activity was the key factor for lowering strawberry firmness. Furthermore, plants were cultivated under controlled conditions in growth cabinets in order to evaluate the effects of temperature and light on quality of strawberries. It was noticed that the effect of higher preharvest temperature over longer periods of time (weeks) was more important for reducing firmness when compared to the effect of higher temperature over shorter periods (hours). In addition the impact of force on development of bruise and the positive relationship that this has with reduced firmness of fruits was investigated. It was shown that firmer fruits grown at lower preharvest temperature had reduced levels of dry bruise development when exposed to artificial transport stress. Finally, the changes in quality profile of fruits through the harvest season and the way that these changes affect consumer acceptability were also evaluated. Consumers were able to relate sweeter strawberries to increased TSS and sugar levels and they also showed preference for them when compared to fruits with lower levels of sugars and TSS.

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LIST OF SYMBOLS/NOTATION

µl	Microlitre
µmol	Micromoles
2-MCE	2-mercaptoethanol
ABA	abscisic acid
ANOVA	Analysis of Variance
AA	ascorbic acid or ascorbate
ca.	Approximately
Ca	Calcium
$CaCl_2$	calcium chloride
DEFRA	Department for Environment, Food and Rural affairs
D.F.	dilution factor
d.w.	dry weight
E.C.	eliminative cleavage
EU	European Union
FAO	Food and Agriculture Organization
f.w.	fresh weight
GLM	General linear model
HCl	hydrochloric acid
HPLC	High Performance Liquid Chromatography
kg	Kilogram
kPa	kiloPascal
L	Litre
LSD	least significant difference
M	molarity
m	Metre
Min	Minute
MeOH	Methanol
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
MT	Metric tonnes
MW	molecular weight
N	Newtons
Nm	Nanometer
NaCl	sodium chloride
NaOH	sodium hydroxide
nm	nanometre
O_2	oxygen
P	Probability
PC	principal component
PCA	principal component analysis
PEL	pectate lyase
pg-3-gluc	pelargonidin-3-glucoside

ppm	parts per million
ppb	parts per billion
RHMAX	maximum relative humidity
RHMEAN	mean relative humidity
RHMIN	minimum relative humidity
RuBp	Rubisco
S.D.	standard deviation
S.E.	standard error
SP1	farm located at Lepe
SP2	farm located at Moguer
TA	total anthocyanins
TMAX	maximum temperature
TMEAN	mean temperature
TMIN	minimum temperature
TSS	total soluble solids
TTA	total titrable acidity
UK	United Kingdom
UKH	farm located at Hereford
UV	ultraviolet
VPD	vapour pressure deficit
v/v	volume by volume
WHO	World Health Organisation
w/v	weight by volume
w/w	weight by weight
µl	microlitre
µmol	micromoles
2-MCE	2-mercaptoethanol
ABA	abscisic acid
ANOVA	Analysis of Variance
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f.w.	fresh weight
GLM	General linear model
HCl	hydrochloric acid
HPLC	High Performance Liquid Chromatography
kg	kilogram
kPa	kiloPascal
L	litre
LSD	least significant difference
M	molarity
m	metre

Min	minute
MeOH	methanol
mg	milligram
min	minute
mL	millilitre
mm	millimetre
MW	molecular weight
N	normality
Nm	nanometer
NaCl	sodium chloride
NaOH	sodium hydroxide
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UV	ultraviolet
VPD	vapour pressure deficit
v/v	volume by volume
WHO	World Health Organisation
w/v	weight by volume
w/w	weight by weight

Chapter 1 INTRODUCTION

Strawberry fruits are cultivated and consumed in most places around the world, and are greatly appreciated for their taste and aroma, as well as for their dietary characteristics. Despite their popularity and the need for retailers to supply consumers constantly, strawberries cannot be cultivated all year round in field conditions in any country. For this reason, extensive movement of fruit is observed. UK markets demand strawberries throughout year round. However, locally produced fruits cannot cover demand. The UK is one of the main importers of strawberry fruits globally. Spain is one of the major producers of strawberries globally. The main volumes of strawberry fruits grown in Spain are imported into the UK during winter and spring, when the weather conditions for strawberry cultivation are not favourable in UK. During the summer months and until late October, the demand for fruits in the UK is covered by local production. The gap between late October and the commencement of the Spanish growing season is when the major volume of strawberry fruits is imported from Israel and Egypt into the UK for example. The current research was mainly focused on Spanish production.

Strawberry is a high value, perishable horticultural product. The combination of short shelf-life as well as high production and transportation cost underlines the significance of minimizing postharvest losses and further understanding mechanisms that contribute to determination of postharvest quality of fruits. Strawberry production, overall quality and postharvest losses are highly dependent on growth conditions amongst other factors such as cultivar and cultural practices. Environmental conditions and seasonal climate variability, and their effects on strawberry production are a source of concern for producers, consumers and retailers. According to several climatic scenarios, the temperature of our planet is expected to rise, furthermore other climate variables that can influence physiology of strawberries are expected to be altered. These variables include the number and severity of extreme weather phenomena, levels of precipitation, availability of solar radiation available for plants, evapotranspiration and ozone levels.

In light of the future predicted variability in climate, it is becoming increasingly important to understand the impact of climate on strawberry quality to ensure that supply chains and fruit quality can be maintained. The research presented in this thesis aims to understand and identify the interaction between growing conditions and postharvest quality attributes of strawberry fruit and to develop models that will predict overall quality of strawberries.

SPECIFIC RESEARCH OBJECTIVES

To develop a model to predict the quality of strawberry fruit in terms of growing environment (Chapter 3).

Research was focused on monitoring the changes in levels of key environmental parameters on two Spanish and one UK commercial farm that are expected to change in the future and which also vary through the growing and harvesting season. Temperature, humidity, solar radiation and ozone levels were monitored by data loggers placed in the sites and local weather and climate stations. Furthermore, analyses of quality parameters of strawberry fruits such as colour, firmness, individual sugars and acids, total anthocyanins, size, visual deformities and shelf-life took place, in order to correlate growing environment to postharvest characteristics and to develop models that will predict overall quality of strawberries.

To determine the period prior to harvest during which the fruit quality is most sensitive to environmental conditions, specifically temperature and light (Chapter 4).

For understanding short-term effects of environment on strawberry quality, controlled experiments took place within growth chambers at the Natural Resources Institute (NRI) facilities. The objective of this experiment was to provide information on the critical time period and the contribution of individual environmental variables on strawberry quality. This experiment was also designed as a verification trial for the field findings.

To investigate the effect of growth environment on activity of Pectate Lyase, and how this relates to fruit firmness (Chapter 5).

Research was also focused on further understanding and explaining physiological mechanisms that are responsible for quality deterioration of strawberries. One of the aims of the project was to investigate the action of PEL in relation to firmness of fruits. PEL is an enzyme whose activity is responsible for cell wall breakdown and contributes to loss of strawberry firmness. Further understanding of the action of PEL in response to environmental factors and firmness of fruits was one of the objectives of the undertaken research.

To understand the relationship between fruit firmness and propensity to develop dry bruise (Chapter 6).

A method for simulation of transport stress was used to create conditions that could lead to the development of artificial dry bruising on fruits. Dry bruise is a significant problem contributing

to quality deterioration of strawberries. The main objective of this part of the research was to prove initial findings which related increased amount of dry bruise on fruits at the end of the season to reduced firmness of fruits and vice versa ability of fruits to withstand dry bruise development at the beginning of the season due to increased firmness.

To determine how consumer acceptance of strawberry fruit changes with harvesting season, and to understand the main quality factors that contribute to acceptance (Chapter 7).

Taste panels to assess consumer acceptability and sensory evaluation were undertaken. Quality of strawberry fruits is changeable through season and alterations of environmental conditions are one of the main reasons for the variability of quality. The objective of this part of the research was to evaluate consumer acceptance of strawberries harvested at different points across the harvesting season. Results of this research can help growers and retailers understand consumer behaviour and satisfy their need for better quality of fruit when environmental conditions are not favourable, by the adoption of appropriate cultivars and cultural practices. An additional objective of the study was to provide information acquired by sensory evaluation and relate these to instrumental readings to gain an enhanced understanding on evaluation of strawberry quality attributes.

Developing a model that will be able to predict postharvest quality characteristics depending on available data of growing conditions is of great importance for consumers, retailers and producers. Strawberries are shipped from farms in apparently perfect condition, as they pass quality control procedures, however they develop problems when they arrive at packhouses and/or markets. For instance, it is quite often that consignments are rejected because of high levels of bruised fruits or the presence of waste fruits. These fruits are sold as second class fruits in wholesale markets or have to be reselected. The above practices lead to the loss of profit for growers and increased cost for retailers. The repeated handling of fruits also further reduces the potential shelf life. Identification of parameters that positively or negatively affect strawberries will help in waste reduction and increased consumer satisfaction. Furthermore cost reduction as well as increase of profit for producers and retailers can be achieved through the selection of appropriate growing and commercial strategies, like selection of proximate markets when conditions are favourable for the development of quality problems and reduced shelf-life, or harvesting of higher quality product when weather conditions are favourable for production of strawberries of better quality. The current research is expected to highlight effects of environmental variables and identify possible effects of climate variability on strawberry quality.

Chapter 2 LITERATURE REVIEW

2.1. IMPORTANCE OF STRAWBERRY PLANT

2.1.1. Economic importance of strawberries

Strawberries are highly important both for their contribution to agricultural income and the dietary and nutritional characteristics. Spain is the fourth largest strawberry producing country (Table 2.1), with the UK in 12th place (FAO, 2014).

Table 2.1: World strawberry production for 2012 (FAO, 2014)

Rank	Country	Production Value (,000)$	Production (MT)
1	USA	1855196	1366850
2	MEXICO	489198	360426
3	TURKEY	479354	353173
4	SPAIN	393475	289900
5	EGYPT	328864	242297
6	REP OF KOREA	260787	192140
7	JAPAN	251096	185000
8	RUSSIA	236166	236166
9	GERMANY	211502	155828
10	POLAND	203796	150151
11	MOROCCO	189588	139683
12	UK	129891	95700

The UK continues to increase its strawberry imports (Table 2.2) both in economic value and quantity. Spain is the leading export country (Table 2.3) into the UK (FAO, 2014). In 2011, the area cultivated with strawberries in Spain was 6,896 ha and in 2012, 7,600 ha, while in the same year in UK 4,650 ha were harvested. Huelva, Spain has been considered for a long period the major strawberry production area in Spain due to its favourable climate conditions (Francisco Gonzalez per.com., 2011) with a regional production of 250,000 mt/annum. In 2010, despite a

reduction in total cultivated area, the total production was still around 250,000 MT (Francisco Gonzalez per. com., 2011). The average strawberry price per tonne in 2012 was $3,021 in UK and $1,428 in Spain (FAO, 2014). It should be mentioned that on average, the year to year increase of berry sales in UK supermarkets has been around 20% up to 2011. Local UK producers cover 85% of the total demand from May to October, with most (~80%) grown under polytunnels.

'Candonga' represents 25% of total strawberry plantation in Huelva region. It is estimated that from 400 million plants cultivated in Huelva region 100 million plants belong to Candonga cv. (Francisco Gonzalez per. com., 2011). Elsinore is a very promising cultivar according to UK growers (Vogels per. com. 2010) with a balanced taste, sugar and acid ratio, increased production over the cropping season.

Table 2.2 : UK strawberry imports (FAO, 2014)

Rank	**Year**	**Imports (,000 US $)**	**Imports (MT)**
5	2011	192,559	47,077
5	2010	146,087	38,057
4	2009	158,494	39,254
4	2008	193,315	44,894
4	2007	185,462	66,589
4	2006	168,959	47,823
4	2005	155,287	46,794
4	2004	138,621	39,946
4	2003	105,214	35,900
3	2002	108,502	36,657
4	2001	65,234	28,493
4	2000	61,539	29,047

Table 2.3: Spanish strawberry exports (FAO, 2014)

Rank	Year	Exports (,000 US$)	Exports (MT)
1	2011	621,831	231,732
1	2010	526,131	360,204
1	2009	526,001	224,618
1	2008	503,849	188,042
1	2007	423,968	186,377
1	2006	397,074	207,974
1	2005	423,931	216,641
1	2004	417,357	226,821
1	2003	329,274	212,327
1	2002	284,236	184,668
1	2001	256,771	212,081
1	2000	213,391	195,336

2.1.2. Strawberry origin and domestication

The strawberry fruit belongs to the genera of berries of the family Rosaceae, genus Fragaria. The species are distributed worldwide with *Fragaria vesca* being the most common. *Fragaria x ananassa* is the most important cultivated strawberry (Njuguna *et al.*, 2013). *Fragaria x ananassa* is a hybrid between the two American octoploid species, *F. chiloensis* from South America and *F. virginiana* from Eastern America (Hancock *et al.*, 2010).

Strawberries have been domesticated over the last two thousand years. There is evidence that *F. vesca* were cultivated by ancient Greeks and Romans. It is also known that Chileans were using strawberry fruits of *F. chiloensis* (Hancock *et al.*, 1999). During the Middle Ages, Europeans were planting strawberries also. Clones of *F. virginiana* did not migrate into Europe until the sixteenth century. During the last centuries, distinct cultivars for different regions appeared.

Many other cultivars and varieties were developed in order to adapt to different climatic conditions (Darrow, 1966).

2.1.2. Anatomy and morphology of strawberry

Strawberry can be characterized as a ¨false¨ fruit formed by simultaneously ripening ovaries all belonging to the same flower (Manning, 1993). The true fruit of the strawberry is the seed, called “achenes”, and the swollen receptacle composes the berry. Strawberry is a herbaceous perennial plant (Fig. 2.1). It has a central compressed stem known as the crown. Leaves, runners, roots and inflorescences emerge from the crown which consists of a central core surrounded by a vascular ring. The core consists of pith with a cambial layer around it. At the base of each leaf a bud exists which can produce runners, branches, crowns or remain dormant. Leaves follow a spiral arrangement around the stem. Each leaf consists of three leaflets. A strawberry plant develops the main part of its roots at a depth of 15 cm, therefore it is considered a shallow rooted plant. Roots emerge from the crown and a have a typical anatomy of dicotyledons. Runners or stolons in most species consist of two nodes. The first produces a daughter plant and the second remains in a dormant stage, or develops another runner (Galletta and Himelrick, 1990).

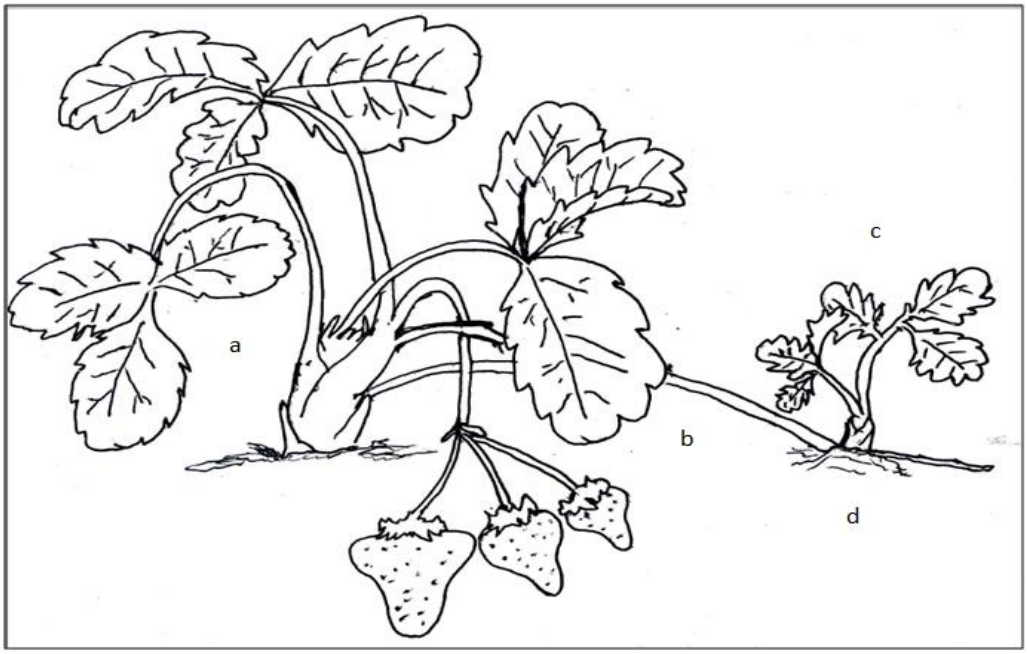

Figure 2.1: Plant habit: a) crown and leaf bases, b) stolon (runner), c) daughter plant, d) secondary runner. Figure adapted from Darrow (1966).

2.1.3. Fruit growth, development, maturation and ripening

Strawberry maturation stages are generally classified according to colour development of the fruit and size. The colour of strawberry fruits starts from green at the immature stage, proceeds to white, gradually turning pink, red, and finally purple red. Generally, there is no clear distinction between stages of maturity, however according to literature (Ferreyra *et al.*, 2007) there are four to five main stages with the most distinctive being small green (SG), large green (LG), white (W) and red (R). In commercial farms strawberries are harvested at late pink-early red stage where they fulfill minimum quality requirements for consumers and retailers, and are quite resistant to withstanding transport stress. Depending on climate conditions, around 30 days are required to reach the fully ripe stage from fruit flowering. The development of colour occurs during the last 5-9 days (Palencia and Martínez, 2013).

It was proposed that the strawberry growth pattern follows a double sigmoid model (Perkins-Veazie and Huber, 1988; De la Fuente *et al.*, 2006). There is a first stage of accelerated growth from zero to six days after flowering, mainly attributed to receptacle growth. Afterwards, a lag period is observed between twelve and sixteen days after flowering. During this phase the embryo develops. The second growth period is characterized by receptacle ripening. Around 21 days after anthesis are required for the fruit to reach white stage and 5 to 10 from white stage to fully mature stage. In total 30 - 40 days are required for the fruits to reach maturity. Single sigmoid growth patterns have however also been reported, depending on the growth measurement method and cultivar (Perkins-Veazie and Huber, 1988).

Strawberry fruits act as a sink for carbohydrates. That is, assimilates from the plant are transported to the fruit and seem to be directed mainly to receptacle tissue. Green fruits have the ability to photosynthesize although the impact of fruit photosynthesis on carbohydrate accumulation is not clear yet. Total sugars increase through ripening and starch is completely degraded after 21 days of petal fall (Perkins-Veazie and Huber, 1988). It was observed that auxins produced by achenes play a very important role in fruit growth (Nitsch, 1955). Fruits that had their achenes removed stopped growth. When β-naphthoxyacetic acid was applied to fruits, growth resumed. Removal of achenes from strawberry fruits also stimulated higher rates of anthocyanin development. It was also noticed that the application of synthetic auxins on the de-achened fruits delayed ripening as defined by loss of firmness, anthocyanin accumulation and loss of chlorophyll. Generally, it is accepted that during growth, auxins produced in high levels stimulate fruit development. A reduction of auxins levels at later stages triggers ripening (Given *et al*., 1988; Manning, 1994; Aharoni *et al*., 2002). Gibberellin, cytokinin and abscisic acid are

thought to have a much less important role in strawberry fruit growth compared to auxin (Perkins-Veazie and Huber, 1988). Achenes have an important effect on size of fruits among other parameters such as the position of fruit on the plant, or order of fruit set. Primary fruits are often larger in size (Moore *et al.*, 1970). Auxin formation is dependent on YUC genes related to tryptophan pathway (Liu *et al.*, 2012).

Strawberry is classified as a non-climacteric fruit. Fruits are characterized as climacteric or non-climacteric according to ethylene production at different stages of development. Climacteric fruits such as tomato, banana and apple are characterized with increased ethylene production during their climacteric stage of ripening, whereas non-climacteric fruits do not. In climacteric fruits there are three stages pro-climacteric, climacteric and post climacteric. The higher production of ethylene which takes place during the climacteric phase is considered an autocatalytic reaction, since ethylene besides being a product acts also a catalyst (Atta-Aly *et al.*, 2000). In strawberry the production of ethylene does not follow that model (Barry and Giovannoni, 2007). Strawberry maturation proceeds regardless of ethylene production. However low amounts of ethylene and carbon dioxide have been detected during ripening - a characteristic of climacteric fruits (Iannetta *et al.*, 2006). Furthermore, reducing ethylene levels in strawberry punnets was found to prolong postharvest shelf-life of fruits (Wills and Kim, 1995). Methyl jasmonate (MJ) with its inhibiting role was also found to increase the formation of ethylene with increasing doses (Mukkun and Singh, 2009).

Strawberry fruit ripening is a complex phenomenon that includes many steps. Strawberry ripening involves processes that are connected to characteristics desirable for consumers such as taste, aroma and texture (Martínez *et al.*, 2004). Sugar levels increase during ripening, as starch is converted to sugar. In addition, the formation of polyphenols, antioxidants, flavour, aroma compounds and volatiles occur (Giovannoni, 2001), and cell walls modify leading to softening of fruits (Heng Koh and Melton, 2002; Bianco *et al*., 2009). During strawberry maturation and ripening, the action of hormones such as auxins associated with fruit size increase, and enzymes such as PELs and pectin methylesterases related to fruit softening, are expressed (Perkins-Veazie and Huber, 1988; Kafkas *et al.*, 2007; Draye and Van Cutsem, 2008).

Strawberry senescence leads to deterioration of fruit quality characteristics as well as reduced inherent disease resistance. Normally fruits are less susceptible to grey mould between flowering and mature stage and more susceptible to infections during senescence (Terry *et al.*, 2004).

2.2. LONG AND SHORT TERM VARIABILITY OF ENVIRONMENTAL CONDITIONS

2.2.1. Importance of climate variability

Short-term weather changes including the short term of variability of environmental conditions affect human activities and life on planet. Environmental conditions can vary significantly on Earth through seasons and most forms of life have managed to adapt to this variability. However, a severe change in climatic patterns expected to take place in the near future can increase the incidence of severe short-term weather changes (climatic variability) and long term alteration of climate on regions of planet. Climate change has been defined by the United Nations (2013) as "*a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods*". Long term predictions of climate change are very useful and many models including several parameters have been developed.

2.2.2. Temperature

Mean global temperature, despite fluctuations, is expected to rise throughout the planet according to the majority of predictions (Fig. 2.2). Wine grapes have been used as an indicator plant in order to monitor the impact of temperature alterations since they are considered plants that show great sensitivity to temperature variations. This given, temperature increases have led to better quality wines in areas of France and the US given the now more favourable conditions (Parry, 2007).

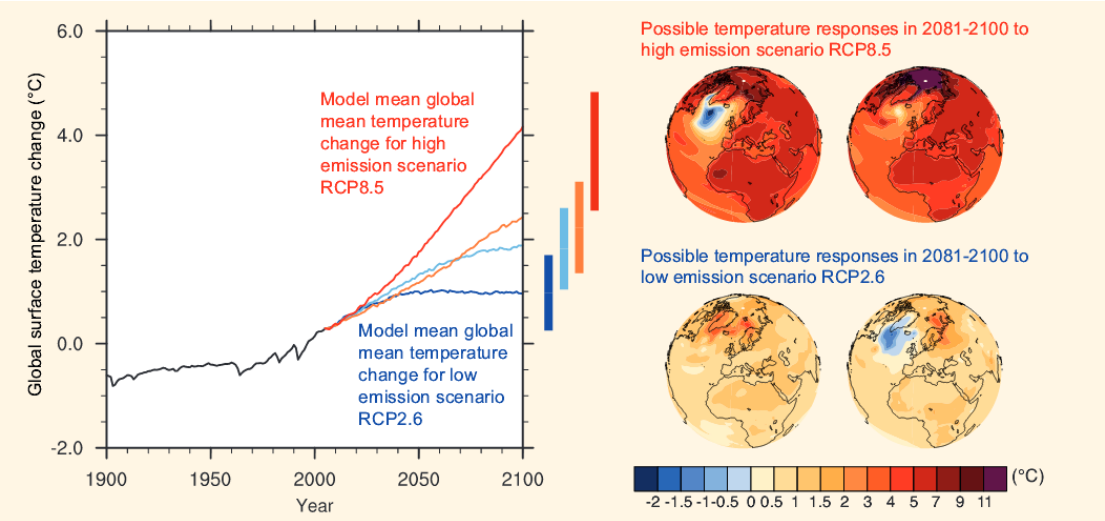

Figure 2.1 : Temperature increase as projected by the IPCC Working Group for scenarios by *Collins et al.,* (2013).

It is expected that in Europe southern countries will have to face mainly negative effects due to the predicted increase of temperature, whereas northern countries could have both negative and beneficial effects (Iglesias *et al.*, 2011). There have been models developed aimed at predicting temperature elevation and implications on plant growth cycles. A temperature impact model suggested it is possible that dates of bloom and maturity for apples in general will be affected (Warrick *et al.*, 2001). There will be impacts of rising temperature on total production and yields of crops such as strawberries. Higher growing temperatures, and solar irradiation levels may lead to earlier production of strawberries (Palencia *et al.*, 2008). Farmers may also face increased costs in order to fight against increased and/or new pests and diseases. Models that were developed to estimate epidemics of Downy mildew indicated that in future, up to two more sprays maybe required (Salinari *et al.*, 2007). Due to elevated temperatures, evapotranspiration of plants might be enhanced, resulting in greater demands for water supply (Rosenzweig *et al.*, 2004).

2.2.3. Water availability

According to different predictions, rainfall is expected to increase in some areas, whilst decreasing in others (Sivakumar *et al.*, 2005). Changing of precipitation levels due to climate change is a factor that creates great concern for the future of agricultural production. Scientists have again attempted to define the level of future precipitation by developing models (Fowler and Ekström, 2009) - not only the quantity of rainfall, but also the quality. Even if the total amount of water that soil receives during the year does not vary significantly, problems may occur due to unbalanced occurrences of rainfall. Drought, followed by extreme precipitation may have catastrophic effects for farmers, since, apart from destroying plant production, erosion could also take place, resulting in deterioration of agricultural land (Scholz *et al.*, 2008). Pest and diseases are also influenced by alterations of rain patterns. In addition, elevated humidity may increase the amount of pathogens that occur in an area and may prove a significant factor for diminishing production and crop quality. Precipitation levels may vary from place to place. In some areas, floods, due to increased rainfalls could take place, as some models predict (Sivakumar *et al.*, 2005).

2.2.4. Ozone (O_3)

It is suggested that changing ozone (O_3) levels are due to human activities. It is expected that ozone concentrations in the troposphere will increase (Fuhrer, 2009; Melkonyan and Wagner, 2013). As warmer conditions occur during summer it is expected that ozone levels near Earth's

surface will also increase. This effect will be enhanced by other conditions predicted to take place more frequently, such as less cloudiness and reduced wind presence. Models predict higher levels of ozone above the European continent in the future (Meleux *et al.*, 2007). A strong increase in surface ozone expressed as the mean of daily maximum over southern and central Europe and a decrease in northern Europe is also predicted by other models (Langner *et al.*, 2005). However, it should be noted that there is no extended analysis on the economic impact of ozone in areas of agriculture or agroforestry (Reilly *et al.*, 2007).

2.2.5. Irradiation

Despite increases in tropospheric (layer near Earth's surface max altitude 8-15 km) ozone, human activity has led to a decrease in stratospheric (layer after troposphere maximum altitude 60 Km) ozone. Reduced concentrations of the ozone layer have resulted in increased levels of UV-B (280-315 nm) radiation intensities. The levels of UV-B have reached a peak, although there are estimates that this will remain high for several decades (Mira de Orduña, 2010). The higher the percentage of cloud cover, the less UV radiation is available for plants (Parisi *et al.*, 2010). Data show that levels of UV radiation reaching ground surface have generally increased with a geographic pattern following the observed reductions in stratospheric ozone.

2.2.6. Increase of extreme climate incidents

Over the last few decades several extreme climate events have taken place. Houghton and Firor (1995) emphasizes that changes in climate do occur. However, only the extreme situations are particularly noticeable by ordinary people who do not monitor climate in detail. There are numerous events that can be characterized as extremes that have been reported throughout the globe, most of which have led to populations facing great catastrophes in terms of loss of life and damage to property. If predictions of a rapid and severe climate change can be made, society will be better placed to mitigate against it (Iglesias *et al.*, 2011). In the UK there are estimations of models for warmer summers with the existence of higher temperatures being more frequent and a reduction of very cold winters. In the UK over the past 50 years there has been a decrease in the number of spring frost events (Sunley *et al.*, 2006). However, for precipitation, there is conflicting evidence. Models, predict a variation of -10% up to an increase of 30% in precipitation over summer period (Wilby *et al.*, 2006). There are also estimations for higher ozone and UV levels which are also not clear. It should be mentioned that precipitation and amount of cloudiness are strongly correlated with ozone and radiation levels (Langner *et al.*,

2005). Increased levels of clouds are associated with lower ozone levels (Niatthijsen and Builtjes, 1997).

Spain is an area that has only 40% of its land suitable for agricultural activities. There is great concern about climate change consequences within the Spanish agricultural sector. From existing models, there are estimations of a mean temperature rise, decreased precipitation, and development of favourable conditions for increased UV and ozone levels (Olesen and Bindi, 2002; Iglesias *et al.*, 2010). Of sixteen different scenarios predicting future levels of temperature and precipitation between present and 2071-2100, fifteen suggest a reduction of precipitation levels, and all estimate a temperature increase of between 1.5°C - 5.8°C.

2.3. EFFECTS OF ENVIRONMENTAL VARIABLES ON GROWTH & DEVELOPMENT OF STRAWBERRY FRUIT

2.3.1. Photosynthesis, transpiration and plant growth

Plant growth is significantly affected by different temperatures (Fig. 2.3). Photosynthesis and respiration show a nonlinear relationship as temperature levels are increased. Both physiological functions increase until they reach an optimum, and above that decrease (Porter and Semenov, 2005). Similar results were also observed for strawberry plants grown in plastic tunnels where the optimum temperature for leaf photosynthesis was 26-34°C (Carlen *et al.*, 2009). Increased levels of ozone had an effect on plant photosynthetic rate. The direct effect of increased ozone levels (78ppb $\approx$ 156 $\mu g/m^3$) was not significant; however, strawberry plant cultivars (cvs.) Korona and Elsanta showed a decrease of net photosynthesis. Indirect effects of ozone on photosynthesis were more significant.

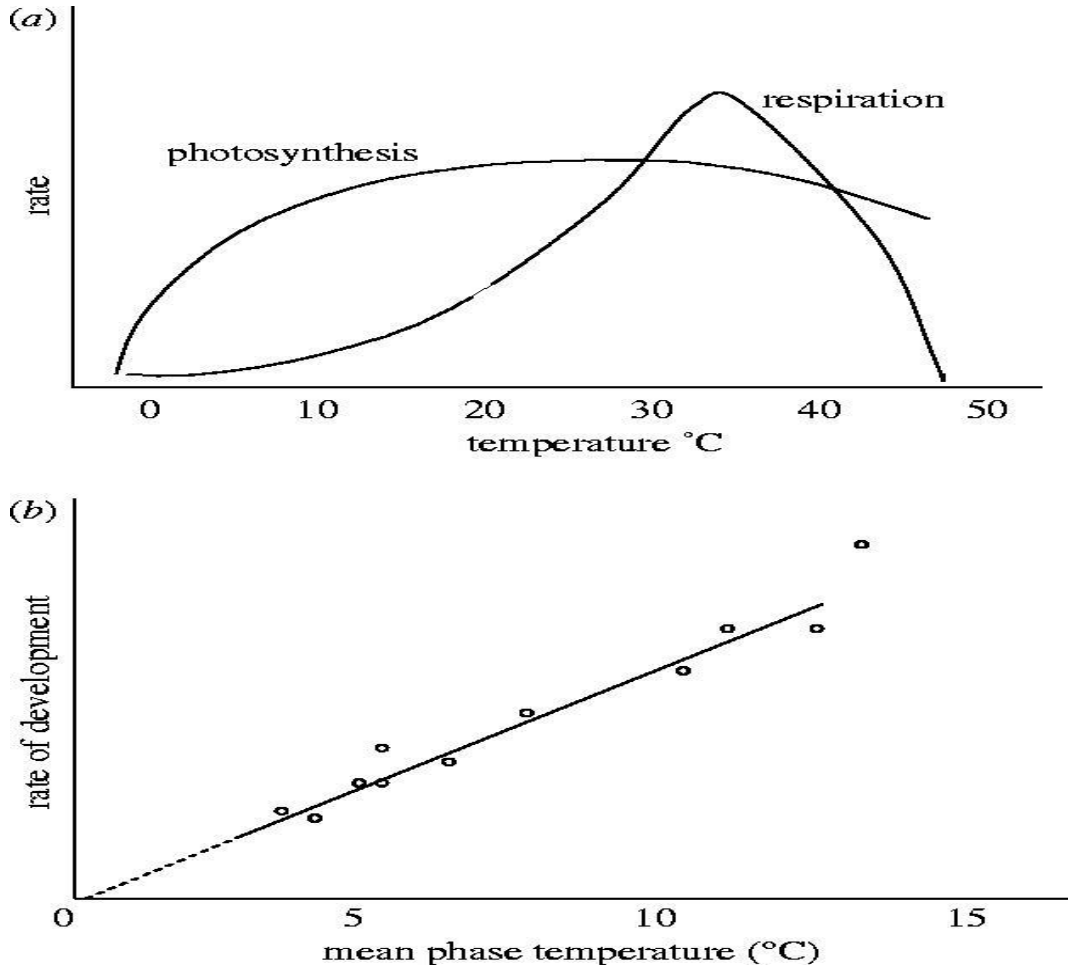

Figure 2.2: Changes in the rate of (a) C3 photosynthesis and respiration and (b) rate of crop development as a function of temperature as cited by Porter and Semenov (2005).

Plants showed lower activity of Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase, an enzyme important in carbon fixation) and decreased chlorophyll content in older leaves. Furthermore, total leaf area was reduced. Different cultivars show different responses. Cultivar Korona was less affected by increased ozone levels (Drogoudi and Ashmore, 2000; Keutgen and Pawelzik, 2008). The need for multi-factorial, open-air experiments that will provide further information on ozone tolerance effects on plants was also discussed, along with the effect of a combination of parameters (ozone, vapour pressure deficit and carbon dioxide) on plants. Generally a threshold for ozone is set at 40ppb, below that no damage to plants was observed (Harmens *et al.*, 2007). Strawberry was considered a rather neutral plant with regards to effects of ozone (Keutgen *et al.*, 2005). There is some evidence UV-radiation has a significant negative effect on Photosystem II and RuBP. Damage that follows this is a reduction in formation of photosynthetic and accessory pigments, modification in canopy morphology and function of

stomata, thus causing inhibition in photosynthetic carbon assimilation (Teramura and Sullivan, 1994).

The impacts of increased CO_2 on plants are already known. Enrichment of glasshouse with CO_2 is a common practice for increasing productivity of plants including tomatoes, cucumbers and strawberries (Enoch *et al.*, 1976; Besford *et al.*, 1990). Elevated carbon dioxide levels are known to reduce stomatal conductance as well as transpiration. Improved water use efficiency, higher rates of photosynthesis and increased light-use efficiency were also reported. Generally, long-term exposure to elevated CO_2 reduces enzymes of the photosynthetic carbon reduction cycle, resulting in increased nutrient use efficiency (Drake *et al.*, 1997).

Imposed water stress on strawberry plants, showed that photosynthesis was less affected by drought or flood conditions compared to transpiration (Blanke and Cooke, 2004). However, a reduction of leaf photosynthesis was observed after four days of water stress in the form of drought and flood conditions.

2.3.2. Dormancy, photoperiod and flowering

The exact moment of dormancy induction in strawberries is affected by temperature and day length (Robert *et al.*, 1999). Increased temperature can prevent dormancy break, however, longer day length combined with elevated temperatures has an effect on strawberry cultivation and dormancy. Sønsteby and Heide (2006) observed that plants of cvs. Elsanta and Korona maintained their ability to grow under short day periods when grown in elevated temperatures. According to climate change scenarios increased temperatures for strawberry production in Spain indicated a reduction in crop cycle duration. Furthermore, a correlation between elevated temperature and early production was noticed (Palencia *et al.*, 2008). These observations indicate that some areas may benefit from climate change whilst other areas currently cropped for berries might be under stress.

Ozone was found to play a role in reproductive growth of strawberry plants. Elevated ozone levels (92 ppb) accelerated inflorescence at the beginning of application, but later reduced inflorescence number and fruit set (Drogoudi and Ashmore 2000; 2001). This response could be mainly explained by reduction in photosynthesis and not by effects of ozone on carbon partitioning.

2.3.3. Root development

Several studies across cultivars have shown that for strawberry cultivars the optimum temperature for root growth was 12.8 °C (Fig. 2.4). The fact that lower night temperatures 12°C are more favourable for root development compared to higher night temperatures (18°C) was also verified by experiments with cultivars "Kent" and "Earlygrow" (Wang and Camp, 2000). Indirect manipulation of root temperature by application of different mulching materials was also found to increase root growth. Hay and black polyethylene increased minimum soil temperature between 0.4°C and 2.4°C and decreased maximum between 2.7°C and 5.8°C (Kumar and Dey, 2011). Changes in day temperature of roots can have a negative effect on their dry mass. When roots were developed in a range between 10°C to 30°C and mean temperature 20°C had reduced dry mass of roots by 30% when compared to roots grown under constant temperature at 20°C (Gonzalez-Fuentes *et al.*, 2016).

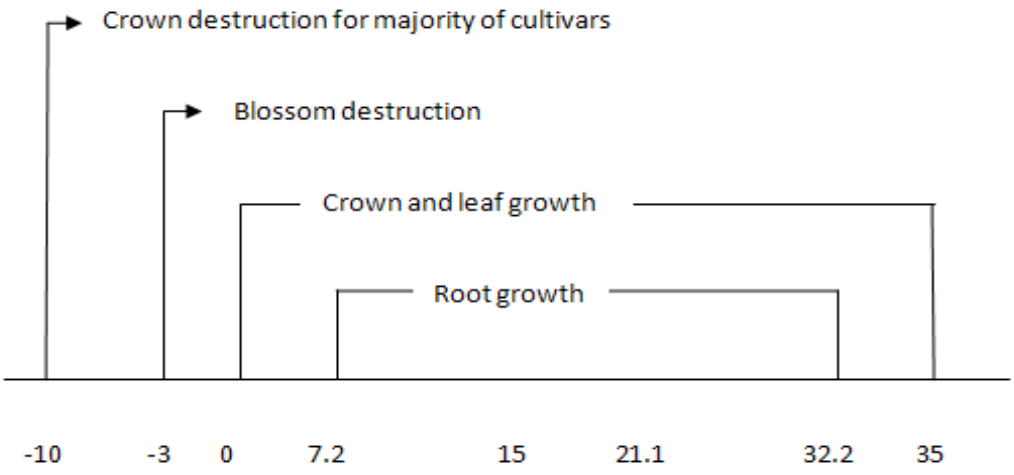

Figure 2.3: Key temperature (°C) ranges affecting strawberry plants during production. Based on data provided by Darrow 1966.

Elevated ozone levels were found to have an effect on root growth. Increased levels of ozone played a negative role on development of root systems as was observed on two wild cultivars of Finish strawberries (Manninen and Siivonen, 2003). However these cultivars were found to have different grades of ozone sensitivity. Water quality and availability play an important role for root development of strawberry plants (Saied *et al.*, 2005). Plants that were subjected to irrigation with increased NaCl levels showed a reduction in root development. Shoot/root ratio did not show a constant trend. During the first year of experiments it was greater than one. However, during the second year, roots constituted a higher proportion compared to shoots

developed during growth, resulting a shoot /root ratio of less than one. The fact that in high salinity environments plants tend to decrease shoot /root was also highlighted in experiments on raspberry plants (Neocleous and Vasilakakis, 2007).

2.3.4. Pests and diseases

Strawberry is considered to be the most vulnerable crop among soft fruits. It is expected that due to altered climate conditions, new pests will arrive and pose a serious threat for strawberry production. Pests such as the vine weevil and Spanish slug are anticipated to cause potential challenges in terms of plant protection (Tuovinen, 2008). Other regions are also anticipated to face problems. For example, it is estimated that there will be an increase in diseases such as powdery mildew, *Phytophthora* species and bacterial pathogens in Finland's strawberry fields, due to temperature increase and precipitation fluctuations (Parikka and Lemmetty, 2008). These changes could affect strawberry production and outweigh the possible effect of growing season elongation. There is also evidence that warmer conditions that may occur in some areas will facilitate the spreading of *Verticillium* wilt. When conditions of the warm summer of 2004 were simulated, an increase in *Verticillium* activity was noticed in plants of strawberry *cv.* Elsanta (Schubert *et al.*, 2008). The risk of migrating new pest in northern areas is also visible. Below, Table 2.4 provides an overview of the main impacts of increasing/decreasing environmental variables on strawberry fruit. Grey mould a pathogen (*Botrytis cinerea*) developed under high humidity and temperature conditions, is expected to require more sophisticated management in order to maintain cost of pesticide applications at low level. Therefore, the development of models for disease prediction and control is very useful (Skevas *et al.*, 2016).

2.3.5. Models for prediction of strawberry quality in relation to environmental factors

Ability to predict quality of crops and more specific strawberries is of major importance in order to decrease postharvest waste, improve quality and decrease consumer acceptability problems. Models that have been developed in the past included regression models, known as black-box, empirical or statistical models. Such models examine relation between input and output, without taking into account physiological processes. Another group are the mechanistic or explanatory models (Heuvelink *et al.*, 2003). Examples of models developed include prediction of keeping quality of strawberries in relation to precursors levels (proanthocyanins). The model estimated the infection of Botrytis levels at postharvest storage in relation to precursors levels. It is known that precursors levels are affected by environmental conditions in various ways as it was discussed, however a more detailed model linking directly preharvest factors and postharvest

quality parameters for strawberries is missing (Schouten *et al.*, 2002). Other model developed linked tomato firmness to the number of days fruits stayed on the plant and found that tomatoes became softer as they spend more time on plants, probably due to action of pectin degrading enzymes (Lana *et al.,* 2005). Models developed for strawberries evaluated the effect of genotype and environmental factors on quality characteristics of strawberries and found a strong relationship between quality and preharvest factors and aroma characteristics (Samykanno, 2012).

Table 2.4: Main effects of preharvest climatic parameters on strawberries

	Levels increase	Effect	Reference
Ozone			
		↓ Photosynthesis	Drogoudi and Ashmore 2000; Keutgen *et al*. 2005
	↑	↓ Inflorescence & fruit set	
		- Yield, size, antioxidant capacity, secondary products (Anthocyanins, phenolics)	Drogoudi and Ashmore, 2000; 2001
Temperature		↑ Photosynthesis	Carlen *et al*., 2009; Porter and Semenov, 2005
		↑ Early production	As above
	↑	- Increased harvest period, earlier flowering	As above
		↑ Pests and diseases	Schubert *et al*,. 2008; Parikka and Lemmetty 2008; Tuovinen 2008
		↑ Flavonoids	Wang and Zheng, 2001
Humidity		↓Photosynthesis	Blanke and Cooke, 2004
	↑	↑Pests and diseases	Parikka and Lemmetty, 2008
		↓Firmness	Lieten, 2000
UV		↑Secondary products (Anthocyanins, phenolics)	Tsormpatsidis *et al*., 2008
	↑	↑Firmness	Ordidge *et al*., 2012
Irradiation		↑ Fruit damage	Rose *et al.* 1934; Sams, 1999
	↑	↓Firmness	
		↑Ascorbic acid, Ellagic acid	Atkinson *et al.*, 2006
CO_2		↑Photosynthesis	Besford *et al.* 1990; Enoch *et al.*, 1976
		- Anthocyanins	Wang *et al*. 2003; Moretti *et al*. 2010
	↑	- Phenolics	As above
		↑Ascorbic acid	As above

Symbols: ↓decrease; ↑ increase; - no change

2.4. POSTHARVEST QUALITY OF STRAWBERRY FRUITS

2.4.1. Strawberry quality aspects

Quality is 'a term frequently used but rarely defined' (Shewfelt, 1999). Strawberry quality is based on several characteristics. These characteristics can be intrinsic or extrinsic. Features such as colour, shape and size as well as absence of defects can be categorized as intrinsic attributes and they are connected to external characteristics of the fruit. Internal characteristics of the fruit such as sweetness, acid composition, texture, flavour, nutritional value and shelf-life are also characterized as intrinsic. Extrinsic characteristics of quality include production system (conventional, integrated, organic, soilless etc.), sustainability of the production and distribution system, environmental impact etc. (Kidd, 2010). Some of the above parameters can be accurately measured objectively (sugars, acids, weight) whilst some others can be evaluated, but not necessarily quantified. For instance firmness can be quantified when a certain technique is used, but not the overall experience of texture that the consumer feels during consumption of strawberries. However, objective scales and measurements can be used to evaluate quality characteristics like taste panels and sensory evaluation of products (Gruda, 2005).

The quality as perceived by consumers, retailers, and producers can vary in definition. Producers are mainly interested in strawberry cultivars that can meet market demands, and more importantly increase yield, and thus profit for them. Product oriented quality is based on the needs of distributors and retailers. Therefore, appearance and postharvest handling characteristics are mainly appreciated. Parameters that are product – oriented include absence of visual deformities, colour and shelf-life (Shewfelt, 1999; Kidd, 2010). Main breeding programmes in the past were focused on improving quality in terms of yield and robustness of fruit, whilst parameters desirable by consumers were set aside. Increasingly, the need to understand and satisfy consumer expectation from products has led to focus also on consumer oriented quality. Fresh produce is considered by supermarkets as a destination category. This means that consumers may change store if they are not satisfied by the quality of the products or if they can find better quality elsewhere (Kidd, 2010). Consumer-oriented quality apart from visual and shelf-life characteristics is also focused on taste, texture and other internal characteristics as well as extrinsic characteristics such as environmental impact and sustainability of production system. However, consumers have great variability when it concerns their needs, demands and preferences (Shewfelt, 1999). Harvesting time is critical for optimizing quality characteristics and maintaining acceptable shelf-life. The key aim of the producers is to

maximize the volume of production and meet retailers' specifications. Harvesting time should take place at a point where maximum fruit size is achieved and stage of maturity satisfies market specifications. Furthermore, harvesting should take in account an appropriate shelf-life in order to maintain acceptable edible characteristics when the product reaches the consumer. The fact that strawberries cover long distances from farm to final consumer, and are exposed to stress during transportation underlines the necessity of an accurate quality prediction model.

2.4.2. Texture and Firmness

Numerous processes take place during strawberry ripening. The final outcome is a fruit that is desirable by consumers since it provides them with valuable nutrients as well as exceptional taste. Strawberry ripening is a mechanism that turns fruits more attractive to animals in order to increase seed dispersal. Accumulation of antioxidants, colorants and sugars is accompanied by loss of textural firmness (Fry, 2004). In strawberry, softening is accompanied by simultaneously growth of fruits and it is mainly an effect of cell wall disassembly (Goulao and Oliveira, 2008). Cell swelling is observed. This observation is common in fruits with soft melting textures like strawberry and avocado and it comes in contrast with other fruits such as apples, that have a crisp texture at the ripening stage with cell swelling absent (Redgwell *et al.*, 1997). Parts that undergo major structural changes and are connected to softening of fruits are the primary cell wall and middle lamella (Brummell, 2006; Payasi *et al.*, 2009). Various biochemical mechanisms contribute to softening of strawberry fruits such as loss of turgor, enzymatic (Table 2.5) and non-enzymatic processes such as loss of pectin stabilization by Ca^{2+} (Medina-Escobar *et al.*, 1997).

Turgor changes have been related to loss of firmness. It has been shown that changes in hydrostatic pressure of tomatoes were observed during their ripening. The reduction of cell turgor could possibly be attributed to fruit water loss and increased levels of osmotic solutes in the apoplast (Shackel *et al.*, 1991). Furthermore, cell expansion has been observed in raspberries during maturation and it is thought to be related to their softening (Sexton and Palmer, 1997).

Plant primary cell wall is the outer wall layer. Plant cell wall can be divided in two distinctive categories. Strawberries belong to type I category as most flowering plants, unlike category II which include monocotyledonous plants like barley and rice. (Yokoyama and Nishitani, 2004). Type I primary cell walls are composed of approximately a 50% cellulose-xyloglucan framework, followed by pectic polysaccharides (approximately 30%) (Figure 2.5). Structural proteins are the third major component of primary cell wall. The main difference between type I

and II primary cell walls is that in type I xyloglucans are the major components, instead in type II it is replaced by glucuronoarabinoxylans (Carpita and Gibeaut, 1993).

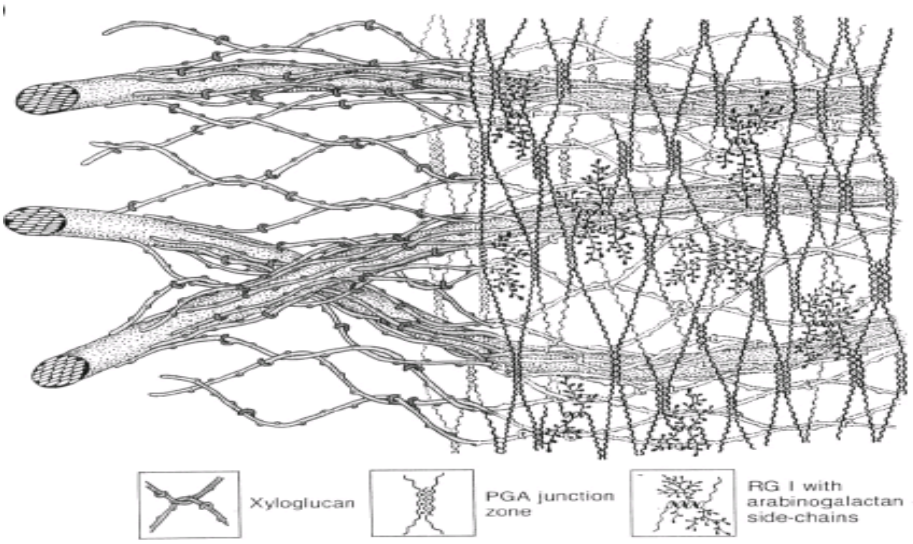

Figure 2.4: Type I primary cell wall (Carpita and Gibeaut, 1993).

The middle lamella is the second layer of cell wall and its degradation is also associated with softening of strawberries. Whereas the primary cell wall contains homogalacturonan, ramnogalacturonan I and II as well as well as cellulose, in the middle lamella only homogalacturonan and structural proteins exist. Cleavage of homogalacturonan can occur by the action of PEL and endo-polygalacturonase (Brummell, 2006).

Enzymes that are thought to contribute to strawberry softening are pectin methylesterases (PMEs) that are mainly active in early ripening stages and prepare plant cell wall for the catalytic action of PGs and PELs (Draye and Van Cutsem, 2008). Their action mechanism is de-esterification of pectin. PGs were also related to softening of strawberries since their decreased activity was related to firmer fruits (Lefever *et al.*, 2004). Other enzymes connected to strawberry are B-galactosidase since they are responsible for loss of galactan (Trainotti *et al.*, 2001). Another component of pectin is arabinan and its structure can by modified by the enzyme α-l-arabinofuranosidase (Carpita and Gibeaut, 1993; Rosli *et al.*, 2009). Endo-β-1,4-glucanases (EGases) can also act on xyloglucan, cellulose and glucomannan at plant cell wall (Harpster *et al.*, 1998; Brummell and Harpster, 2001). β-xyloxidase action can also be responsible for plant cell well disassembly (Martínez *et al.*, 2004; Bustamante *et al.*, 2006) . Breaking down of mannans by exo-manasses (Bourgault *et al.*, 2001) as well as that of xyloglucan by Xyloglucan endotransglucosylase (XTH) are processes related to strawberry loss of firmness (Fry, 2004).

Table 2.5 Enzymes contributing to fruit softening.

Enzyme	Action mechanism	Reference
PELs,	Cleavage of polygalacturonic acid	(Medina-Escobar *et al.*, 1997; Benitez-Burraco, 2003; Figueroa *et al.*, 2008; Wang *et al.*, 2014)
polygalacturonases (PGs)	hydrolytic activity and depolymerisation of pectin	(Lefever *et al.*, 2004; García-Gago *et al.*, 2009)
pectin methylesterases (PMEs)	catalysis of pectin de-esterification	(Draye and Van Cutsem, 2008)
B-galactosidase	depolymerisation of pectin and hemicellulose by side-chain loss of galactan	(Trainotti *et al.*, 2001)
α-l-arabinofuranosidase	Catalysis of polymeric arabinan loss	(Rosli *et al.*, 2009)
endo-β-1,4-glucanases (EGases)	active against the xyloglucans which coat the cellulose microfibrils	(Harpster *et al.*, 1998; Manning, 1998) (Mercado *et al.*, 2010)
β-xyloxidase	decrease of hemicelluloses at strawberry cell wall	(Martínez *et al.*, 2004) (Bustamante *et al.*, 2006)
exo-mannanases	mannan degradation at strawberry cell wall	(Bourgault *et al.*, 2001)
Xyloglucan endotransglucosylase (XTH)	break and remake glyco-sidic bonds in the backbone of xyloglucan in primary cell wall	(Fry, 2004) (Opazo *et al.*, 2013)
Expansins	cleave hydrogen bonds of cellulose microfibrils (not conventional enzyme action)	(Harrison *et al.*, 2001) (Dotto *et al.*, 2006, Nardi *et al.*, 2013)

Not only enzymatic action is responsible for fruit softening. Fry (2004) reviewed phenomena related to fruit softening. Hydroxyl group is perceived as a cause of pectic polysaccharide disassembly. Hydroxyl groups can be produced through Fenton reactions that require a metal ion and H_2O_2 (Schopfer, 2001). It was also proposed that apoplastic active oxygen could make cell wall polysaccharides vulnerable to hydrolysis (Gómez *et al.*, 1995). The role of apoplastic calcium chelators could also explain pectin solubilisation, since calcium is responsible for links between galacturonates (Brady, 1987). Boron is also proposed to contribute to cell wall structural

stability. Borate cross linking is another mechanism supposed to play a role in loss of firmness however the mechanism is not fully understood (Fry, 2004).

2.4.2.1. Mechanism of action of PELs

Disassembly of the strawberry cell wall is one of the main causes of fruit softening. PEL (E.C. 4.2.2.2.) is considered one of the enzymes contributing to breakdown of fruit cell walls resulting in firmness loss. PEL has recently being examined for its role in cell wall degradation and fruit softening since it was originally thought as mainly excreted by plant pathogens (Marin-Rodriguez *et al.*, 2002). PEL action was first highlighted in 1962 in a study on phytopathogenic bacteria *Erwinia carotovora* and *Bacillus sp.* (Starr and Moran, 1962) and its role is the catalytic cleavage of de-esterified pectin through the disassembly of polygalacturonate (Yoder *et al.*, 1993). PELs have a similar mechanism to polygalacturonases, however instead of a hydrolytic depolymerisation of pectate they act on them by β-or trans elimination (Figure 2.5). The products derived from PEL action are oligosaccharides with unsaturated galacturonosil residues at their non-reducing ends. Despite the fact that PELs were initially thought to target only pectates and they were distinguished from Pectin lyases (PNLs), a further study showed that they can also catalyse pectin depolymerisation (Bartling *et al.*, 1995). PELs were also proposed as responsible not only for cell wall modification, but as enzymes contributing to triggering defence mechanisms of plants, by release of oligogalacturonides which have a defence elicitor role (Bourquin *et al.*, 2002).

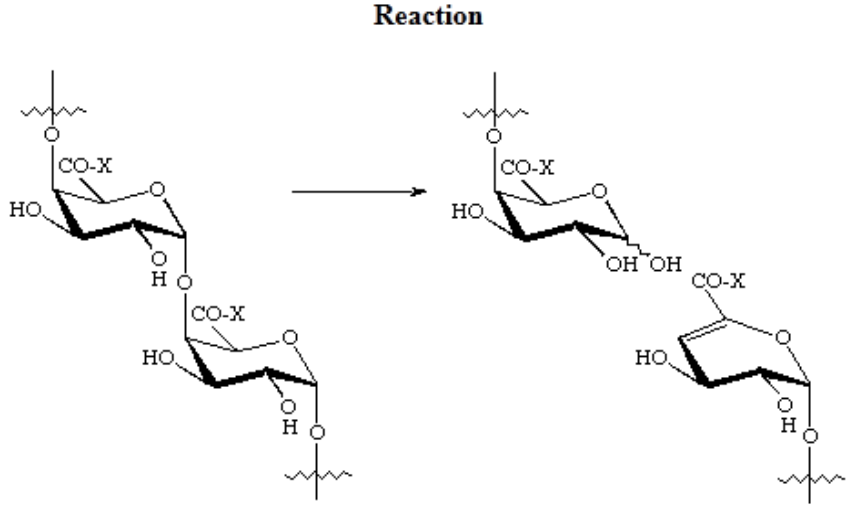

Figure 2.5: Eliminative cleavage of (1→4)-α-D-galacturonan to give oligosaccharides with 4-deoxy-α-D-galact-4-enuronosyl groups at their non-reducing ends. Source: http://www.chem.qmul.ac.uk/iubmb/enzyme/EC4/2/2/2.html

The role of PELs in fruit softening was reviewed by Marin-Rodriguez (2002b). The presence of both endo- and exo- PEL activity has been reported with endoenzymes showing increased maceration activity when compared to exo-enzymes (endo-enzymes are the enzymes that show activity inside the cell, exoenzymes are active outside their source cell) (Marin-Rodriguez, 2002). The role of PELs is also well appreciated in industry where they contribute to fruit juice quality by controlling their viscosity and concentration and by improving fruit juice yield (Alkorta and Garb, 1998).

2.4.2.2. The origin of PELs

PELs have several sources (Marin-Rodriguez, 2002) (Table 2.6), they are produced by *Colletotrichum gloeosporioides* fungus (Drori *et al.*, 2003) a postharvest pathogen affecting tropical and subtropical crops like avocado, where pH has a role in enzyme secretion by the pathogen, with optimum value for commencing of secretion to be 5.8. It was noticed that PEL activity was not detected when pericarp pH was lower than 5.8 and the pathogen could not colonize the host (Yakoby *et al.*, 2000). PEL was also secreted by *Bacillus subtilis*. Carrots (Chesson and Codner, 1978), cucumbers and olives were reported to be affected by development of soft rot symptoms. PELs are also produced by enterobacterium *Clebsiella* (Chatterjee *et al.*, 1979). Soft rot bacteria *Pseudomonas sp.* was a source of PEL (Zucker and Hankin, 1970; Liao, 1991). *Erwinia amylovora* a pathogen that is responsible for fire-blight development on many top fruits belonging to Rosaceae, also secres PEL (Kim and Beer, 1998). *Erwinia carotovora* was also identified as a pathogen in potato and the interaction between host and pathogen was noticed since it is believed that secretion of PEL is triggered by molecules found in potato that activate its synthesis in the pathogen. PEL extracellular activity reported from potato extracts ranged between 0 and 200 μ/mg dry weight (Tarasova *et al.*, 2013). Another source of PEL is *Clostridium botylicum –beijerinckii*a bacterium found in human intestines involved in digestion of pectic substances (Nakajima *et al.*, 1999). PEL is also produced by *Yersinia sp.* an invasive human pathogen related to development of gastroenteritis (Liao *et al.*, 1999). A well-studied source of PEL is *Erwinia chrisanthemi*, a pathogen that is macerating plant tissues (Kelemu and Collmer, 1993). PELs were also observed in plant tissues (Marin-Rodriguez *et al.*, 2002). Two PEL genes were reported in pollen of tomato flowers (Wing *et al.*, 1990). It is proposed that PEL loosens pollen cell wall and allows pollen tube growth and penetration transmitting tissue (Marin-Rodriguez *et al.*, 2002). PEL was also reported in alfalfa (Wu*et al.*, 1996) cedar pollen allergen (Taniguchi *et al.*, 1995) and *Papaver soniferum* (Pilatzke-Wunderlich and Nessler, 2001). In ripening fruits, PEL expression has been detected in grapes (Nunan *et al.*, 2001),

mango (Chourasia *et al.,* 2006) banana, (Dominguez-Puigjaner *et al.*, 1997; Medina-Suarez *et al.,* 1997). Activity of bananas was assessed and an increase in spectrophotometric readings was in the range of 0.015 to 0.030 OD at 235 nm (Marín-Rodríguez *et al.*, 2003). PEL of bananas was also expressed in terms of units/mg of protein and it was in the range of 0 to 25 units/mg of protein (Payasi and Sanwal, 2003). However, in other research, the range of activity as expressed in units/mg of protein was between 0-6 (Lohani *et al.,* 2004). In mango its activity was in a range of 0.1 to 0.7 units/Kg of fresh weight (Chourasia *et al.,* 2006). PEL activity was also assayed in apples and the difference in absorbance was at the range of 0.1 to 0.25 nm at 548 nm (Goulao and Oliveira, 2008). PEL activity was also detected in navel oranges (Lei *et al.*, 2010).

Table 2.6: Sources of PEL

Found in	PEL secreted by microorganisms	Described by	Related to
Avocado	fungus *Colletotrichum gloeosporioides*	Yakoby *et al.*, 2000	Anthracnose
carrot, cucumber and olive	*Bacillus subtilis*	Chesson & Codner, 1978	Soft rot symptoms
Human intestines	enterobacterium *Clebsiella*	Chatterjee *et al.*, 1979	Digestion of pectic substances
Potato	*Pseudomonas fluorescens*	Zucker & Hankin, 1970; Liao, 1991	Soft rot
Rosaceae fruits	*Erwinia amylovora*	Kim & Beer, 1998	Fire-blight
Potato	*Erwinia carotovora*	Tarasova *et al.*, 2013	Soft rot
Human intestines	*Clostridium botylicum – beijerinckii*a	Nakajima *et al.*, 1999	Digestion of pectic substances
Human intestines	*Yersinia sp* *Erwinia chrisanthemi*	Liao *et al.*, 1999 Kelemu & Collmer, 1993	Gastroenteritis
Found in	PEL secreted by plants	Described by	Related to
Pollen of tomato flowers	*Lycopersicon esculentum*	Win *et al.*, 1990	Pollen cell wall loosening
Alfalfa	*Medicago sativa*	Wu et al., 1996	Pollen cell wall loosening
Cedar	*Cryptomeria japonica*	Taniguchi *et al.*, 1995	pollen allergen
Opium poppy	*Papaver soniferum*	Pilatzke-Wunderlich & Nessler, 2001	Breaking down the transverse or lateral laticifer walls
Grape	*Vitis vinifera cv muscat*	Nunan *et al.*, 2001	Cell wall breakdown
Mango	*Mangifera indica var. Dashehari*	Chourasia *et al.*, 2006	Cell wall breakdown
Banana	*Musa acuminata* *cv Dwarf Cavendish & Grand Nain*	Dominguez-Puigjaner *et al.*, 1997; Medina-Suarez *et al.*, 1997	Cell wall breakdown

2.4.2.3.Conditions affecting PEL activity

PEL activity is affected by temperature, pH, the presence of co-factors, amount of substrate, and product of reaction (Hugouvieux-Cotte-Pattat *et al.*, 1992; Charkowski *et al.*, 2012). In most cases PEL requires Ca^{+2} as a cofactor (Nagel and Wilson, 1970), however there are occasions where other chemical elements were found to be involved in PEL pectolytic role. In the case of plant pathogenic bacterium *Dickeya dadantii* (formally known as *Erwinia chrisanthemi*) causing soft rot, Fe^{2+} acts as a cofactor (Hassan *et al.*, 2013), manganese was also found to increase

activity of PEL produced by *Bacillus pumilus* (Basu *et al.*, 2008). The optimum pH value for PEL in β-elimination cleavage of polygalacturonic acid (PGA) is in the range of 7.5-10 (Kobayasi *et al.*, 1999; Jayani 2005). However, there are exceptions such as *Bacillus sp.*, which responds with highest activity at pH 10.5 (Marin-Rodriguez, 2002). Furthermore, there are observations where high activity (more than 45% of highest activity) takes place in a wide range of pH values 3-12 (Yuan *et al.*, 2012). Temperature optima for the majority of PEL is at the range of 40-50 ^{o}C (Jayani *et al.*, 2005).

2.4.2.4. PEL in strawberry fruits

PELs share the same basic structure known as the 'parallel β helix' according to which β-strands are forming a larger superhelix by right-handed folding, however they may be differentiate in size and conformation of the folding (Marin-Rodriguez *et al.*, 2002). In strawberries as in other higher-plants, PELs' protein is relatively hydrophilic with a hydrophobic amino-terminal region indicating that this protein interacts with cell walls (Medina-Escobar *et al.*, 1997).

Three PEL genes have previously been reported in strawberries, FaplA (plA), FaplB (plB), and FaplC (plC). While the plB gene is a single copy gene, plA is probably encoded by a multigene family. All three genes are connected to strawberry firmness and cell wall degradation and they are detected in fruits but not in vegetative tissues. Their presence is increased during the later stages of ripening (Benitez-Burraco, 2003). It is believed that auxin negatively regulates the activity of the above mentioned genes. Furthermore, PEL activity was reduced under controlled atmosphere conditions where high levels of CO_2 existed (Benitez-Burraco, 2003; Manning, 1998). Other observations made on transgenic strawberries indicated the presence of FaplC was reduced whilst an increase in strawberry firmness took place. It was observed that fruits with reduced expression of FaplC where firmer even when they were tested three days after storage at 25 ^{o}C. Histological analysis of fruits revealed lower intercellular spaces and a higher degree of cell to cell contact area in fruits with reduced FaplC expression (Youssef *et al.*, 2009). On researching gene transcript accumulation associated with physiological and chemical changes during developmental stages of strawberry cv. Camarosa, there was an increase of plA and plB transcription at the early ripening stages and a decrease at later stages. plC followed the opposite path with increased transcription at later stages of maturation. The above observations led to the assumption that plA and plB are associated with cell division, while wall disassembly is the main process that plC is involved in (Severo *et al.*, 2011). In agreement with the previous research comes a study on *Fragaria chiloensis* and *Fragaria x ananassa*, showing that in mid ripening stages *Fragaria chiloensis* and *Fragaria x ananassa* had increased levels of plA gene expression,

when compared to later stages. Furthermore, harder fruits produced by *Fragaria x ananassa* plants had reduced levels of plA when compared to fruits coming from *Fragaria chiloensis* (Figueroa *et al.*, 2008). Increased firmness of strawberry fruits when PEL gene was suppressed between 30-100% compared to control lines was also observed. The above observations were related to reduced cell wall swelling and reduced amount of ionically bound pectins because of suppression of PEL. The fact that reduced amount of electrolytes were released when fruits were incubated in distilled water was additional evidence of reduced cell wall dissociation (Jiménez-Bermúdez *et al.*, 2002).

2.4.3. Visual attributes

The visual appearance of strawberries is a key parameter for determination of quality. Consequently, bruising is an important visual defect and fruits are often considered of inferior quality by marketers where bruising (wet or dry) exceeds 5% of a consignment (Fig. 2.7).

Figure 2.6: Dry bruised fruits

Dry bruise can be caused by pressure impact or vibration, inappropriate harvesting, deterioration of strawberry quality by increasing mechanical damage during transport (Martinez Romero *et al.*, 2004; Ferreira *et al.*, 2008). The mechanism of bruising of strawberries could be the result of cell breakage caused by disorder of cells due to stress factors (Ferreira *et al.*, 2008). Little data exists about dry bruised fruit and further research that will give more information about implications of growing conditions on the presence of dry bruising is necessary. Wet bruised and mouldy fruits can be caused by development of *Botrytis* on strawberries. *Botrytis cinerea* is a major pathogen infesting strawberries and can dramatically reduce their quality. Infestation of *Botrytis* can start in the field, however symptoms can be expressed later when fruits are at retail shelves or within a day following purchase by consumers. For maintenance of strawberry postharvest quality, several methods have been adopted by the industry that include appropriate packaging and maintaining the cool chain typically at 3 °C. Shape which is typical for each

variety was not evaluated, since strawberries arriving in pack house were already quality controlled by pickers and fruits of abnormal shape were not sent to markets.

2.4.4. Taste

The amount of sugars as well as acids and their ratios is important in determining the eating quality of fruit. For cultivars Festival and Ventana, the dominant sugar compound was glucose. Fructose and sucrose comprised 50% of the total levels when compared to glucose (Basson *et al.*, 2010). Total sugar concentration was five times higher than total acid concentration in fully ripe fruits. The main enzymes possibly connected with sugars which increased their activity during development are invertase (Qin *et al.*, 2008), pyrophosphate-dependent phosphofructokinase (PFT) and fructose-1,6-bisphosphatase (FBPase). Sucrose synthase had very low or undetectable activity (Basson *et al.*, 2010). The presence of other sugars like xylose, sorbitol and xylitol was also identified in traces (Makinen and Soderling, 1980; Bordonaba, 2010). The ratio of fructose to glucose has been shown to be about 1:1 (Bordonaba, 2010). It was observed that total amount of sugars increased during ripening with the maximum levels occurring 35 days after fruit set. Fructose and glucose followed similar trends and their presence was enhanced up to 35 days after fruit set. However, sucrose formation followed a different trend. Sucrose levels were increased until the 28^{th} day after fruit set then decreased reaching the lowest values during growing season after 42 days of fruit set (Montero and Mollá, 1996) a result that was also verified by other studies (Watson, 2002). In other studies it was found that the three major sugars increased, however there were differences between the developmental pattern of sugars in achenes and strawberry receptacle. In the achenes the amount of sugars decreased over time, whilst in the receptacle the opposite trend followed (Fait *et al.*, 2008).

Besides taste, acids can contribute to the formation of off-flavors during processing. They are also responsible for the gelling state of pectin and pH of fruits (Montero and Mollá, 1996). Organic acids present in strawberry fruits include citric, malic, ascorbic (vitamin C), shikimic, glycolic succinic and tartaric, with citric being predominant (Montero and Mollá, 1996; Sturm *et al.*, 2003; Keutgen and Pawelzik, 2008; Bordonaba, 2010). It is generally accepted that total titratable acidity of fruits decline at the fully ripened stage. However, the concentration of ascorbic acid was found to be steady or slightly increased when fruits were ripe (Montero *et al.*, 1996). Ascorbic acid (AA), a major acid contributing to taste, can be affected by preharvest climate conditions. Temperature and light can affect the formation of AA (Atkinson *et al.*, 2006). The total amount of AA that will be formed during the season is highly dependable on the

amount and quality of light that plants receive and the location of cultivation (Lee and Kader, 2000).

2.4.5. Colour

Strawberry colour is a major attribute influencing consumer purchasing behaviour. Many compounds contribute to the colour of strawberry fruit. The predominant compounds are simple phenols, anthocyanins and flavonols, with the most important found to be the anthocyanins. A sub group of polyphenols are flavonoids, and a sub category of flavonoids with benefits for human health are anthocyanins (Terry, 2011). The predominant anthocyanin pigment in strawberries of three different cultivars was found to be Pg3-gluc (77-90%) followed by Pg 3- rut (6-11%) and Cya 3-gluc (3-10%) (da Silva *et al.*, 2007). Anthocyanins are also known for their contribution to human health. A rich diet in anthocyanins is associated with healthy vision, increased protection of cardiovascular and neural systems and also show benefits for human skin (Verbeyst *et al.*, 2010). Anthocyanin metabolism within the human body is not completely understood. However, studies have shown that the main metabolite excreted from human body after the consumption of strawberries is a monoglucuronide of pelargonidin (Felgines *et al.*, 2003). Phenolic consumption is connected with lower rates of cancer, strokes and cholesterol levels. These phytochemicals present in strawberries are also connected with reduced brain damage due to aging (Törrönen and Määttä, 2000; Joseph *et al.*, 2009; Giampieri *et al.*, 2013). Phenolics are considered to be antioxidants since they reduce the presence of free radicals connected to human aging. Recent studies indicate that the contribution of strawberry phenolics to antioxidant activity of plasma might not be as significant as originally thought. It is suggested that the antioxidant activity of strawberry phytochemicals is not connected to their direct present in plasma, but in their ability to increase presence of phenolics metabolites which result in higher levels of uric acid. Uric acid is known for its antioxidant activity. Phenolic compounds that were present in strawberries and were characterized as having antiproliferative cancer cells properties, are cyanidin glucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, kaemfperol, quercetin, kaempferol-3-6-coumaroylglucoside, 3-4-5-trihydroxyphenyl-acrylic acid, glucose ester of (E)-p-coumaric acid and ellagic acid (Zhang *et al.*, 2008).

Genes known to influence strawberry colour development mainly belong to the super family of reductase or 2-oxoglutarate-/Fe^{2+}-dependent dioxygenase (Almeida *et al.*, 2007). Other enzymes that are connected to the formation of strawberry anthocyanins are phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS). The role of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHP) was also monitored since it is related to secondary metabolism products, such

as anthocyanins. Experiments involving cell suspension cultures of PAL and CHS activity were observed to increase by elicitor treatment, wounding and UV irradiation (Mori *et al.*, 2001).

Several theories have been published for the formation of phenolics and their role in plants (McKey, 1974; Herms and Mattson, 1992; Jones and Hartley, 1999). All of them accept the fact that phenols produced from secondary metabolism come at a cost to growth, since secondary metabolism requires resources which are allocated from primary metabolism and can limit growth in favour of phenolics formation (Lattanzio *et al.*, 2012). It is proposed that plants form secondary metabolites in order to defend themselves against biotic and abiotic stress factors. Increased UV radiation levels (Tsormpatsidis *et al.*, 2008; Tsormpatsidis *et al.*, 2011; Ordidge *et al.*, 2012), low temperature and limited availability of nutrients (Lillo *et al.*, 2008; Olsen *et al.*, 2008) as well as water stress (Bordonaba and Terry, 2008) were found to enhance phenolics levels in plants. Furthermore, plants produce phenolics in order to defend themselves against pests (Lattanzio and Cardinalli, 2006). Since formation of secondary metabolites requires allocation of resources, plants try to avoid it if possible in order to focus on growth. However, it is proposed that plants which are frequently attacked have to produce metabolites in advance. On the other hand plants that face threats more rarely are adapted to produce defensive metabolites after the initial damage; this is riskier since the pathogen could expand rapidly at a stage which host would not be able to cope with it (Koricheva *et al.*, 2004). Phenolics have an inhibitory role against fungi and can reduce or eliminate damage in many plant species such as olive and citrus (Arcas *et al.*, 2000; Del Río, 2003). In strawberries a negative relationship between the amount of proanthocyanidins and the development of grey mould was noticed and it was proposed that phenolics had an inhibitory effect on the activity of polygalacturonase produced by fungus (Schlosser, 1988). Furthermore, phenolics play a significant role in plant insect interaction. Plants can produce secondary compounds that inhibit oviposition by insects and larval growth (Chapman, 2009; Lattanzio and Cardinalli, 2006). Phenolics have also an effect on plant growth as they act as plant physiological regulators and chemical messengers. They act as photoreceptors and they are involved in changes in cell wall structure and can influence turgor and growth (Turner *et al.*, 1993).

2.5. EFFECTS OF CLIMATE VARIABLES ON POSTHARVEST QUALITY OF STRAWBERRY FRUIT

The positive effect of higher growth temperatures on softening of fruits it is well known, and such an effect was observed with strawberries (Rose, *et al.*, 1934; Sams, 1999). However it has not always been verified (Diamanti *et al.*, 2008). Strawberry fruits exposed to ambient levels of UV radiation in poly tunnels were found to be firmer compared to fruits that were grown in the absence of UV radiation (Tsormpatsidis *et al.*, 2011). The effect of humidity on firmness (cv. Elsanta) was evaluated on glasshouse grown strawberries (cv. Elsanta). Fruits that were grown under high relative humidity (90%) tended to be softer than fruits grown in lower humidity (50% and 70%). Lower, humidity levels also increased the shelf-life of fruits (Lieten, 2000).

Anthocyanin and total phenolic levels were found to be influenced by temperature. Increased night and day temperatures enhanced the levels of anthocyanins and flavonoids. When strawberry fruits of cv. Kent were grown under three day/night temperature treatments (18/12, 25/12, 25/22, and 30/22 °C), the highest amount of flavonoids was found in strawberries grown in the 30/22 °C scheme, followed by the 25/22°C temperature pattern. The lowest amount of phenols and anthocyanins were found in fruits grown in the 18/12°C scheme (Wang and Zheng, 2001). Higher temperature and irradiation levels during the last period of fruit development (cv. Elsanta) were also related to increased total phenol content (Krüger *et al*., 2008). In the absence of UV light, strawberries were found to produce less total phenolics and anthocyanins (Tsormpatsidis *et al.*, 2011). However, other experiments indicated that only individual anthocyanins were affected and not the sum of phenolics (Josuttis *et al.*, 2010). The effect of light on formation of anthocyanins was also evaluated when dark treatment was applied during later stages of growth of strawberry fruits (cvs. Nyoho and Toyonaka). Fruits that were grown in the dark accumulated lower amounts of anthocyanins and it was noticed that the effect of treatment was greater at later stages of fruit development. Fruits were covered with a black cloth from flowering until harvest day (Kawanobub *et al*., 2011). Increased air temperature (30/15°C, 14/10h, day/night) was found to decrease expression of anthocyanin formation related genes compared to (20/15°C, 14/10h, day/night) air temperature scheme (Matsushita *et al.*, 2016).

Increased photosynthetic active radiation levels, enhanced by reflective mulching, were found to enhance ellagic acid content of fruits (Atkinson *et al.*, 2006).

Preharvest application of ozone (156 μg m^{-3}) on cvs. Elsanta (ozone sensitive) and Korona (less ozone sensitive) were found to affect only the sensitive culivar. Elsanta fruits showed a decrease

in *L** value (became more dark), chroma levels were decreased (less intense) and more yellow since °hue values decreased. However, no effect of ozone on total anthocyanins was identified (Keutgen and Pawelzik, 2008).

Increased temperatures was found to reduce total soluble content and amount of sugars in strawberry fruits (cvs. Kent and Earlygrow). The lowest amount of TSS was found in strawberry fruits grown under the 30/22 °C (day/night temperature) scheme, when compared to 18/12, 25/12, 25/22 °C patterns. The highest amount of TSS was recorded at low temperatures 18/12°C (Wang and Camp, 2000). Furthermore, increased night temperatures were found to decrease soluble solids content. Strawberries (cvs. Nyoho and Toyonaka) had lower °brix levels when grown under the 23/20 °C day/night temperature scheme compared to 23/10 °C pattern (Matsuzoe *et al.*, 2006). The amount of total soluble solids was not found to be affected by UV radiation levels (Tsormpatsidis *et al.*, 2011). The effect of light on strawberry soluble solids accumulation was tested by subjecting fruits into shading. The fruits (cv. Ostara) that received the higher levels of light (74% of light penetration on shading material) had the highest amount of TSS. On the contrary, fruits that received low levels of light (5% light penetration on shading material) had the lowest soluble solids levels (Osman and Dodd, 1994). Similar results were also found when strawberry fruits (cv. Elsanta) were subjected to three shading levels (0, 25 and 47%). Fruits that were grown under increased shade one week prior harvesting, showed a decrease in sucrose and fructose (Watson *et al.*, 2002). Ozone preharvest application did not affect sucrose and glucose concentrations. However, with the ozone sensitive cv. Elsanta, fructose content decreased after ozone treatment, Korona (less sensitive) did not show such effect (Keutgen and Pawelzik, 2008).

Increased temperature were found to have an negative effect on formation of ascorbic acid (Wang and Camp, 2000). The amount of acids was not found to be affected by UV radiation when strawberries of cv. Elsanta were grown at different levels of UV radiation (Josuttis *et al.*, 2010). However, it is generally appreciated that increased light levels will have a positive effect on formation of Ascorbic Acid (AA) of horticultural crops and it was noticed that parts of fruits facing the sun have increased amounts of AA (Lee and Kader, 2000). Preharvest ozone effect on strawberries was recorded at strawberry fruits of two cultivars Elsanta (sensitive) and Korona (less sensitive). For both cvs., ascorbic acid levels decreased after ozone treatment (Keutgen and Pawelzik, 2008)

The size of strawberries is affected by environmental conditions. It was found that lower temperatures enhance the production of larger strawberry fruits (Wang and Camp, 2000). Fruit

weight was also found to decrease in atmospheres with no UV radiation (Casal *et al.*, 2009). Increased radiation levels resulting through the use of reflecting mulching films also increased fruit size, and the number of fruits bellonging to category I was higher (Atkinson *et al.*, 2006). Preharvest application of ozone (156 μg m^{-3}) on cvs. Elsanta (ozone sensitive) and Korona (less ozone sensitive) were not found to affect yield, fruit weight or dry matter content (Keutgen and Pawelzik, 2008).

Generally it is accepted that warm fruits show increased elasticity, a fact that leads to enhanced resistance to impact bruise and decreased ability to withstand vibration stress (Sommer, 1960; Paull, 1999). Quality traits of octaploid strawberries mainly affected by environmental conditions as it was shown by genetic dissection are mainly related to sugar and acid formation as well as firmness of fruits. It was shown that colour traits was less affected by environmental conditions (Lerceteau-Köhler *et al.*, 2012). The complex interatctions between hormone signaling Abscisic acid (ABA), environmental stress such as temperature, light and water stress and the way that these plant stresses affect fruit ripening and quality was also highlited. Increased ABA levels can result to increased sugar levels and phytochemicals through expression of related genes (Ayub *et al.*, 2016).

Chapter 3 : A STUDY OF PREHARVEST ENVIRONMENTAL EFFECTS ON POSTHARVEST QUALITY OF STRAWBERRY FRUITS AT COMMERCIAL FARMS IN ENGLAND AND SPAIN.

3.1 INTRODUCTION

Postharvest quality of strawberries is affected by several variables such as genotype, agricultural practices and environmental conditions. Earlier studies indicated that preharvest environmental variables such as temperature, ozone, vapour pressure deficit (VPD) and solar irradiation contribute to postharvest quality of strawberries (Wang and Zheng, 2001; Atkinson *et al*., 2006; Tsormpatsidis *et al*., 2011).

The aim of this research was to examine the effects of preharvest factors by modelling postharvest quality of strawberry fruits in terms of preharvest environmental growing conditions. The objectives were to identify individual effects of environmental variables and the range beyond which negative impacts on postharvest quality manifest themselves. During the three year study the effects of environmental variables on strawberry quality characteristics (total soluble solids, colour, weight, visual deformities, firmness, total anthocyanins, organic sugars and acids) were assessed.

One of the major challenges of the study was to evaluate effects of simultaneously changing conditions which are not independent of each other on strawberry quality. Furthermore, since observations were undertaken on commercial farms, there were influences of individual harvesting and marketing strategies that each enterprise followed owing to fluctuations in consumer demand.

This research was driven by the desire of commercial businesses to offer a more consistent quality product which will be more acceptable to consumers. Current practices and/or strawberry cultivars are not able to offer sufficient flexibility or resistance to changes in growing conditions to allow growers to meet their objectives in terms of quality and quantity.

An additional aim of the research was to model the effects of environmental variables in order to produce a useful tool for growers for quality prediction. Such a tool could improve the efficiency of strawberry supply chain. The cost of transporting strawberries from a distance of several thousand miles is quite high, and the intensive use of pesticides, plastic, water and land resources in their production has been criticised. These issues highlight the necessity of a more effective

strategy for improving postharvest life and quality of strawberries as well as reducing postharvest waste. Specifically it is estimated that losses of strawberry fruits within the at UK supply chain -excluding household- can be more than 8% (Mena *et al.*, 2014).

3.2. MATERIALS AND METHODS

3.2.1. Plant material and growing sites

Two cultivars of strawberry were examined during the study: the short day Sabrosa-Candonga™ in Huelva, Spain, and the ever bearer (day-neutral) Elsinore™ in the UK. Sabrosa-Candonga is a relatively new variety that was established in EU in 2004 and in U.S.A in 2006 (Arias, 2006). The variety was introduced by Plantas de Navarra S.A., Planasa. It is described as a plant with spherical growth habit, medium density but strong vigour and tolerance to low temperature exposure. Sabrosa-Candonga is a mid-season flowering variety. Compared to cv. Camarosa it produces flowers later. The normal productivity season in Huelva, Spain is from December up to the middle of May. Sabrosa-Candonga requires chilling in order to flower and normally a few hours below 7°C is sufficient. It was observed that primary fruits have a length of about 5.5cm and width 4cm, whilst secondary fruits have length of 4.5cm and width 3cm. Fruits are characterized as red, very sweet, medium acid with maintenance of quality characteristics when stored at 2°C for two days (Arias, 2006). Both Spanish sites had sandy soil allowing good drainage and aeration, however they were medium fertility soils and nutrients should be applied. Nutrients were mainly applied through drip irrigation. The main forms of fertilizers used were potasium nitrate, folliar application of calcium and quite often depending on stage of development micronutrints such as zinc and boron. Typically per hectare the application of N was between 170-200 units, phosphorus 100-120 units, potassium 300-350 units and calcium 30-50- units. Soil pH was slightly acidic 5,5-6,5. Organic matter was 1,5-2.5%. The farms were located close to Lepe and Moguer in Province of Huelva (Figure A.1). At SP1 farms approximately 1,000,000 Candonga plants were grown and the SP2 site 2,750,000. All sites were planted at a density of 55,000 plants per hectare.

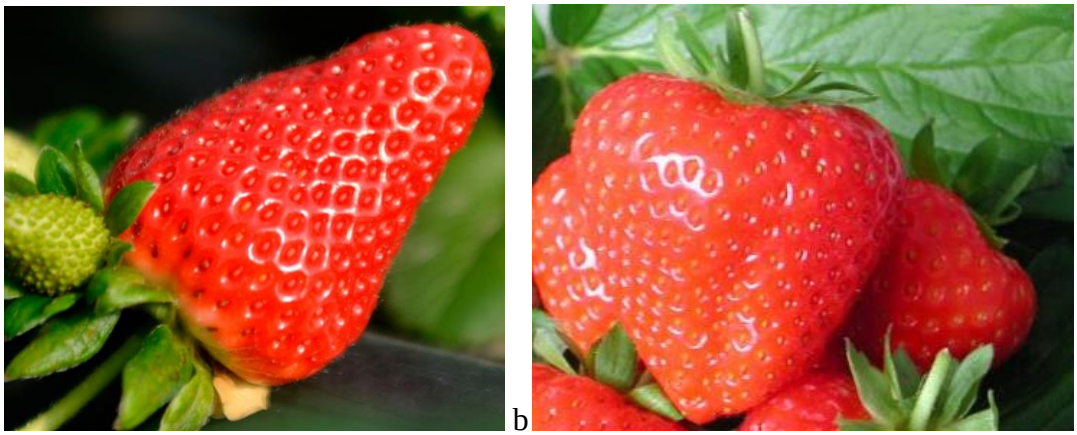

Figure 3.1: Candonga fruits (a) and Elsinore fruits (b).

Elsinore strawberry plants are planted the end of March to mid-April in the UK. UK production starts about 10 weeks after planting, depending on climate conditions. As a general pattern it is accepted that production starts around beginning of July, with an early harvest pick and a late pick (Vogels, pers. com). Elsinore derives from the crossing (Elsanta x Muir) x Sweet Charlie (INRA, 2013). The plant is characterized as of medium vigour, with vibrant green leaves and high yields. It re-flowers with the flower level above the foliage. The flowers are large and have increased amounts of pollen. Fruits of Elsinore are conical shape or truncated-cone, having large size. Colour is orangey-red, bright and even throughout the fruit. The flesh is red, even, firm and has a very nice flavour (Mazzoni group, 2011). The UK site that was selected was near Hereford (Figure A.2) and strawberry plants were grown in peat bags under protection, drip irrigation was provided. Density of plants was 55,000 per hectare. The soil was of average pH (6-7) and typically per hectare the application of N was between 150-180 units, phosphorus 110-130 units, potassium 280-320 units and calcium 30-50 units.

The selected varieties had different shape characteristics with the Candonga being less sperical and Elsinore being more rounded. Shape is an important factor for bruise development among other parameters (fruit layer in the box, box position in the truck etc.). Generally fruits with small sphericity values are more susceptible to bruise development (Aliasgarian *et al.*, 2015). In addition the two different varieties could provide useful information about behaviour of short day plants (Candonga) and day neutral plants (Elsinore) since the flowering habit can have an impact on quality characteristics, as it affects crop load and harvesting time.

3.2.2. Fruit sampling

It was decided to collect samples 3-4 times per month in order to have representative image of the growing season. A challenge to be addressed was that of spatial sampling. The aim was to obtain representative samples. The Spanish sites have areas of 50 and 100ha respectively. The sampling method was decided after taking into account the area of the farms, estimated quantity of fruit harvested and exported to the retailer, and number of tunnels expected to be harvested. Preliminary experiments were performed prior to the main data collection in order to determine the amount of variation across and within tunnels for selected variables. For the UK site the sampling was done over fewer tunnels.

3.2.2.1. Preliminary sampling regime

The sampling of strawberries was designed in a way that would enable capturing variability of fruits inside the tunnels. Pre-sampling was done in order to investigate effect of variability of different field locations. Three tunnels were chosen per site. Inside each tunnel a punnet of strawberries was collected from five different places (Figure 3.1).

Figure 3.2: Sampling positions inside tunnels.

The reason for conducting pre-sampling test was to be confident that variability between harvesting positions inside tunnels and between different tunnels was not significant and the changes in quality could be attributed only to environmental factors and physiological stage of plants as well abiotic and biotic effects. All quality parameters in the were evaluated, colour, firmness, sugars, acids etc. as described in detail earlier in the chapter.

3.2.2.2. Pre-sampling results

Significant ($p<0.001$) differences were not found across tunnels at either site for firmness, brix, colour, quality assessment, or weight of fruits. However, significant ($p<0.001$) differences were found between sites (Table A.1).

3.2.2.3. Main sampling regime

Spanish Fruit

Based on preliminary sampling results, the main sampling regime was adopted as it indicated differences could be adequately captured and that data from loggers placed in commercial fields were valid when compared against weather stations within those radii (figures A.3, A4 and A.5). Spanish fruit strawberry fruits (cv. Candonga) were grown using standard polytunnel practices (Figure 3.2) on two commercial production sites, SP1 and SP2 in Huelva, Spain. Ripe fruit were harvested by hand, packed into 400g punnets containing bubble film, moved from field to pack-house within 30 mins and cooled to remove field heat. Fruits were then transported to the UK by road over 2 days within refrigerated containers at +3ºC±1 and subsequently stored under standard pack-house conditions at +3ºC±1. Randomized samples were taken on average on a weekly basis through the harvest period from each production site. On the third sampling year (2012) fruits from SP1 were not assessed. Fruits were sampled from 3 boxes per pallet (from a total of 85 boxes per pallet), with 3 punnets taken from each box (from a total of 12 punnets per box) for further sub-sampling. In total 9 punnets were sampled per consignment.

At each sampling date, a batch of fruit were harvested according to normal commercial practice, from which sub samples for the analyses described below were undertaken, with the same fruit being used for various assays in some cases. The remaining fruits in the punnets were placed under shelf life conditions for three days.

Figure 3.3: Commercial strawberry polytunnels in Huelva, Spain.

UK fruit

Strawberry fruits (cv. Elsinore) were grown on a commercial site UK1. Ripe fruits were harvested, packed into 400g punnets and immediately cooled to remove field heat. Strawberry boxes were randomly sampled after fruits had been subjected to postharvest cooling, according to standard pack house practice. Fruits were then transported to the Natural Resources Institute within 24 hours from harvest in a sealed polythene cool-box containing ice. Samples were sent on a weekly basis from September until the end of October 2010. Twelve to fifteen 400g boxes were sent at each outturn. Nine boxes were selected randomly and three fruits per box were sub-sampled for further analysis.

All fruits (UK and Spanish) upon their arrival to NRI were randomized and 3 fruits per punnet were used for quality assessment (colour, TSS, weight, firmness) and subsequently frozen for further chemical analyses. The remaining fruits in the punnets were placed under shelf life conditions for three days.

3.2.3. Colour

The colour of individual strawberry fruits was determined by the use of CIE coordinates. *L*, a*, b** values were recorded during the research. Positive *a** value represents the degree of red colour on fruits. Higher *a** values represent red (magenta) colour, while negative values represent green. Increased *L** values represent brighter colour and positive *b** values represent the degree of yellow colour on fruits. For the measurements a chroma meter was used (Croma-meter CR 400/, Minolta Japan). A white plate was used for calibration. Colour coordinates were also used for calculation of chroma (Chroma = $[a^{*2} + b^{*2}]^{1/2}$) and hue angle (h^0 = arc tangent [*b*/a**]). 0^0= red-purple, 90^0 = yellow, 180^0 = bluish green and 270^0 = blue (Holcroft and Kader, 1999; Keutgen *et al*., 2005). The mean of two readings on opposite sides of the equatorial axis of the fruits was calculated. In total 27 strawberries were tested at each sampling date from each growing site.

3.2.4. Firmness

At each sampling date, fruit firmness (Newtons) was assessed using a hand-held penetrometer mounted on a stand (Bishop Instruments Ltd) with an 8mm diameter probe. Strawberry fruit firmness was measured for whole fruits on one side of each fruit. Fruits were sampled in a completely randomized design. The number of fruits (n=27) per week was decided upon in order to have a number of replications that would enable an accurate prediction of firmness (with low standard error), without increasing significantly the duration of analysis. A number between 10 and 30 fruits was used in order to reduce error for firmness determination (Døving and Måge, 2002).

3.2.5. °TSS

The amount of total soluble solids was recorded with a digital refractometer (Model AR 200, Reichart ophthalmic instruments, USA). Half strawberry fruits were squeezed and fruit juice was used for the determination of brix° (an indication of % content of water-soluble solids in fruit juice). At each sampling date (n=27) fruits were analysed from each growing site and values recorded. For calibration of the refractometer, distilled water was used according to manufacturer's instructions.

3.2.6. Freeze drying

Following colour, weight and firmness measurements on fresh fruits, the individual fruits were cut in halves along the vertical axis. One half was weighed, snap-frozen in liquid nitrogen and stored at -80ºC. Frozen samples were stored in plastic bags suitable for -80˚C freezer (New Brunswick Scientific, Ltd. UK) until freeze drying in order to stop any biological activity. After 36 hours in the freeze dryer (Super Modulyo, Edwards Ltd. Crawley, UK) at 0.015kPa fruit tissue was removed, the weight was recorded on a balance (A 2005, Sartorious Analytic Ltd. Germany) and placed in a -80˚C freezer until further chemical analyses took place (approximately 6 to 12 months after sampling). Moisture content was measured using the initial weight of half fruits and the weight recorded after freeze drying.

3.2.7. Carbohydrates

Freeze-dried strawberry samples were removed from a -80˚C freezer and ground into powder using a pestle and a mortar. Aliquots of freeze dried powder (0.05g) were extracted with 1ml of aqueous ethanol for two hours in a shaking water bath at 70ºC. Samples were vortexed every 20 minutes to avoid formation of residue. Afterwards, samples were centrifuged at 13,000 rpm for 4 minutes. Cooled samples were filtered through a 0.45 μm syringe filter. Samples (10μl) were injected into an Agilent 1200 series HPLC, fitted with an Agilent Zorbax carbohydrate column (150mm x 4.6mm x 5μm) and an Agilent Zorbax NH_2 guard column using 75/25 acetonitrile/water mobile phase maintained at 30˚C. Flow of the sample was 1 ml/min. Fructose, glucose and sucrose standards were used for determination of sample concentrations. For detection of peaks a refractive index detector was used and data processing and analysis was performed by an Agilent EZChrom Elite version 3.3. Results were expressed as mg/g dry weight. Sweetness Index was also calculated using the following equation S.I. = Glucose*1 + Fructose*2.3 + Sucrose*1.35 (Bordonaba and Terry, 2008; Crespo *et al.*, 2010).

3.2.8. Organic acids

Freeze-dried strawberry samples were removed from the -80˚C freezer and ground into powder using a pestle and a mortar. Aliquots of freeze dried powder (0.05g) were extracted with 1ml of deionised water and left for 20 min. at room temperature. Samples were vortexed and centrifuged at 13,000 rpm for 4 minutes. Afterwards, samples were filtered through a 0.45 μm syringe filter. Samples (10μl) were injected into an Agilent 1200 series HPLC, fitted with an Agilent Zorbax SB-Aq column (250mm x 4.6mm x 5μm). Oxalic, fumaric, citric and malic acid standards were used for determination of sample concentrations. For detection of peaks a

refractive index detector was used and data processing and analysis was performed by an Agilent EZChrom Elite version 3.3. Results were expressed as mg/g dry weight (Bordonaba and Terry, 2008).

3.2.9. Total anthocyanins

Samples of the lyophilized powder (0.1 grams) were extracted with 10 ml methanol:HCl (99.9:0.1) and placed in a vial. Then, the mixture was homogenized using a hand held stirrer for 1-2 min. The supernatant was then transferred to a screw-cap vial and placed in a cold room, at a temperature of 4 °C, overnight to facilitate extraction. There is evidence that a period in the cold room facilitates extraction and reduces the coefficient of variation compared to immediate measurement of extracts (Tsormpatsidis *et al.*, 2008).

The determination of the total monomeric anthocyanin content was achieved by the use of the pH differential method. The anthocyanin content was estimated with a spectrophotometer at 510 nm and 700 nm and buffers at pH 1.0 and 4.5 using a spectrometer (Cecil, CE 9200) (Giusti and Wrolstad, 2001; Meyers *et al*., 2003). Using the equation:

Monomeric anthocyanin pigment (mg/litre) = (A x MW x DF x 1000)/(E x 1)

Where:

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

MW is the molecular weight of the standard (443.2)

DF is the dilution factor (in our case 6 because 0.5 ml sample were diluted with 2.5 ml of buffer and

E is the molar absorptivity (17330).

All values were estimated and expressed as pelargonidin-3-glucoside and expressed as mg anthocyanin per 1 g DW. The method was chosen because it is more rapid compared to HPLC, with similar accuracy (Lee and Kader, 2000).

3.2.10. Visual evaluation - shelf-life

The quality of strawberries was assessed visually (Hertog *et al*., 1999). The number of fruits with dry and wet bruise was recorded, as well as waste (Figure 3.3). Percentage of fruits developing bruising and characterized as waste was also calculated for each box. No categorization of severity took place. Assessment of strawberry quality was conducted on the

arrival day and on the expiry day (three days after arrival). Between the arrival and expiry day (sell by) the fruits were stored in incubators at 4 °C in order to simulate house refrigerator conditions.

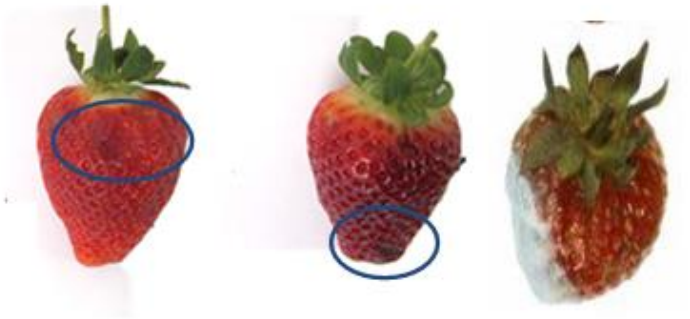

Figure 3.4: Fruit quality problems detected by visual evaluation. From the left, wet bruise development, dry bruised and waste fruits.

3.2.11. Statistical analysis

Results were analyzed using general linear models (GLM). Further statistical analysis for tests of significance of the significantly different means, Pearson's correlation, linear models and principal component analyses (PCA) were undertaken using software packages R statistics 2.10, Genstat 13th edition and XL stats. Analyses were undertaken in triplicate unless otherwise stated.

3.2.12. Recording of environmental data

In Spain, levels of precipitation (mm/day), mean temperature (˚C), average humidity (%RH) and solar irradiation amount (MJ/m^{2}day) were recorded from two weather stations belonging to Institute de Investigacion y Formacion Agraria y Pesquera (IIFAP) approximately 5Km from the production sites at Lepe (SP1 site) and Moguer (SP2 site). Mean temperature (˚C), average humidity (%RH) were recorded at the fields (Figure 3.) and within polytunnels (using Tinytag Plus 2 and Tinytalk data loggers every 15 minutes). Ozone (µg/m^{-3}) data were recorded from stations belonging to National Institute for Aerospace Technology (INTA) research institute, in Huelva.

a  b 

Figure 3.5: Temperature and humidity loggers' positions outside (a) and inside (b) tunnels.

Climate data for the UK commercial growing site was also provided from several local climate stations. Temperature and relative humidity data were obtained from Hereford weather station approximately 9.4 Km from the site. For solar irradiation values, a station at Sobton was used approximately 18 Km from the growing site. The above stations belong to the Meteorological Office. Data on ozone levels data were provided from a station based at Leominster approximately 10 Km away from the growing site. Calculation of daily Vapour Pressure Deficit (VPD) was calculated from relative humidity and temperature data recorded, using the formula (Howell and Dusek, 1995; Castellvi *et al*., 1997):

$VPD=e^*T_{dew} \times [(1- (RH_{mean}/ 100)]$

e^*T_{dew} was calculated from the following formula:

$e^*T_{dew}=0.611 \exp [(17.27\ T_{mean})/(T_{mean} +237.3)]$ kPa

where $T_{mean} = (T_{max} + T_{min}) / 2$ and $RH_{mean} = (RH_{max} + RH_{min}) / 2$.

Maximum (T_{max}) and minimum temperature (T_{min}) values, as well as maximum and minimum relative humidity values were provided from local weather stations.

At both Spanish sites, temperature recorded in the tunnels was higher than in the field approximately 3°C (Figures A3, A4). It should be also mentioned that station had the lowest temperatures compared to field at all times. At the UK farm, the trends were similar, with minor exceptions (Figure A5). Mean temp and average RH used in the research were computed from the data recorded at the weather stations and not measured in the growing sites.

3.3. RESULTS AND DISCUSSION

The results of the three-year study that took place in two Spanish and one farm in UK are presented below. Strawberry quality characteristics were evaluated against environmental variables recorded two days, one week and three weeks prior to harvest. The period of seven days prior to harvest is known to be a critical period for fruit maturation since development of pigments, sugar accumulation and softening takes place at this time, and furthermore mid-range environmental effects could be monitored. Three weeks before harvest time was also selected to identify the long term contribution of the growing environment to strawberry fruit quality. Finally, the two day interval was selected in order to investigate potential short-term effects of environment on fruit quality. All of the climatic and atmospheric variables (i.e. temperature, VPD, relative humidity, solar radiation and ozone concentration) were measured at all of the three production sites (two in Spain and one in the UK) over the stated periods prior to harvest.

There are several options for targeting environmental variables e.g. air temperature, soil temperature, diurnal temperature etc. (Samykanno, 2012) as a metric in order to relate environmental changes with that of changing fruit quality . Mean temperature during the growing season was used as a tool to evaluate changes in fruit quality since initial work on temperature difference (Max-Min) did not provide any additional information or improved models.

A combination of statistical methods was used in order to extract as much information as possible from a multivariate system such as open field strawberry growing trials.

Apart from General Lineal Models (GLM) that were developed by the use of R statistics (R 3.2.5.)in addition PCA was used. PCA is an unsupervised data evaluation method where no information about data classification is required. PCA can provide useful information about the complex relationships of biological systems in a 2 dimensional graph where all the variables and factors are presented. PCA loadings can also provide information of the contribution and importance of factors on the overall changes that occur in a multivariate biological system.

Table 3.1: Average monthly values of environmental variables as recorded by weather stations across years and farms.

Month/ Year	Site	Medium temperature °C	RH %	Ozone (µg/m^3)	Solar irradiation MJ/m^2	VPD KPa
01/2010	SP1	11.3	80.0	37.8	7.3	0.27
02/2010	SP1	12.2	80.1	45.1	10.8	0.28
03/2010	SP1	13.5	73.5	39.4	15.6	0.41
04/2010	SP1	17.2	68.8	44.0	23.8	0.61
05/2010	SP1	18.4	59.5	86.9	28.3	0.85
Mean 2010 Harvest period	**SP1**	**14.5**	**72.4**	**50.6**	**17.2**	**0.49**
01/2011	SP1	11.5	84.8	53.3	8.1	0.20
02/2011	SP1	12.2	77.5	70.9	13.1	0.32
03/2011	SP1	13.6	78.3	79.1	16.2	0.34
04/2011	SP1	17.7	75. 7	87.1	19.8	0.49
05/2011	SP1	20.4	71.0	86.1	23.8	0.70
Mean 2011 Harvest period	**SP1**	**15.1**	**77.9**	**75.3**	**16.2**	**0.41**
01/2010	SP2	10.4	83.5	38.1	7.8	0.20
02/2010	SP2	11.83	85.4	45.1	10.1	0.20
03/2010	SP2	13.2	77.4	39.4	15.2	0.34
04/2010	SP2	17.0	72.7	44.0	20.9	0.59
Mean 2010 Harvest period	**SP2**	**13.1**	**79.8**	**41.7**	**13.5**	**0.32**
01/2011	SP2	10.6	88.9	53.5	8.0	0.14
02/2011	SP2	10.9	83.5	70.9	13.0	0.22
03/2011	SP2	12.84	82.3	79.1	16.3	0.26
04/2011	SP2	17.0	78.5	87.1	19.0	0.41
05/2011	SP2	20.0	74.6	86.1	23.4	0.59
Mean 2011 Harvest period	**SP2**	**14.3**	**81.6**	**75.3**	**16.0**	**0.33**
01/2012	SP2	9.2	82.0	59.1	11.0	0.21
02/2012	SP2	7.9	65.3	64.0	16.0	0.37
03/2012	SP2	12.8	71.3	66.6	18.0	0.42
04/2012	SP2	14.3	73.0	54.9	21.1	0.44
Mean 2012 Harvest period	**SP2**	**12.8**	**73.2**	**66.1**	**17.9**	**0.36**
08/2010	UK	15.1	79.2	42.3	13.4	0.36
09/2010	UK	13.5	83.2	37.6	10.0	0.26
10/2010	UK	10.0	85.0	37.9	6.6	0.18
Mean 2010 Harvest period	**UK**	**12.9**	**82.5**	**39.2**	**10**	**0.26**

3.3.1 Fresh fruit measurements and their relationship with environmental factors

3.3.1.1. Firmness

The main finding was that increased temperature before harvest were found to have a negative impact (i.e. firmness is reduced) on strawberry fruits (cv. Candonga) at both Spanish farms located in Lepe and Moguer. As it will be presented later on in this chapter at the part of harvesting period where higher temperatures were recorded (mainly at the final part of picking season) fruits were becoming softer.

At the UK farm, despite the fact that there was a reduced number of observations (six), a negative trend between temperature and strawberry fruit (cv. Elsinore) firmness was also observed.

Strawberry fruit firmness at harvest varied throughout each cultivation year, with the highest values of firmness recorded at the beginning of the season for Spanish farms (Figures 3.6 and 3.7). For the UK site firmer fruits were observed at the end of the season (Figure 3.8). In both countries firmer fruits were harvested when recorded temperature before harvest was at its' lowest value.

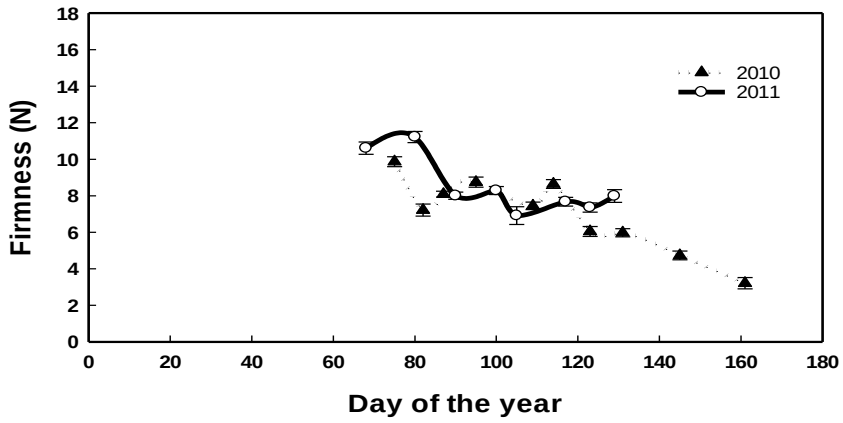

Figure 3.6: Average firmness in Newtons (N) at harvest of cv. Candonga strawberry fruits (n=27) at each sampling week at the SP1 site during 2010 and 2011.

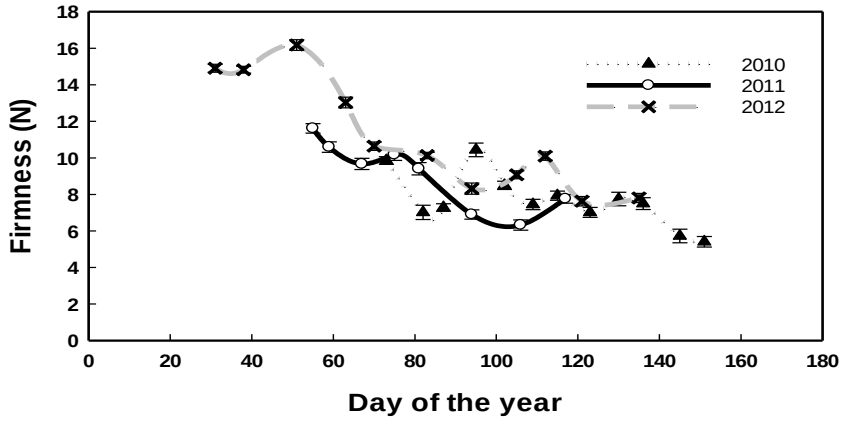

Figure 3.7: Average firmness in Newtons (N) of cv. Candonga strawberry fruits (n=27) at each sampling week at the SP2 site during 2010, 2011 and 2012.

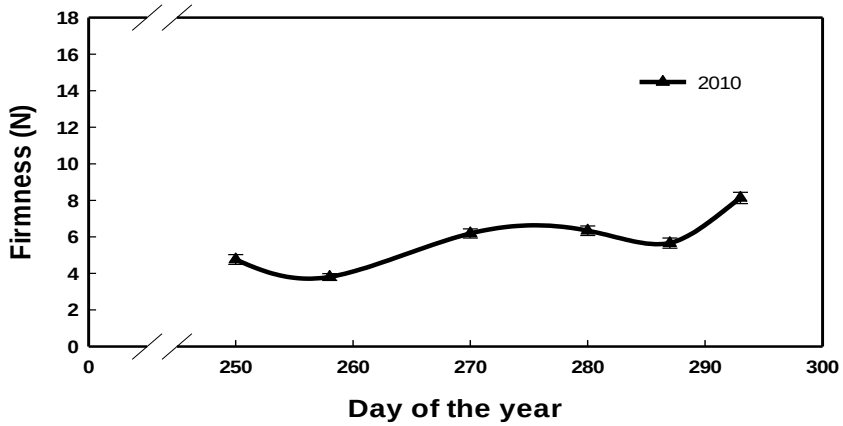

Figure 3.8: Average firmness (N) of cv. Elsinore strawberry fruits (n=27) at each sampling week at the UK site during 2010.

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There were also variations in fruit firmness between harvest seasons and sites (Table 3.2). The lowest firmness was observed at the UK site where strawberries of cv. Elsinore were grown. Firmer fruits were produced in Spanish farms where cv. Candonga strawberries were cultivated. Between Spanish sites there were differences. However, no direct comparisons could be made since the length of each season varied and sampling dates for each site were not identical.

Table 3.2: Mean firmness of strawberry fruits over sites and harvest seasons ±se. (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	Firmness (N)
SP1	1	7.00 ± 0.14
SP1	2	8.51 ± 0.15
SP1	1,2*	7.67 ± 0.10
SP2	1	7.66 ± 0.12
SP2	2	9.06 ± 0.15
SP2	3	11.15 ± 0.13
SP2	1,2,3*	9.25 ± 0.08
UK	1	5.82 ± 0.18

* Mean across years

Application of linear models show the effect of temperature on firmness was statistically significant ($p<0.001$) at 2 days (Table 3.3), 1 week (Table 3.4) and three weeks (Table 3.5) prior to harvesting. The negative effect of temperature on strawberry firmness was consistent apart from exceptions at the UK site which could be explained by limited number of observations

Table 3.3: Linear firmness model and significance level of environmental variables 2 days prior to harvest (Candonga in SP1 & SP2, Elsinore in UK).

Site/ Year	R^2	Firmness =	Temperature (T)	VPD (V)	Irradiation (I)	Ozone (O)
SP1/ 1	0.4209	-0.6T+0.2I-3.8V-0.05O+17.6	<.001	<.001	0.010	<.001
SP1/ 2	0.2061	-1.5T +0.3I +5.2V+0.02O+24.1	<.001	0.006	0.017	0.422
SP1/ 1,2*	0.1617	-0.5T-0.1I +0.6V+0.02O	<.001	0.574	0.046	0.110
SP2/ 1	0.2303	-0.4T +0.03I+1.2V-0.02O +14.2	<.001	0.108	0.290	0.085
SP2/ 2	0.3898	0.2T+1.0I -24.6V-0.2O +14.6	0.281	<.001	<.001	<.001
SP2/ 3	0.4040	-0.6T -0.4I+8.1V-0.03O+23.8	<.001	<.001	<.001	0.079
SP2/ 1,2,3*	0.3943	-0.7T -0.1I +5.2V-0.02O +20.5	<.001	<.001	<.001	0.001
UK/ 1	0.4209	0.7T+0.3I-77.5V+0.2O+5.0	<.001	<.001	<.001	<.001

* Mean across years

Table 3.4: Linear firmness model and significance level of environmental variables 7 days prior to harvest (Candonga in SP1 & SP2, Elsinore in UK).

Site/ Year	R^2	Firmness=	Temperature (T)	VPD (V)	Irradiation (I)	Ozone (O)
SP1/ 1	0.4309	-0.4T-4.4V+0.2I -0.03O +14.9	<.001	0.003	0.042	0.021
SP1/ 2	0.1964	-0.4T-0.3I+6.8V -0.009O+19.8	0.051	0.003	0.056	0.886
SP1/ 1,2*	0.3161	-0.5T+0.1I -3.8V-0.0008O+15.5	<.001	<.001	0.040	0.943
SP2/ 1	0.2726	-0.5T+0.08I+0.2V-0.02O+15.0	<.001	0.850	0.146	0.246
SP2/ 2	0.3693	-0.3T+0.2I -10.7V -0.1O+ 22.7	0.002	0.005	0.125	<.001
SP2/ 3	0.4294	-0.8T+0.02I+7.2V -0.06O+21.5	<.001	0.889	0.003	0.012
SP2/ 1,2,3*	0.446	-0.7T -0.07I+4.2V-0.015O+ 20.0	<.001	<.001	0.164	0.103
UK/ 1	0.3878	0.04T+0.1I-22.3V+0.03O+7.5	0.825	0.257	0.025	0.624

* Mean across years

Table 3.5: Linear firmness model and significance level of environmental variables 21 days prior to harvest (Candonga in SP1 & SP2, Elsinore in UK).

Site/ Year	R^2	Firmness=	Temperature	VPD (V)	Irradiation (I)	Ozone (O)
SP1/ 1	0.3486	-0.2T -0.09I+0.8V- 0.07O+15.0	0.032	0.110	0.648	<.001
SP1/ 2	0.1981	-0.3T - 0.3I+1.7V+0.01O+18.0	0.417	0.467	0.368	0.917
SP1/ 1,2*	0.2204	-0.3T-0.02I- 3.5V_0.007O+15.0	<.001	0.003	0.664	0.518
SP2/ 1	0.2016	0.1T-0.2I+3.1V-0.05O +10.4	0.026	0.073	<.001	<.001
SP2/ 2	0.4347	0.7T-1.0I-3.7V+0.07O+10.9	0.059	0.025	0.012	0.556
SP2/ 3	0.4963	-0.4T+0.02I+9.8V -0.2O +25.2	<.001	0.138	0.921	<.001
SP2/ 1,2,3*	0.3774	-0.5T -0.09I+1.4V - 0.04O+20.5	<.001	0.297	0.038	<.001
UK/ 1	0.3822	-1.8T-1.9I+144.8V-0.8O +40.9	<.001	<.001	<.001	<.001

* Mean across years

At all three sites in both the UK and Spain, and across all harvest seasons there was a negative relationship between firmness of fruits and temperature one week prior to harvest (Figures 3.9, 3.10 and 3.11). Despite the fact that harvest seasons extended for different time periods across years, and there were also temperature variations, the negative trend between increased temperature and reduced strawberry firmness was observed constantly.

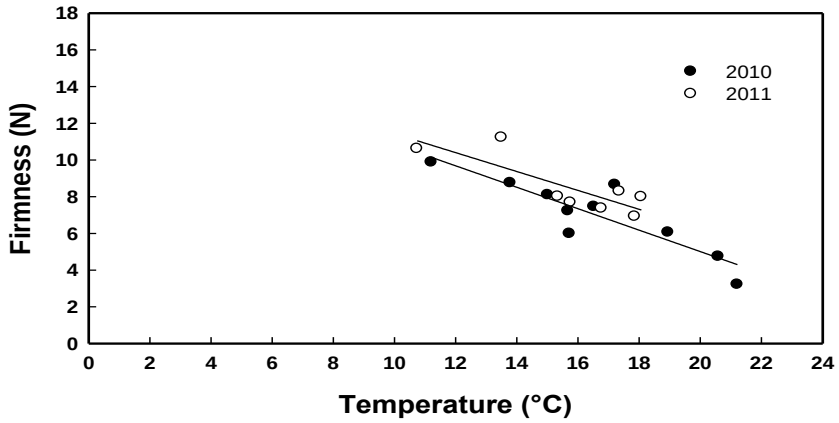

Figure 3.9: Relationship between mean firmness (N) of cv. Candonga fruits, (n=27) per sampling week (R^2 2010 0,69 and 2011 0,78) and mean temperature one week prior to harvest at the Spanish (SP1).

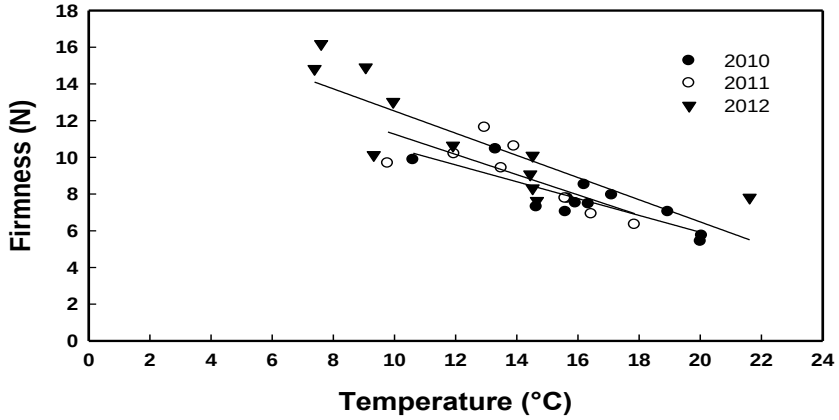

Figure 3.10: Relationship between mean firmness (N) of cv. Candonga strawberry fruits, (n=27) per sampling week (R^2 2010 0,68, 2011 0,73 & 2012 0,58), and mean temperature one week prior to harvest at the Spanish (SP2).

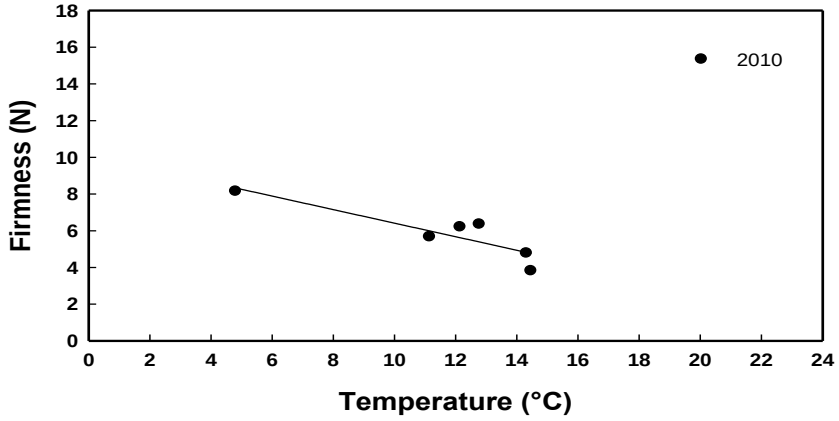

Figure 3.11: Relationship between mean firmness (N) of cv. Elsinore fruits, (n=27) per sampling week (R^2 for 2010 0,79), and mean temperature one week prior harvest at the UK site.

Principal Components Analysis (PCA) for examination of the relationship between environmental variables was performed (Figure 3.12, 3.13 and 3.14). More than 85% of variability of monitored environmental variables was explained by two components, with F1 being able to explain more than 65% at all three strawberry farms in the UK and Spain. F1 was related to increased temperature, irradiation, VPD and reduced relative humidity (% RH) values, leading to the assumption that positive values of F1 could be connected to weather patterns more likely to occur in late spring and summer. On the other hand, negative values of F1 could be related to weather patterns occurring in winter where colder, cloudy days and low RH and VPD values occur. F2 positive values were related to increased ozone levels at all farms and elevated RH levels in Spanish sites.

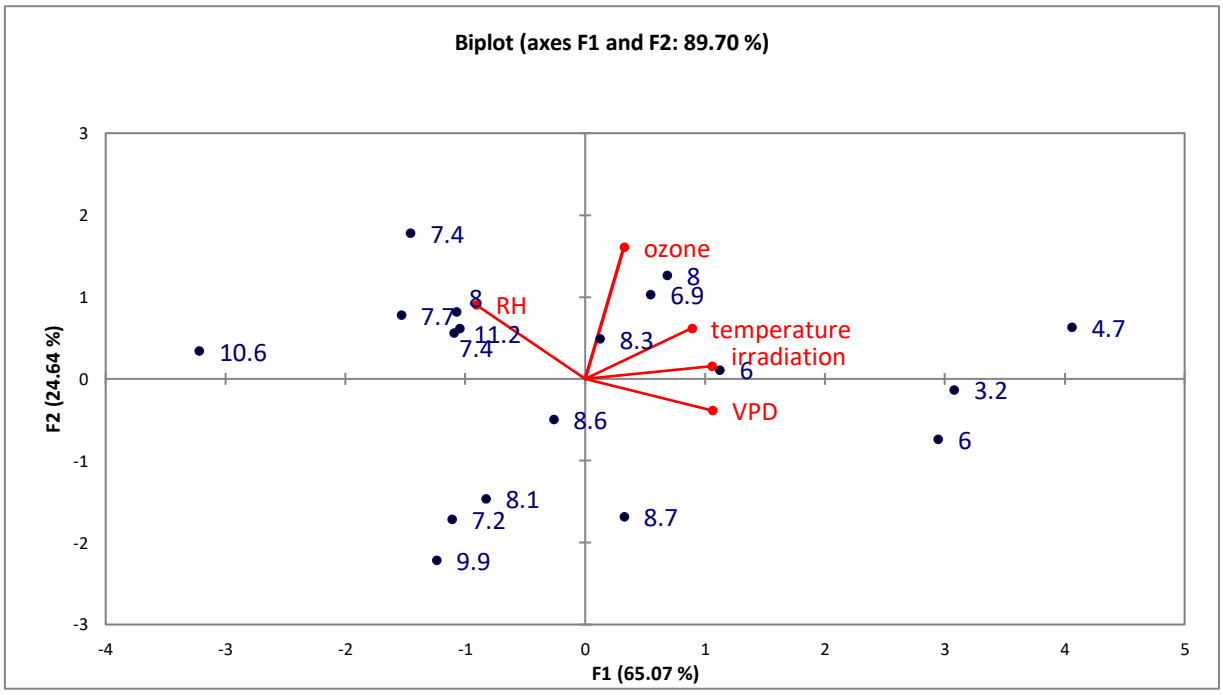

Figure 3.12: PCA of environmental conditions against mean firmness of sampling weeks of cv. Candonga strawberry fruits over two seasons at SP1 site.

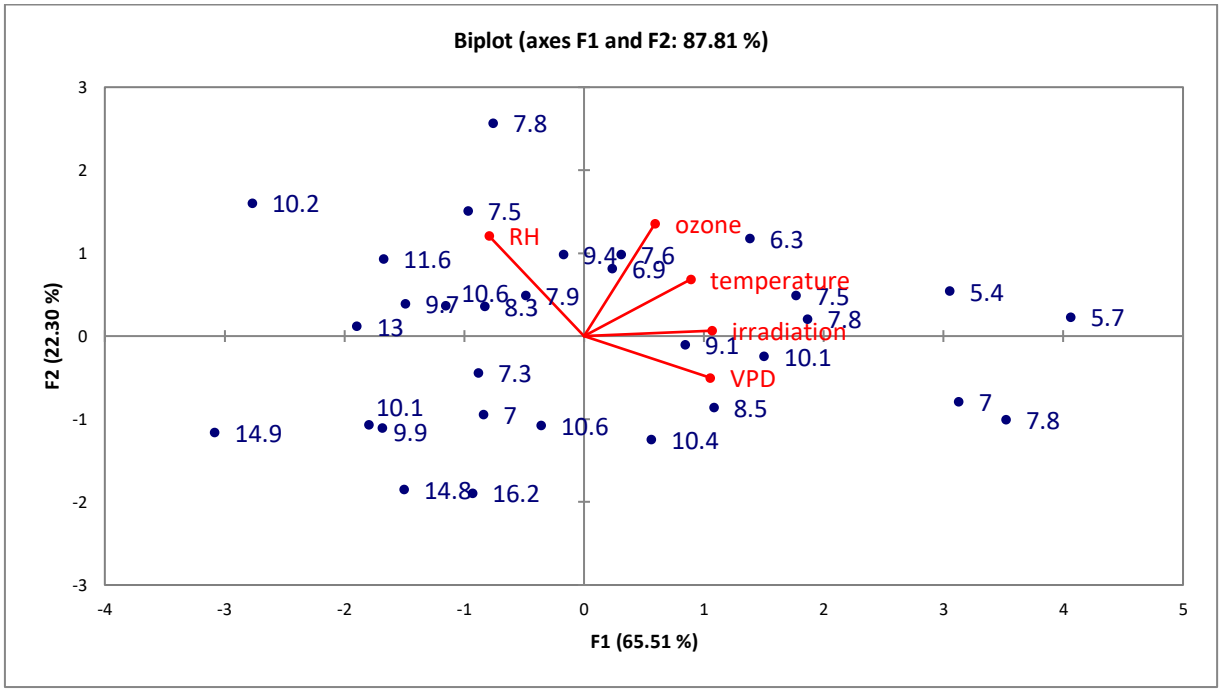

Figure 3.13: PCA of environmental conditions against mean firmness of sampling weeks of cv. Candonga strawberry fruits over three seasons at SP2 site.

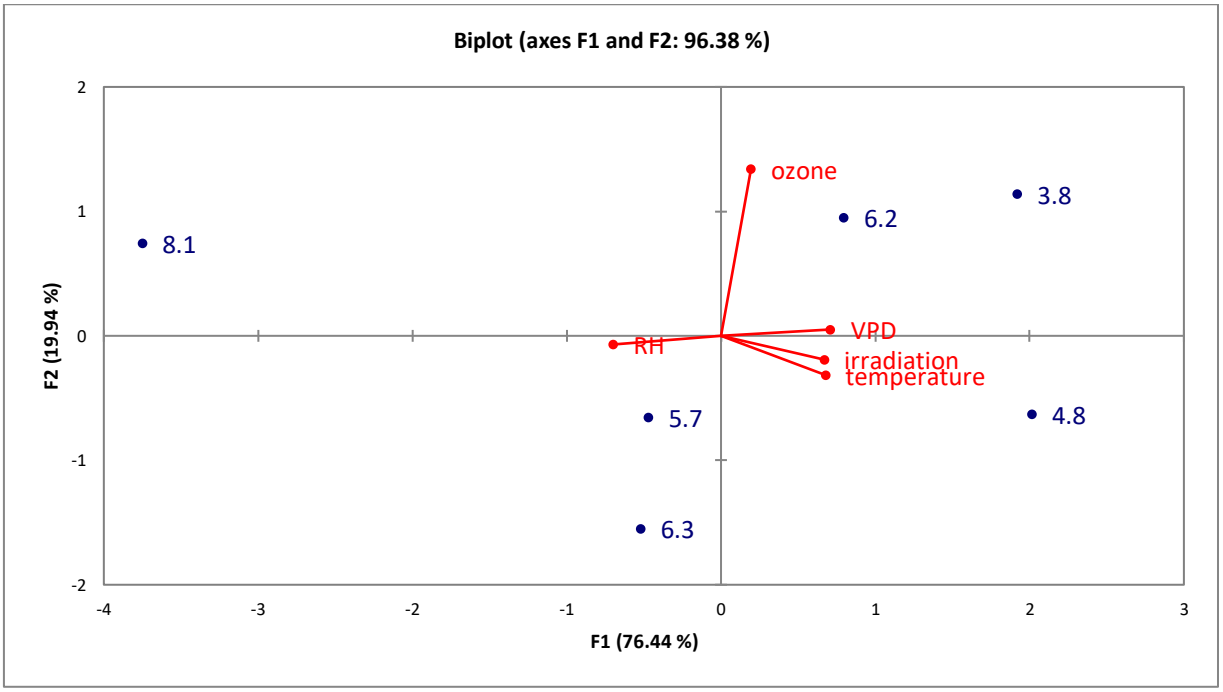

Figure 3.14: PCA of environmental conditions against mean firmness of sampling weeks of cv. Elsinore strawberry fruits over one season of experiment at the UK site.

As expected, there was a negative relationship between VPD and RH values. This is expected since it is much easier for plants to lose water when RH is low, and therefore VPD is high. Furthermore, temperature, irradiation, VPD and ozone had positive loadings in F1. This is also expected as increased solar irradiation and temperature levels occur later in the spring - summer

period, a fact that leads to increased ozone levels (mainly in Spain) due to photochemical breakdown of nitric oxide.

Sampling weeks with the lowest mean firmness were placed at the far right of the PCA graph where F1 axis had positive values. On the contrary, sampling weeks with the firmest fruits were placed at the left site of the graphs where F1 had negative values. There was no clear positioning of the sampling weeks against the F2 axis since firm and soft fruits could be related with both positive and negative values of this axis. The above observation agrees with the linear models (Table 3.3, 3.4 and 3.5) where variables primarily temperature, followed by VPD were found to have significant effects on firmness.

The negative effect of higher growth temperatures on fruit firmness has been observed previously (Sams, 1999). Increased temperature can increase VPD levels and therefore dehydration of fruits. Dehydration of fruits can decrease cell turgor (Shackel *et al.,* 1991) resulting in firmness loss. Furthermore temperature can increase metabolism and enzymatic activity. Enzymes that are related to strawberry cell wall degradation that lead to softening are (pectin methylesterases) PMEs (Draye and Van Cutsem, 2008; Wolf *et al.*, 2009), PGs (Lefever *et al.,* 2004), B-galactosydase (Trainotti *et al.*, 2001) α-l-arabinofuranosidase (Carpita and Gibeaut, 1993; Rosli *et al.*, 2009), Endo-β-1,4-glucanases (EGases), (Harpster *et al.*, 1998; Brummell and Harpster, 2001), β-xyloxidase (Martínez *et al.*, 2004; Bustamante *et al.,* 2006) , exo-mannanases (Bourgault *et al.*, 2001), Xyloglucan endotransglucosylase (XTH) (Fry, 2004) and expansins, (Harrison *et al.*, 2001). Solar radiation plays a role in fruit firmness since light is necessary for fruit development. However, irradiation that exceeds photosynthetic saturation levels can damage fruits directly or can affect them indirectly by increasing temperature, leading to firmness loss (Sams, 1999). Furthermore, absence of UV radiation was found to decrease firmness of strawberry fruits (Tsormpatsidis *et al.*, 2011; Ordidge *et al.*, 2012), although, the mechanism that UV radiation contributes to increased firmness is not completely understood. High humidity levels lead to decreased firmness of strawberry fruits grown in glasshouse. It was suggested that higher humidity prevented movement of calcium from roots to fruits (Lieten, 2000). Lower firmness of fruits was recorded at the part of harvesting season where increased temperature, irradiation and decreased RH levels occurred. Since increased UV radiation and reduced RH levels are related to increased firmness, and yet in this situation the firmness actually decreased it could be assumed that temperature had the predominant role in fruit softening. This fact was also highlighted by linear models where the negative effect of temperature on firmness was classified as significant, compared to other environmental factors.

A decreasing trend in strawberry firmness of cv. Candonga was also reported (Correia *et al.*, 2011) through harvesting season for cv. Candonga. Fruits were getting softer when temperature rose. That trend was constant even when different applications of calcium took place. Firmness of strawberry fruits decreased through the season when they were sampled between the 22nd February and 28th May 2008 (Correia *et al.*, 2011).

3.3.1.2. Colour measurements (Chroma, °Hue, *L**, *a** and *b**)

Chroma, ° Hue, *L**, *a** and *b** mean weekly values were recorded at the two Spanish and the English farms. At the UK farm the highest *a** values were recorded. *a** value, an indication of red colour of strawberry fruits, was found to have low variation across years and sites in Spain. However, differences were observed for *b** value across sites and sampling seasons. At both Spanish sites *b** was higher in 2011when compared to 2010. Fruit from the UK farm had higher *b** values than Spanish fruit (Table 3.6).

Table 3.6: Colour of strawberry fruits over sites and harvest seasons ±se (Candonga in SP1 & SP2, Elsinore in UK)..

Site	Year	Chroma	°Hue	*L**	*a**	*b**
SP1	1	44.4 ± 0.22	32.2 ± 0.28	37.7± 0.20	37.4 ± 0.15	23.8 ± 0.27
SP1	2	45.8 ± 0.26	34.0 ± 0.34	40.1 ± 0.27	37.8 ± 0.16	25.7 ± 0.34
SP1	1,2*	45.0 ± 0.17	33.0 ± 0.22	38.8 ± 0.17	37.5 ± 0.11	24.6 ± 0.22
SP2	1	43.7 ± 0.19	30.6 ± 0.20	36.8 ± 0.15	37.5 ± 0.13	22.3 ± 0.20
SP2	2	48.1 ± 0.24	37.0 ± 0.26	38.2 ± 0.18	38.4 ± 0.17	28.9 ± 0.26
SP2	3	46.5 ± 0.22	35.6 ± 0.24	39.2 ± 0.20	37.6 ± 0.14	27.1 ± 0.27
SP2	1,2,3*	45.8 ± 0.14	34.0 ± 0.17	38.0 ± 0.11	37.8 ± 0.08	25.7 ± 0.17
UK	1	49.9±0.27	38.4±0.28	43.5±0.23	39.0±0.21	31.0±0.30

* Mean across years

Chroma had its highest values at the UK farm (Table 3.6). At the UK site the lowest values were observed during early season sampling, however due to limited sampling there is no clear trend and no definite conclusions could be drawn. At both Spanish sites there was increased variability between sampling weeks for all sampling years, and the values for Chroma displayed no clear trend between early and late season harvesting. The lowest Chroma values were recorded in first sampling year (2010) for the SP2 site and the highest in 2011. This could

mainly be attributed to the effect of decreased *b** values that were observed at 2010 and to a lesser extend to decreased *a** values (Table 3.6).

°Hue followed similar pattern to Chroma at all sites. It received its highest values at the UK farm. Lower levels of °Hue were noticed at the two Spanish farms (Table 3.6). At the UK site the lowest values were noticed at early season harvesting weeks. At the Spanish sites variability was observed between sampling weeks at all sampling years. Following a similar pattern to that of Chroma, lowest °Hue values were observed at first sampling year (2010) for SP2 site and the highest °Hue values at the second year (2011).

*L** values generally varied through harvest season at all sites and no obvious effect of time of harvest could be identified, the only exception was noticed at SP1 farm for 2010 where at the second half of the season increased values were observed when compared to the first half. For the UK farm due to limited sampling no definite conclusion should be drawn despite the fact that decreased values were observed at the beginning of the season. However, at the UK farm the highest *L** values were observed when compared to Spanish sites (Table 3.6). At SP2 site the lowest values were noticed during first sampling year (2010) and a similar observation took place for SP1. Application of linear models did not reveal any constant significant effect.

Colour of strawberries can be affected by many environmental variables. At the SP1 site chroma had similar values between years, however at SP2 site the levels of chroma were lower at the first year and highest at the second. At the English farm more intense chroma was observed at late sampling season. °Hue followed similar pattern to chroma and *L** showed lower values at 2010 at Spanish sites. Lower *L** values were found to be affected by amount of UV radiation. Decreased UV radiation levels at decrease *L** value of fruits (Tsormpatsidis *et al.*, 2011). This fact could probably be related with the reduced levels of lightness (*L**) since at 2010 the lowest levels of radiation was observed. Colour of strawberry fruits is affected by many variables apart from genotype and cultural practices. Amount of anthocyanins is related to strawberry colour (Ordidge *et al.*, 2012). Bruise is also another variable that should be taken into account for assessment of strawberry colour. It is expected that all environmental variables that affect the above parameters should contribute to final colour development. The complex mechanism of strawberry colour development and its interaction with environmental parameters will require further study, no clear relationship was identified from application of linear models (Tables B.6 – B.14).

3.3.1.3. Weight

Fruit size varied across the UK and Spanish farms, with the heavier weight fruits being produced at the SP2 site and the lightest at the UK site.

Table 3.7: Mean weight (g) of strawberry fruits over sites and harvest seasons (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	Weight (g)
SP1	1 (10 harvest weeks)	21.9 ± 0.50
SP1	2 (8 harvest weeks)	20.3 ± 0.73
SP1	1,2*	21.2 ± 0.43
SP2	1(12 harvest weeks)	24.8 ± 0.38
SP2	2(8 harvest weeks)	30.5 ± 0.65
SP2	3(11 harvest weeks)	25.7 ± 0.51
SP2	1,2,3*	26.3 ± 0.30
UK	1(6 harvest weeks)	19.4 ± 0.40

* Mean across years

There was a trend of decreasing fruit size over the sampling period at both Spanish sites and throughout all years, with heavier fruits being produced at the beginning of each season (Figures 3.15 and 3.16). For the identical sampling period at SP2 farm, the lightest fruits were produced during the 2012 sampling year (Figure 3.16). At the UK farm strawberry weight did not vary greatly through the sampling period, however heavier fruits were produced at the last sampling week (Figure 3.17). Application of linear models revealed a significant negative effect of temperature on fruit weight at most cases. However, findings should be examined with caution since physiological stage of the fruit was not included in the models and it is quite likely to play a role in fruit size.

Generally, primary fruits are bigger and the size of fruits is getting progressively smaller through the season (Darrow, 1966; Arias, 2006). This trend is aligned with the findings at Spanish farms. In the UK site only the fruits of last sampling week had increased size. However, a clearer picture for the whole season was not possible because of the limited sampling weeks. Furthermore, lower temperature levels (Wang and Camp, 2000), and increased irradiation (Atkinson *et al.*, 2006) were also positively related to increased size of fruits and could probably enhance the seasonal trend for heavier fruits at early season in UK and Spain respectively. In

addition deficit irrigation conditions that could result because of increased VPD levels could decrease the size of fruit at late season in Spain (Giné Bordonaba, 2010).

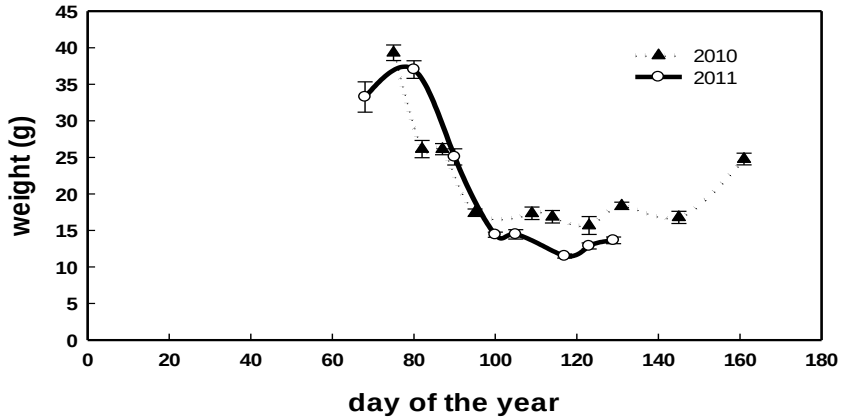

Figure 3.15: Average weight (g) of cv. Candonga strawberry fruits (n=27) at each sampling week at SP1 site during 2010, and 2011.

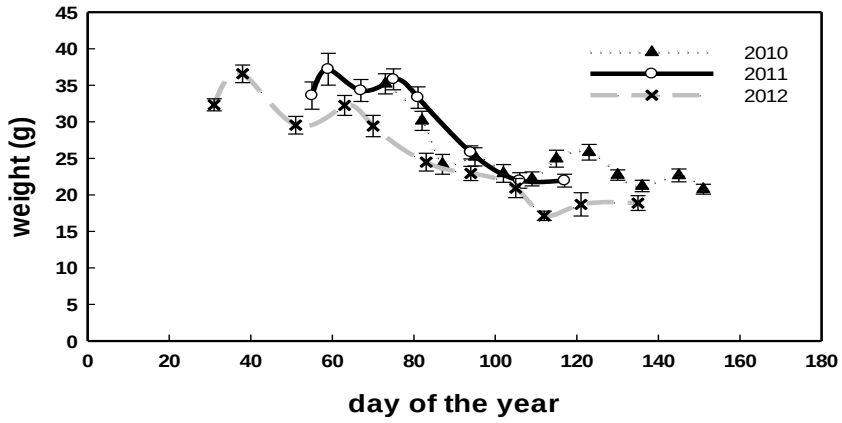

Figure 3.16: Average weight (g) of cv. Candonga strawberry fruits (n=27) at each sampling week at SP2 site during 2010, 2011 and 2012.

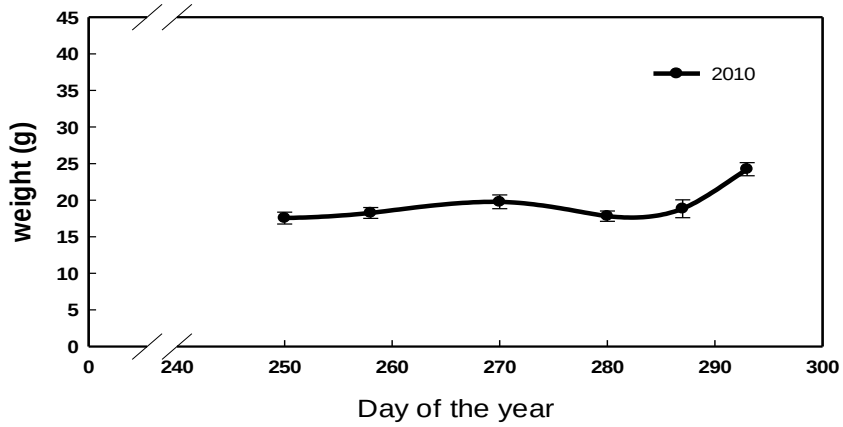

Figure 3.17: Average weight (g) of cv. Elsinore strawberry fruits (n=27) at each sampling week at the UK site during 2010.

3.3.1.4 Total Soluble Solids (TSS)

The amount of TSS recorded in SP1, SP2 showed a double peak pattern where the highest TSS values where observed at the beginning and final part of harvest season (Figures 3.18 and 3.19). At the UK site the sampling period took place at the end of the harvest season and fruits had rather stable profile for TSS content. However, due to limited sampling at the English farm no definite conclusions could be drawn (Figure 3.20). The TSS values for SP1 were similar at both sampling seasons with average fruit TSS content ranging mainly between 6% and 9%. Generally, supermarkets consider any fruits with TSS < 6% to be of poor quality. Fruits that have TSS content above 7% are considered as acceptable quality produce (Rodanto Ltd, pers. comm.). At the SP1 farm the third sampling week of 2010 had TSS levels very close to 5%, a value that is low for supermarket standards, with the sixth sampling week of the same sampling season being borderline (5.9 °Brix). During the 2011 sampling season TSS values exceeded 6% at all sampling weeks (Figure 3.18). At the SP2 site the mean weekly TSS value was always above 7% with the exception of the third sampling week at 2010 (6.3 °Brix). At SP2 it was not uncommon for TSS values to exceed 9%, especially during early and late season (Figure 3.19). This value is an indication of higher quality fruits (Rodanto Ltd, pers. comm.). At the UK farm (Figure 3.20). TSS values were between 7% and 8% with the only exception being noticed at the last sampling week (9.6°Brix).

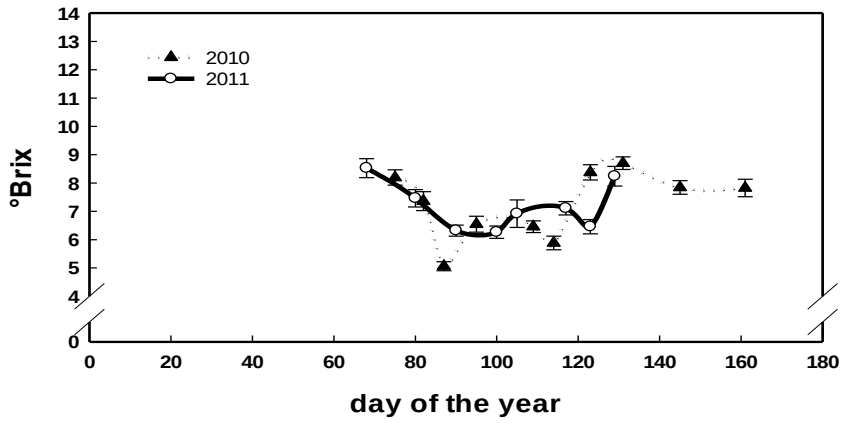

Figure 3.18: Average total soluble solids of cv. Candonga strawberry fruits (n=27) at each sampling week at SP1 site during 2010, and 2011.

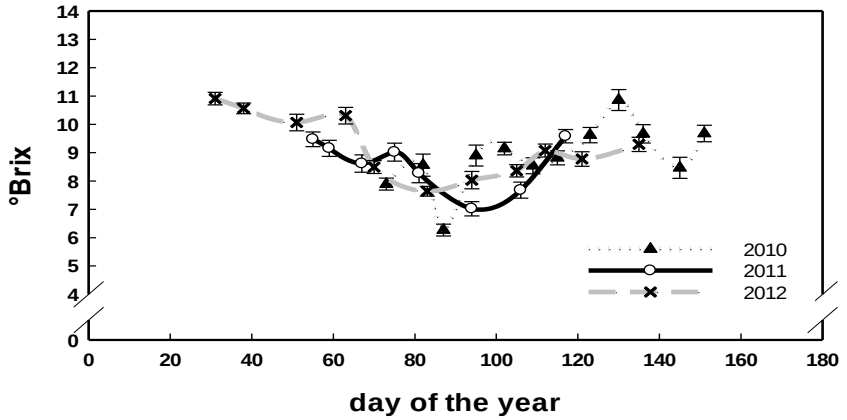

Figure 3.19: Average total soluble solids of cv. Candonga strawberry fruits (n=27) at each sampling week at SP2 site during 2010, 2011 and 2012.

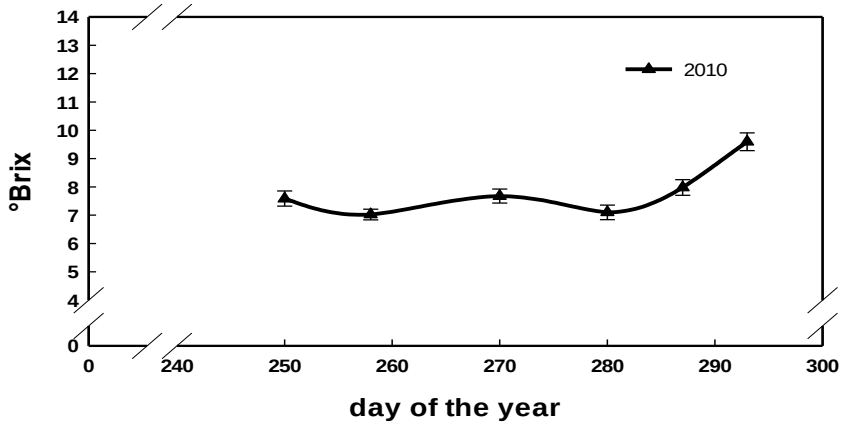

Figure 3.20: Average total soluble solids of cv. Elsinore strawberry fruits (n=27) at each sampling week at the UK site during 2010.

The Spanish (SP2) site had the highest TSS when compared to the other two farms and the sampling season with the highest TSS values, for SP2, was 2012. However, it should be noticed that above average TSS levels were recorded during the first half of the 2012 season (Figure 3.19), with the remainder of the season following values that were similar to previous years at SP2. The application of linear models did not reveal further information about the distribution of TSS over season. In general low correlations were observed and no clear conclusion for the environmental effects could be drawn.

Table 3.8: Mean total soluble solids (°Brix) of strawberry fruits over sites and harvest seasons (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	°Brix
SP1	1 (10 harvest weeks)	7.23 ± 0.11
SP1	2(8 harvest weeks)	7.16 ± 0.12
SP1	1,2*	7.20 ± 0.08
SP2	1 (12 harvest weeks)	8.87 ± 0.10
SP2	2 (8 harvest weeks)	8.60 ± 0.23
SP2	3 (11 harvest weeks)	9.23 ± 0.11
SP2	1,2,3*	8.92 ± 0.06
UK	1 (6 harvest weeks)	7.83 ± 0.12

* Mean across years

3.3.2 Measurements on freeze-dried fruits

3.3.2.1. Carbohydrates

The levels of total sugars varied between sites with the SP1 producing fruits with the lowest carbohydrate content. The highest amount of carbohydrates was found in fruits of the SP2 site.

These results are consistent with the TSS data. Fructose was the dominant carbohydrate at all three sites, followed by glucose and sucrose. However, it should be noticed that the individual carbohydrate ratio varied through seasons. Total amount of carbohydrates varied through the harvesting season. At the two Spanish sites farms lower values of total carbohydrates, expressed on dry weight basis, were observed in the middle of the season. At SP1 increased sugars' content was recorded at the end of the season for both sampling years (Figure 3.21). At SP2,

increased sugar content was also observed at the beginning of 2012 harvest season (Figure 3.22). At the UK site variation of sugar content increased levels of sugars were accumulated at the two final strawberry harvests (Figure 3.23).

Table 3.9: Sugars, (Sucrose + Fructose + Glucose) expressed on dry (DW) and fresh weight basis (FW), and Sweetness Index of strawberry fruits (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	Fresh Weight/ Dry Weight	Total sugars (mg/g)	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Sweetness Index
SP1	1 (10 weeks)	FW	45.9 ± 0.6	13.9 ± 0.2	17.3 ± 0.3	14.7 ± 0.2	73.2 ± 1.1
		DW	388.3 ± 4.8	118.3 ± 2.0	146.2 ± 2. 3	123.8 ± 1.7	
SP1	2 (8 weeks)	FW	42.5 ± 0.7	8.5 ± 0.20	19.6 ± 0.3	14.3 ± 0.2	71.0 ± 1.2
		DW	376.8 ± 5.8	75.9 ± 1.8	173.6 ± 2.5	127.4 ± 1.9	
SP1	1,2*	FW	44.4 ± 0.5	11.5 ±0.2	18.3 ± 0.2	14.5 ± 0.2	72.2 ± 0.8
		DW	383.2 ± 3.7	99.2 ± 1.8	158.5 ± 1.8	125.4 ± 1.3	
SP2	1(12 weeks)	FW	48.4 ± 0.5	16.9 ± 0.3	16.5 ± 0.2	15.0 ± 0.2	75.7 ±0.8
		DW	428.2 ± 3.9	149.6 ± 2.3	145.9 ± 1.4	132.7 ± 1.4	
SP2	2 (8 weeks)	FW	52.6 ± 0.8	10.5 ± 0.2	24.3 ± 0.38	17.8 ± 0.3	87.8 ± 1.4
		DW	439.6 ± 6.7	88.5 ± 2.1	202.5 ± 2.9	148.6 ± 2.2	
SP2	3 (11 weeks)	FW	51.7 ± 0.6	13.9 ± 0.2	20.8 ± 0.25	17.1 ± 0.2	83.6 ± 1.0
		DW	448.6 ±4.7	120.3 ± 1.9	180.4 ± 2.0	147.9 ± 1.6	
SP2	1,2,3*	FW	50.7 ± 0.4	14.2 ± 0.2	20.0 ± 0.2	16.5 ± 0.13	81.7 ± 0.6
		DW	438.4 ± 2.9	123.2 ±1.6	173.0 ± 1.5	142.3 ± 1.0	
UK	1 (6 weeks)	FW	49.2 ± 0.5	14.0 ± 0.4	18.5 ± 0.2	16.7 ± 0.2	78.1 ± 0.8
		DW	427.9 ± 4.4	122.2 ± 3.6	160.7 ± 1.8	145.0 ± 1.6	

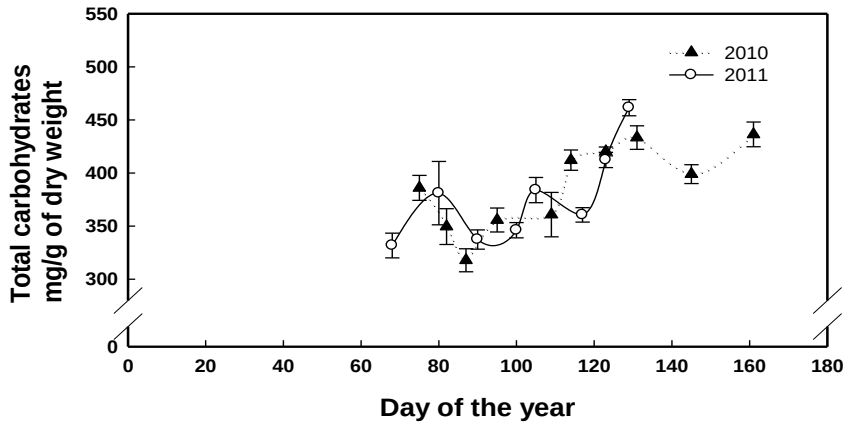

Figure 3.21: Total sugars (Sum of Sucrose + Fructose + Glucose) as mg/g of dry weight of cv. Candonga strawberry fruits at each sampling week (n=9) at the SP1 site during 2010, and 2011 ±se.

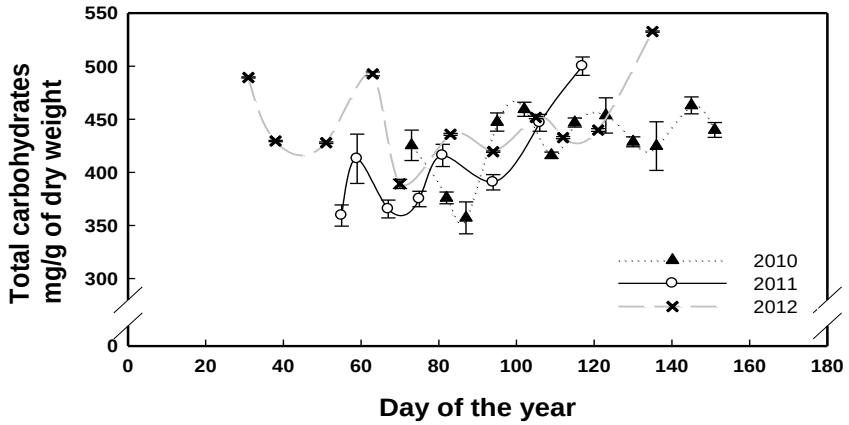

Figure 3.22: Total sugars (Sum of Sucrose + Fructose + Glucose) as mg/g of dry weight of cv. Candonga strawberry fruits at each sampling week (n=9) at the SP2 site between 2010 and 2012 ±se.

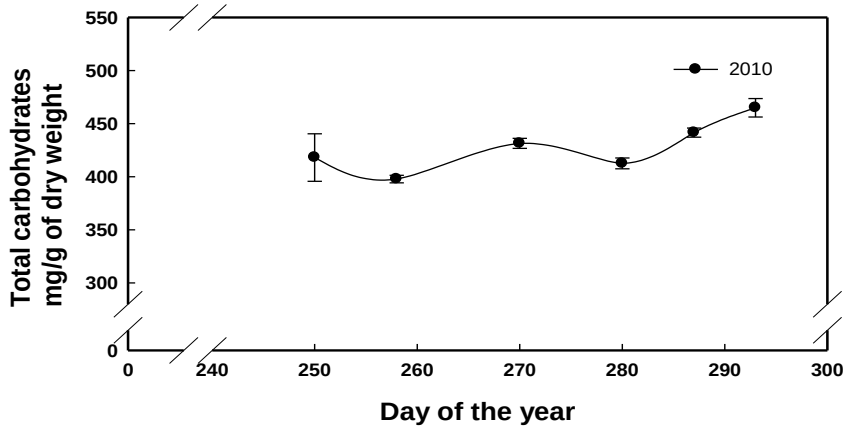

Figure 3.23: Total sugars (Sum of Sucrose + Fructose + Glucose) as mg/g of dry weight of cv. Elsinore strawberry fruits (n=9) at each sampling week at the UK site during 2010 ±se.

The total amount of carbohydrates, expressed on fresh weight basis, followed similar pattern to that of sugars expressed on dry weight basis. Again total carbohydrates decreased during the middle of the season for Spanish sites with increased sugars noticed at the end of harvesting. At SP2 increased levels were also witnessed for 2012 early season. At the UK farm the highest amount of sugars were observed at the end of the season.

Sweetness index (sum of 2.3 x fructose + 1 x glucose + 1.35 x sucrose) followed an increasing pattern towards the end of harvesting for SP1 during both growing years. At SP2 a similar trend was noticed for 2010 and 2011, however for 2012 increased sweetness index values were also noticed at early season. At the UK site lower values of sweetness index were noticed at the earlier sampling weeks. Application of linear models did not reveal any strong relation between environmental factors and total sugars (Tables B.18 to B.21).

TSS and sugars of strawberry fruits play an important role in consumer perception of quality. Within this research, the total soluble solids had a double peak pattern at Spanish sites, with lower values arising during the middle of the harvest season. This finding does not agree with other observations where increased temperatures were found to have a limiting effect on the formation of sugars (MacKenzie and Chandler, 2011). At the UK farm total soluble solids had increased values at the end of the season. However, the sampling period for the English site started late with no data covering the initial part of season. Increased solar radiation levels are related to increased levels of total soluble solids. Fruits grown in shade were found to have decreased values of soluble solids as well sucrose and fructose levels (Osman and Dodd, 1994; Watson, 2002). However, UV radiation was not found to alter the formation of soluble solids (Tsormpatsidis *et al.*, 2011). Increased temperature values, both night and day, were related to decreased amount of sugars and total soluble solids (Wang and Camp, 2000; Davik and Bakken, 2006; Matsuzoe *et al*., 2006). The preharvest effect of ozone varied according to the sensitivity of cultivar. With less sensitive cultivars there was no effect noticed on levels of sucrose and glucose. On the other hand, there was a negative effect of increased ozone levels on formation of sugars on the sensitive cv. Elsanta (Keutgen and Pawelzik, 2008). Increased VPD levels could result in extended dehydration of fruits, if appropriate irrigation is not supplied to plants, and they could lead to deficit irrigation, that could consequently increase levels of sugars due to concentration (Mahajan and Tuteja, 2005;Giné Bordonaba and Terry, 2010). However, in this study °brix levels followed a double peak in the beginning and at the end of the season. Total sugars and sweetness index followed an increasing pattern through season when expressed both

as per dry and fresh weight. This fact shows that the increased levels of sugars at the final harvest could not be attributed only to plant water relations. Physiological stage of plants could probably explain some of the patterns recorded in the research. Since no clear and constant effects of environment could be identified with the application of linear models, a closer look to plant growth pattern could explain present findings. Despite the fact that during the three year study no such measurements took place, it is suggested that total soluble solids content is negatively related to crop load of strawberry and other fruits and positively related with the leaf area/yield ratio (Link, 2000; Morinaga *et al.*, 2003; Atkinson *et al.*, 2006; Potel *et al.*, 2006; Correia *et al.*, 2011). Correia *et al.*, (2011) found the crop load of cv. Candonga was higher at the middle of the harvest season, whereas in this study total sugars, and °brix had the lowest levels at that period. This could be an indication that apart from environmental effects on postharvest quality, physiological parameters play an equally important role, and models that would include both aspects could probably have increased efficiency.

3.3.2.2 Acids

Fruits had variable acid content through harvest weeks. The dominant acid was found to be citric, followed by malic. Ascorbic acid was present at lower concentrations (Table 3.10). Fruits produced at the UK site had higher total acid content, followed by SP1. The lowest amounts of acids were recorded in fruits produced at SP2 farm.

At the SP1 farm total acids expressed as per dry weight followed an increasing pattern towards the end of the season at both sampling years, with the 2011 producing fruits with lower acid content compared to 2010 (Figure 3.24). At the SP2 farm no clear trend was noticed through different sampling years (Figure 3.25). Variation at total acid content, on dry weight basis, was also observed to fruits produced at the UK farm (Figure 3.26).

When total acid content was expressed on fresh weight basis, the trends were similar to that of acid content expressed on dry weight basis. At SP1 farm total acids were increased through season with fruits produced at the second sampling year having lower acid levels. At SP2 and the UK farms there was variation through harvest season and no clear trend was noticed. Application of linear models for total acids expressed per fresh weight did not reveal constant interactions between monitored environmental conditions and total acid content of strawberry fruits.

Table 3.10: Total acids, (Sum of citric + malic + ascorbic) expressed on dry (DW) and fresh weight basis (FW), of strawberry fruits over sites and harvest seasons (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	Fresh Weight/ Dry Weight	Total acids (mg/g)	Citric (mg/g)	Malic (mg/g)	Ascorbic (mg/g)
SP1	1(12 weeks)	FW	15.3 ± 0.5	11.7 ± 0.3	3.5 ± 0.1	0.26 ±0.02
		DW	178.7 ± 5.0	136.2 ± 3.6	41.4 ± 1.3	2.98 ± 0.25
SP1	2 (8 weeks)	FW	12.8 ± 0.3	9.9 ± 0.2	2.6 ± 0.1	0.37 ± 0.00
		DW	152.7 ± 3.2	117.6 ± 2.6	30.7 ± 0.8	4.44 ± 0.10
SP1	1,2	FW	14.2 ±0.3	10.9 ± 0.2	3.1 ± 0.1	0.31 ± 0.01
		DW	167. 1± 3. 3	127.9 ± 2.4	36.6 ± 0.9	3.63 ± 0.15
SP2	1 (10 weeks)	FW	14.1 ± 0.3	9.5 ± 0.3	3.8 ± 0.1	0.32 ± 0.03
		DW	151.2 ± 4.4	107.4 ± 3.1	43.3 ±1.2	3.56 ± 0.32
SP2	2 (8 weeks)	FW	13.6 ± 0. 3	9.9 ± 0.2	3.5 ± 0.1	0.23 ±0.00
		DW	153.3 ±3.1	111.4 ± 2.2	39.3 ± 1.2	2.62 ± 0.06
SP2	3 (11 weeks)	FW	11.9 ± 0.2	9.4 ± 0.2	1.8 ±0.1	0.73 ± 0.06
		DW	136.6 ±2.4	108.0 ± 2.3	20.9 ± 0.7	8.32 ± 0.70
SP2	1,2,3	FW	13.2 ±0.2	9. 6 ± 0.1	3.1 ±0.1	0.44 ± 0.01
		DW	146.7 ±2.1	108. 7 ± 1.5	34.5± 0.9	5.01 ± 0.15
UK	1(6 weeks)	FW	15.4 ± 0.2	10.9 ± 0.2	4.1 ±0.1	0.47 ± 0.02
		DW	176.8 ± 2.2	124. 8 ± 1.8	46.6 ± 1.1	5.41 ± 0.24

* Mean across years

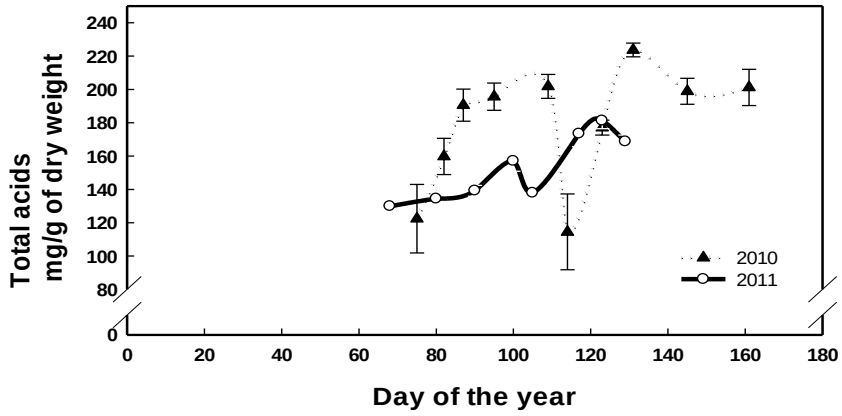

Figure 3.24: Total acids, (Sum of citric + malic + ascorbic) expressed as mg/g of dry weight of cv. Candonga strawberry fruits (n=9) at each sampling week at SP1 site during 2010, and 2011.

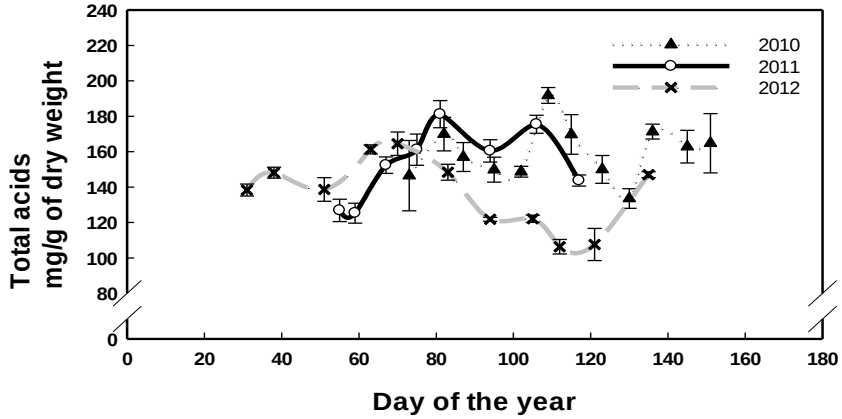

Figure 3.25: Total acids, (citric+malic+ascorbic) expressed as mg/g of dry weight of Candonga strawberry fruits (n=9) at each sampling week at SP2 site during 2010, 2011 and 2012.

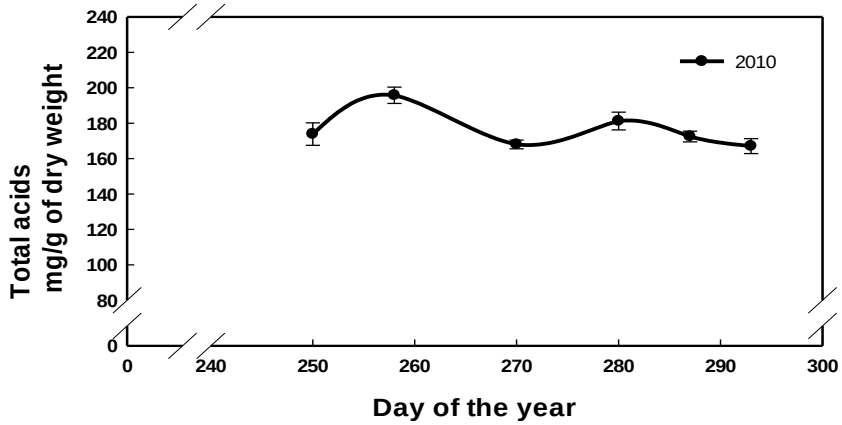

Figure 3.26: Total acids, (Sum of citric + malic + ascorbic) expressed as mg/g of dry weight of cv. Elsinore strawberry fruits (n=9) at each sampling week at the UK site during 2010.

There was an increasing trend of total acids through season at the SP1 farm. At SP2 there were seasonal variations and no clear trend was identified. At the UK site total acid concentrations also did not show any clear pattern through the season. An identical trend to that of SP1 farm as far as it concerns titratable acidity (TA) was also observed in three strawberry cultivars (Candonga, Ventana and Camarosa) when grown in Portugal (Correia *et al*., 2011). Generally it is considered that high temperature decreases ascorbic acid content in strawberry fruits (Wang and Camp, 2000). Increased ozone (Keutgen and Pawelzik, 2008) was also found to have a negative effect on ascorbic acid. However, it is generally accepted that increased solar irradiation levels have a positive effect on formation of ascorbic acid (Lee and Kader, 2000; Atkinson *et al.*, 2006). A negative effect of crop load on TA was identified in strawberry cv. Ventana, such a relationship was not verified for cv. Candonga. However, a positive relation between number of leaves and TA was identified. Above ground biomass is also positively related to TA in strawberries (Correia et al., 2011). The vitamin C content was also negatively related to the average crop per day (Crespo *et al*., 2010). Further work with incorporation of additional variables might improve models from present research.

3.3.2.3 Anthocyanins

Total anthocyanins varied through season when expressed per dry weight. The lowest amount of anthocyanins was found in strawberries produced from the UK site and the highest was found in fruits grown at the SP2 farm in Spain (Table 3.11). At the SP1 farm, anthocyanin content of fruit varied through harvesting period however lower levels were noticed during 2011 when compared to 2010 (Figure 3.27). At the SP2 site there was variation of anthocyanin content of fruits through years and harvesting weeks and no clear pattern could be identified (Figure 3.28). At the UK site there was a decreasing trend of anthocyanin content in strawberry fruits towards the end of the season (Figure 3.29).

When anthocyanin content of fruit was expressed on fresh weight basis at the SP1 site variability through season was noticed. Unclear image was also observed for the SP2 farm with variable anthocyanin levels through seasons. At the UK site farm anthocyanin content of strawberries followed a decreasing pattern towards the end of the season.

Table 3.11: Total anthocyanins, expressed on dry (DW) and fresh weight basis (FW), of strawberry fruits over sites and harvest seasons (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	Total anthocyanins (mg/10g, dry weight)	Total anthocyanins (mg/100g, fresh weight)
SP1	1 (12 weeks)	43.48 ± 0.7	37.68 ± 0.73
SP1	2 (8 weeks)	41.54 ± 0.67	35.02 ± 0.64
SP1	1,2*	42.62 ± 0.50	36.49 ±0.46
SP2	1 (10 weeks)	40.53 ± 0.51	36.53 ±0.46
SP2	2 (8 weeks)	41.92 ± 0.59	36.54 ±0.50
SP2	3 (11 weeks)	40.14 ± 0.30	34.79 ±0.29
SP2	1,2,3*	40.75 ± 0.27	35.91 ±0.24
UK	1 (6 weeks)	30.94 ± 0.54	27.53 ±0.51

* Mean across years

Anthocyanin levels are related to colour of strawberry fruit, however not always very close (Ordidge *et al.*, 2012). During the three year period anthocyanin levels varied and no clear pattern could be identified. According to literature favourable conditions for increased anthocyanin levels could be found at the latest part of the season at Spanish farms and early season for the UK farm. Increased radiation and more specifically UV radiation levels could trigger formation of ellagic acid, anthocyanins and total phenolics (Atkinson *et al*., 2006; Josuttis *et al*., 2010; Tsormpatsidis *et al*., 2011; Ordidge *et al*., 2012;). Decreased light levels could also decrease anthocyanin content when present at last part of the maturation period (Kawanobu *et al.*, 2011). Furthermore, increased growth temperature is related to increased levels of anthocyanins and flavonoids in strawberries. Increased temperature levels have a positive contribution to anthocyanin and phenolic levels when present both throughout growing period (Wang and Zheng, 2001) and at the last part of fruit maturation (Krüger *et al.*, 2008). Increased temperature and low relative humidity levels result in higher VPD levels creating favourable conditions for plants to dehydrate, if appropriate irrigation is not supplied. Environmental conditions that increased VPD were present at the late and early sampling season in Spain and UK respectively. Higher VPD could potentially increase water stress of plants and create deficit irrigation conditions. Deficit irrigation was proved to increase amount of total phenolics in strawberry fruits (Terry *et al.*, 2007).

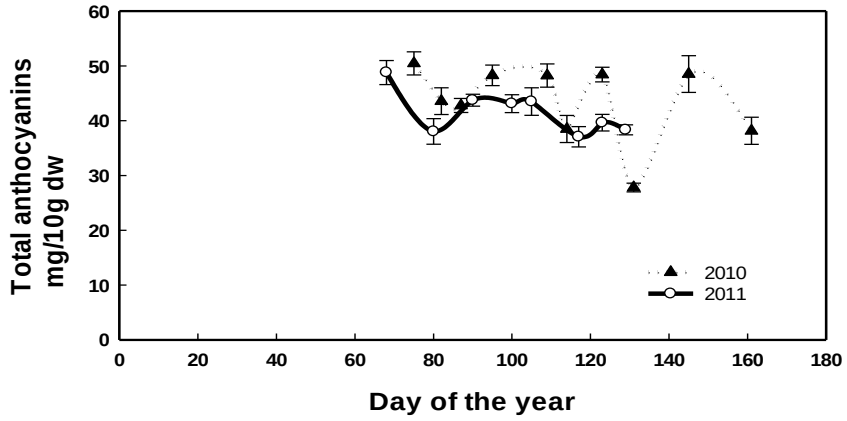

Figure 3.27: Total anthocyanins, expressed as mg/10g of dry weight of cv. Candonga strawberry fruits (n=9) at each sampling week at SP1 site during 2010, and 2011.

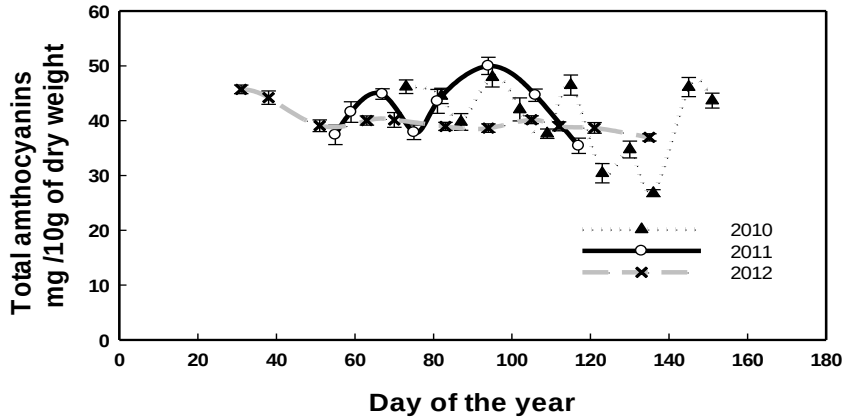

Figure 3.28: Total anthocyanins, expressed as mg/10g of dry weight of cv. Candonga strawberry fruits (n=9) at each sampling week at SP2 site during 2010, 2011 and 2012.

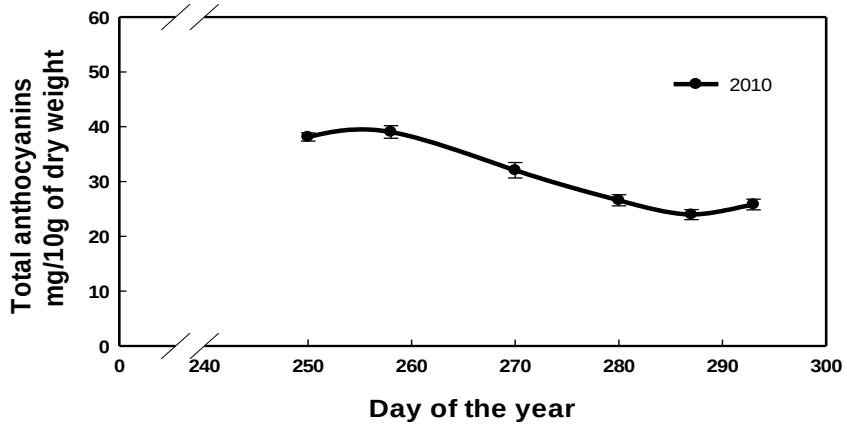

Figure 3.29: Total anthocyanins, expressed as mg/10g of dry weight of cv. Elsinore strawberry fruits (n=9) at each sampling week at the UK site during 2010.

Despite the fact that, according to literature, an increasing pattern for total anthocyanin was expected towards the end of season in Spain and early season in the UK, due to favourable environmental conditions, such a trend was not identified.

A decisive factor for the accumulation of anthocyanin levels through harvest period is also the time interval between flowering and harvest. Fruits with slower development and prolonged ripening period were found to have increased anthocyanin content (Andrianjka-Camps *et al*., 2012). It is known that Spanish strawberries in early season where conditions are not favourable for early production and quick ripening can require up to forty days from flowering to harvesting. On the contrary in late season, where temperature and solar irradiation is higher, twenty days could be a sufficient time interval for strawberries to reach commercial ripe stage (Palencia and Martínez, 2013). Fruits that spent more time on the plant in early season in Spain and late in the UK farm, despite adverse environmental conditions for anthocyanin formation, could have the potential to accumulate similar anthocyanin levels with fruits that were grown under favourable conditions for anthocyanin accumulation since the later ones had decreased ripening period.

3.3.2.4 Visual evaluation- Bruising

The amount of dry bruised fruits was found to be greater from both Spanish sites compared to the English farm (Table 3.12) and it was the main visual defect of fruits at SP1 and SP2. At the UK site the amount of dry bruised fruits was decreased when compared to fruits produced at Spanish farms. However, the amount of wet bruised fruits, for the UK site, was relatively high and on the arrival day it was found to be even higher than the amount of dry bruised fruits. At the Spanish farms the amount of wet bruised fruits was low and it was not consider as a major problem for deterioration of fruit quality. At all sites waste levels were low.

The amount of wet bruised fruits was variable throughout the season, for all sites, and no clear pattern was observed (Figures 3.30, 3.31 and 3.32). For the Spanish sites, wet bruise was not present at high levels. However, the % of dry bruised fruits followed an increasing trend towards the end of the growing season in Spain. The dry bruised fruits for the two Spanish farms were at similar levels for the first two seasons. For the last harvesting season (2012), lower levels of dry bruised fruits were recorded at SP2. Application of linear models revealed significant ($p<0.001$) positive effect of increased temperature on the amount of dry bruised developed on fruits.

Table 3.12: Bruised and wasted (%) strawberry fruits observed on arrival (AD) and expiry day (ED) over sites and harvest seasons (Candonga in SP1 & SP2, Elsinore in UK).

Site	Year	Arrival day/ Expire day	% Dry bruised fruits	% Wet bruised fruits	% Wasted fruits
SP1	1 (12 weeks)	AD	43.8 ± 2.6	<1%	<1%
		ED	78.8 ± 1. 8	<1%	<1%
SP1	2 (8 weeks)	AD	36.2 ± 2.2	<1%	<1%
		ED	69.7 ± 2. 2	1.25 ± 0.38	<1%
SP1	1,2*	AD	40.4 ± 1.8	<1%	<1%
		ED	74.8 ± 1.4	<1%	<1%
SP2	1 (10 weeks)	AD	51.9 ± 2.2	<1%	<1%
		ED	82.3 ± 1.5	<1%	<1%
SP2	2 (8 weeks)	AD	46.5 ± 2.7	1.4 ± 0.40	<1%
		ED	82.4 ± 2.5	<1%	<1%
SP2	3 (11 weeks)	AD	12.8 ± 1.3	3.8 ± 0.6	<1%
		ED	28.1 ± 1. 8	4.4 ± 0.6	<1%
SP2	1,2,3*	AD	36.6 ± 1.6	2.1 ±0. 3	<1%
		ED	63.1 ± 1.9	1.9 ± 0.2	<1%
UK	1 (6 weeks)	AD	9.5 ± 0.8	14. 5±1. 2	<1%
		ED	24.9 ± 1.0	21.7 ±1.5	<1%

* Mean across years

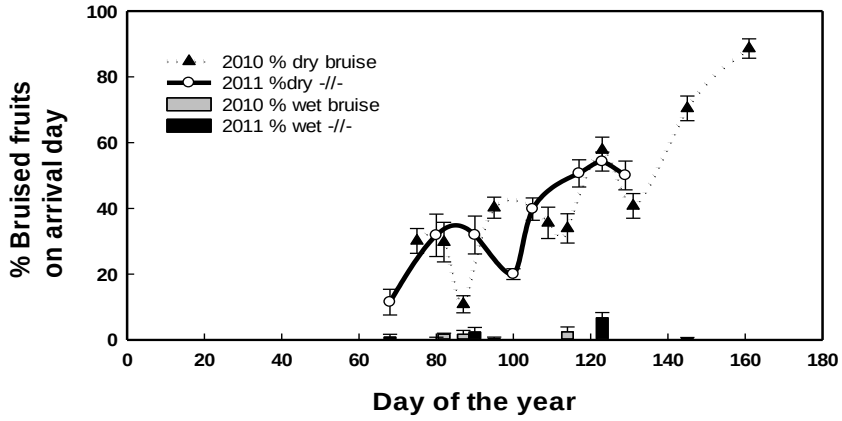

Figure 3.30: Bruised and wasted (%) strawberry fruits observed on arrival day of cv. Candonga strawberry fruits (n=9 punnets) at each sampling week at SP1 site during 2010 and 2011.

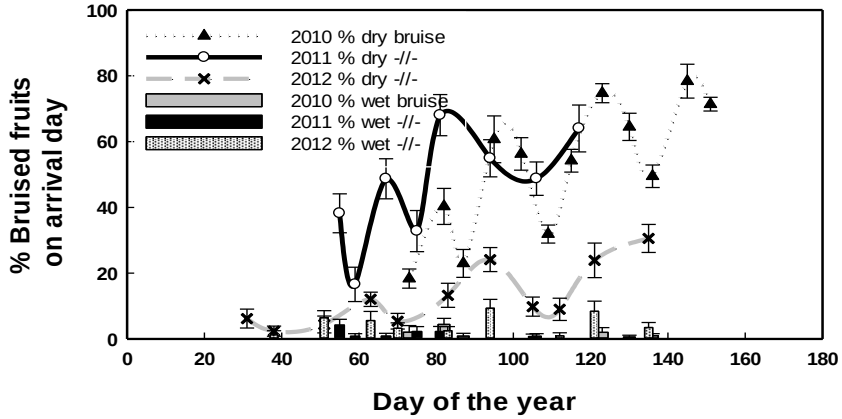

Figure 3.31: Bruised and wasted (%) strawberry fruits observed on arrival day of cv. Candonga strawberry fruits (n=9, punnets) at each sampling week at SP2 site during 2010, 2011 and 2012.

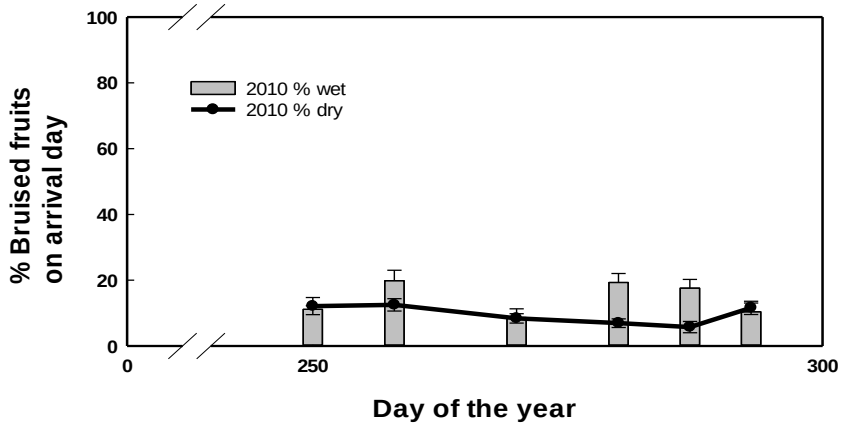

Figure 3.32: Bruised and wasted (%) strawberry fruits observed on arrival day of cv. Elsinore strawberry fruits (n=9) at each sampling week at the UK site during 2010.

Amount of waste and wet bruised fruits was relatively low throughout the season for fruits grown on Spanish farms. Increased amount of wet bruised fruits was observed at the UK site. Wet bruise is related to varietal characteristics such as susceptibility to moulds and the agronomic practices applied in order to prevent mould development. Since data of spraying was not available from the UK farm, the phenomenon could not be evaluated in detail. Fruits arrived from Spain showed increased amounts of dry bruised fruits when compared to fruits grown in the UK farm. An observation that could be explained since Spanish strawberries were subjected to increased transport stress. Warmer fruits can show increased elasticity, resulting in increased ability to withstand impact, but decreased ability to vibration stress (Sommer, 1960; Paull, 1999). Fruits from Spain travelled approximately one and a half days to arrive at the packhouse in Kent, whereas fruits grown in English farms arrived to NRI on the day of harvest and assessed the following day. The decreased amount of dry bruised fruits that was recorded at the SP1 site for the 2012 harvest season could be explained by decreased preharvest temperatures which lead to increased firmness of fruits. Positive relationship between postharvest temperatures in increased bruising was identified for strawberry fruits (Nunes *et al.*, 2003). Strawberries are known for their perishable nature and their susceptibility to increased postharvest temperature. It is a common practice for growers and retailers to import fruits in a cold chain as soon as possible after harvest (Kader, 1991). It is known that fruits can deteriorate rapidly, delay in cooling for two hours can result in postharvest losses up to 93% and decrease in firmness (Mitchell *et al*., 1974; Nunes *et al*., 1995; Paull, 1999). Observation of fruit firmness at harvesting point as well as detailed recording of bruise could offer useful information. Observation of fruit at harvesting could explain if deterioration and increased amount of dry bruise was due to postharvest conditions immediately after harvest or preharvest environmental conditions during growth. It is possible that both preharvest conditions and postharvest conditions - before the entrance of fruits in the cold chain - affect quality at the consumer stage and more specifically dry bruise occurrence. Further research will enhance understanding of mechanisms contributing to loss of firmness and subsequently increased dry bruise of strawberries. Fields of further study could be i) the mechanism that increased temperatures affects fruit softening and ii) the critical period that increased temperature affects fruits. In addition, understanding the mechanisms that contribute to softening of strawberries e.g. role of pectolytic enzymes and dehydration of fruits, could be an insight for enhanced understanding of strawberry postharvest deterioration.

3.3.3 Relationship between environmental factors and quality parameters

A PCA analysis was carried out for each site to examine the relationship between the environmental factors 7 days prior to harvest and the fruit quality characteristics. PCA is a method for pattern identification in data and furthermore expression in a two dimensions of data similarities and differences. PCA is a useful tool for analysing data produced from complex biological systems and reducing the number of dimensions to two (Smith, 2002)

Application of PCA revealed that VPD, average temperature, solar radiation and ozone levels were positively related at the Spanish farms, an observation that was expected since higher solar radiation and temperature levels result in higher ozone levels and increased VPD values. All the above parameters follow an increasing pattern from early towards late season for Spanish farms (Figures 3.33 and 3.34). On the other hand, increased relative humidity values are observed mainly in early season where lower temperature and higher precipitation is expected. At the UK site temperature, solar radiation and VPD were also related positively, however for ozone levels no clear relationship was observed with the above parameters. Relative humidity was negatively related to the values of temperature, VPD and solar radiation (Figure 3.35). At all sites colour values (*L*, a*, b**, chroma and °hue) were positively related. A negative relationship was noticed between colour parameters and total amount of anthocyanins. Increased fruit firmness was also related to decreased amount of dry bruised fruits at all farms.

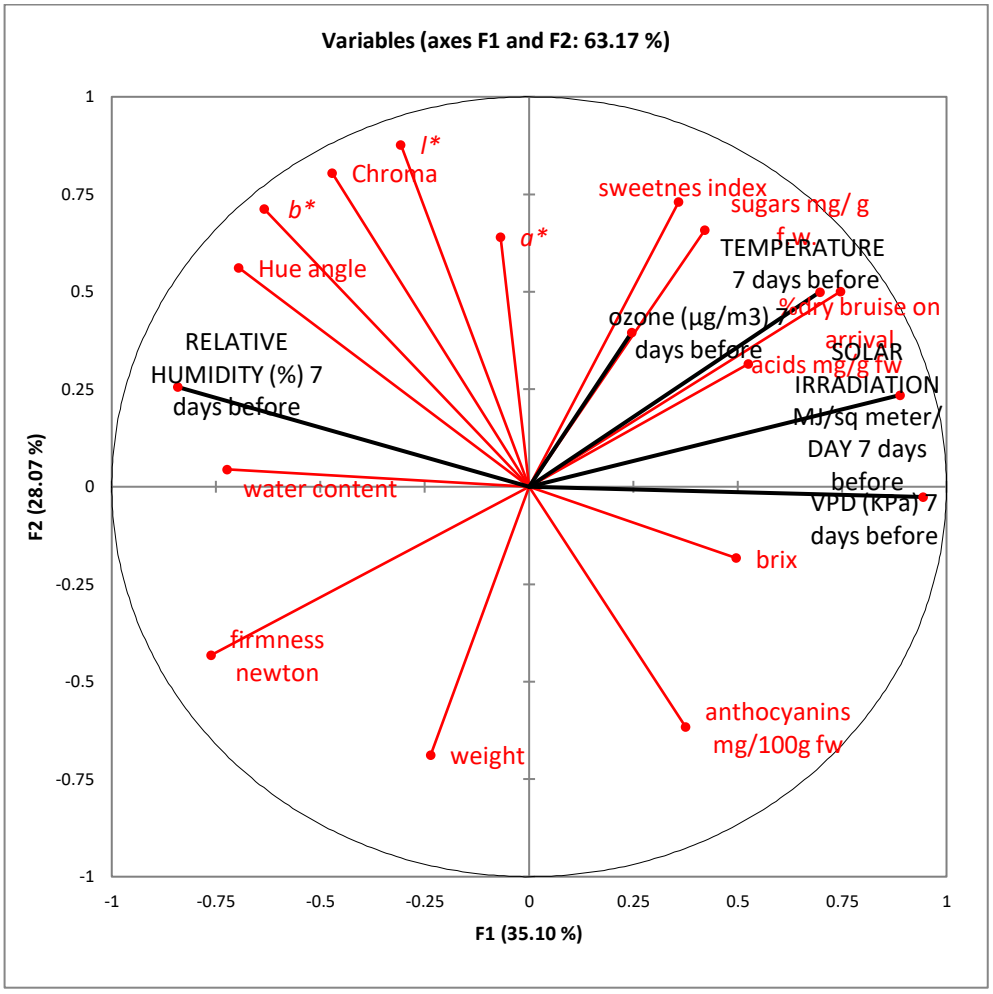

Figure 3.33: PCA of environmental conditions against strawberry fruit quality parameters of cv. Candonga over the duration of experiment at SP1 site.

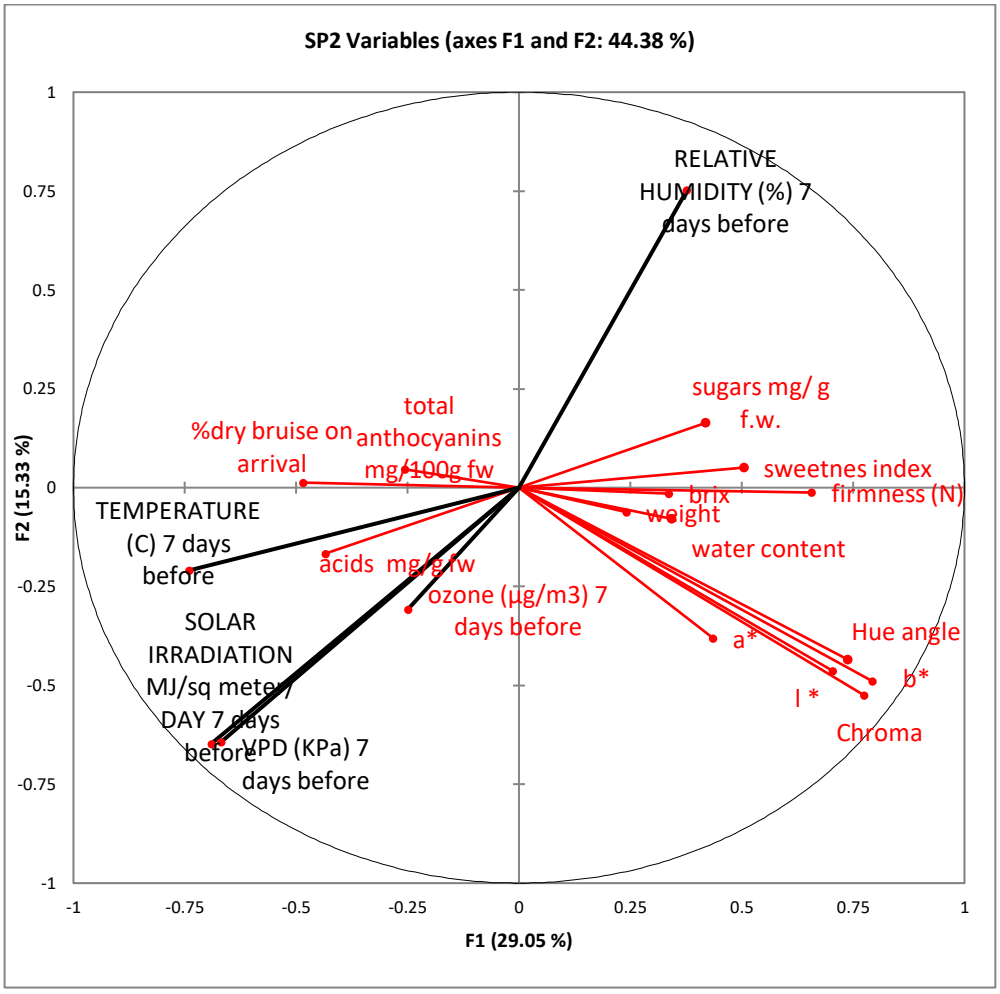

Figure 3.34: PCA of environmental conditions against strawberry fruit quality parameters of cv. Candonga over the duration of experiment at SP2 site.

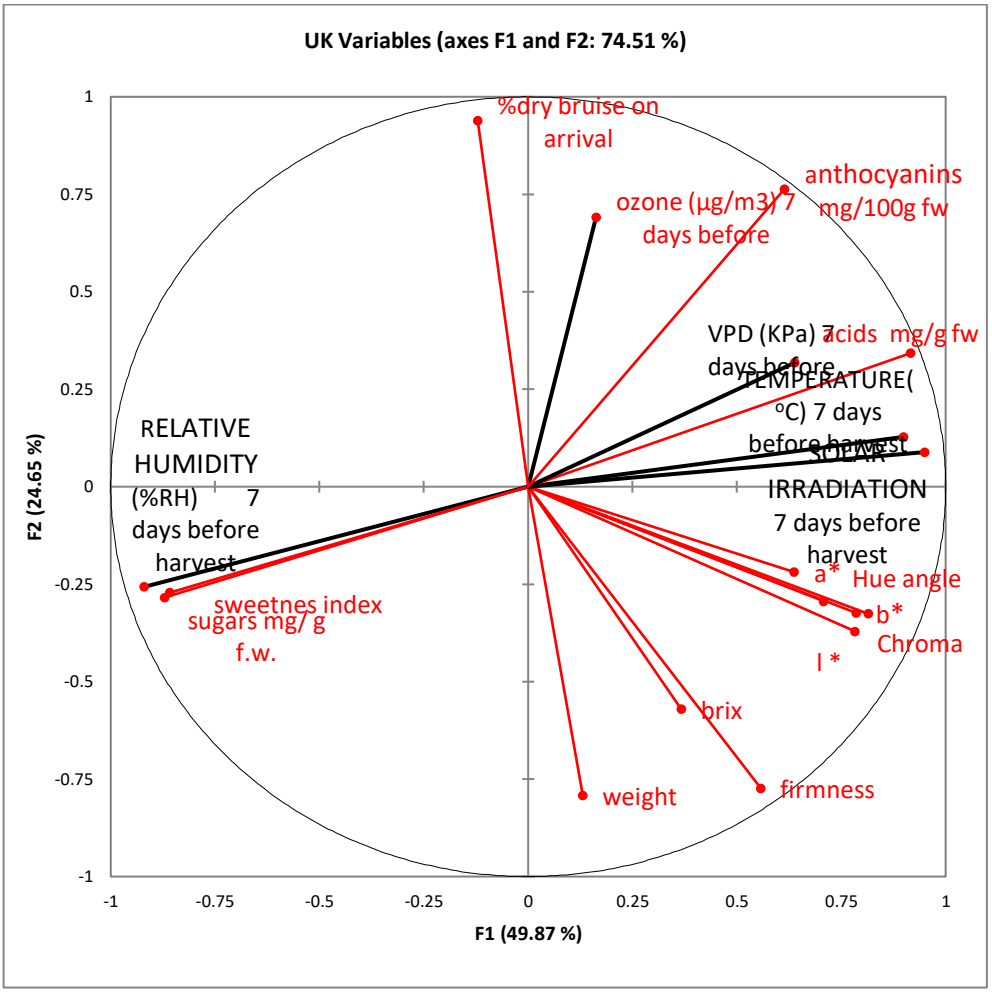

Figure 3.35: PCA of environmental conditions against strawberry fruit quality parameters of cv. Elsinore over the duration of experiment at the UK site.

3.4 Conclusion

The study of preharvest environmental effects on postharvest quality of strawberry fruits at commercial farms in UK and Spain over three years revealed that strawberries were getting softer as temperature, VPD, solar irradiation and ozone levels received their highest values across harvesting season. Despite the fact that models showed a positive relationship between increased preharvest temperature and reduced firmness of fruits the critical period that plays important role for softening of fruits was not clear. Furthermore, it was not proven that temperature was the most significant variable affecting firmness since the rest of the environmental factors that were studied also showed increased values at the time of season where fruits were becoming softer. Further work in a controlled environment that could highlight the effect of temperature on firmness, as well as explain biochemical mechanisms -e.g. PEL - was necessary. Furthermore, verification of the observation that softer fruits developed increased

amount of dry bruise and the way that consumers perceived variable strawberry quality through season were studied in subsequent chapters.

- Present research provided indication that strawberries were getting softer as preharvest temperature increased. However, further research should take place.

- TSS had a double peak pattern in Spanish sites. They received highest values at the beginning and at the end of the harvest season.

- There was a positive relationship between amount of dry bruise and softness of fruits, especially for the Spanish fruits which were imposed to increased transport stress.

Chapter 4 : EVALUATING SHORT TERM EFFECTS OF TEMPERATURE AND LIGHT ON STRAWBERRY QUALITY PARAMETERS UNDER CONTROLLED GROWTH CONDITIONS

4.1. INTRODUCTION

One of the most important factors affecting strawberry quality is firmness (Toivonen and Brummell, 2008). Increased firmness of fruits is related to enhanced ability of fruits to withstand transport stress, and therefore breeders aim to produce varieties with firm berries. Strawberry firmness can be affected by many different factors including preharvest cultural practices (Krüger *et al.*, 2000), cultivar selection, preharvest weather conditions and postharvest manipulation (Aguayo *et al.*, 2006; Nunes *et al.*, 2009). The most profound environmental effect on firmness is that of temperature. It is known that higher temperatures promote the production of softer fruits which are more susceptible to mechanical injury and bruising (Nunes and Emond, 2003). Furthermore, the necessity of maintaining low temperatures during postharvest handling is well appreciated with most producers applying cold chain treatments on harvested strawberries to reduce heat stress on fruits. The quality and quantity of light can also affect firmness. Solar radiation, and especially radiation in the UV range, was found to have an increasing positive effect on strawberry fruit firmness (Sams, 1999; Ordidge *et al.*, 2012). In chapter three evidence of a negative relationship between increased preharvest temperatures and loss of firmness was demonstrated. However, there was uncertainty about the critical period before harvest at which temperature is effective. Also, because of the strong correlation of environmental variables such as temperature, VPD and solar irradiation it was not possible to separate the effects of these variables, and was therefore necessary to examine the effects of the above variables in a controlled environment. The aim of this study is to examine short-term effects of preharvest conditions of temperature and light on firmness of fruits.

The hypothesis being tested is that there is a short-term effect of increased temperature and light levels on strawberry fruit firmness. In the previous chapter it was demonstrated that there was a negative relationship between higher temperature prior to harvesting and firmness of strawberry fruits. The application of linear models in the previous chapter revealed that temperature was classified as a significant climatic variable for firmness reduction two days, one week and three weeks prior harvesting. However, it was not clear which was the critical period before harvesting

that would result in reduced firmness of fruits. In this chapter short-term effects of temperature and light on firmness of strawberries were examined

4.2. MATERIALS AND METHODS

4.2.1. Plant material

Fresh strawberry plants of the ever-bearing (short-day) cultivars Elsinore (described in chapter two) and Capri were supplied by Consorzio Italiano Vivaisti (CIV), Italy. Capri is a variety producing firm fruits of conical shape. The size of primary fruits is about 9-11 cm and secondary fruits are 6-7 cm. In terms of vegetative vigour it is classified as medium-strong and it of yellow-orange colour getting darker at full maturity level (Leis, 2013; Mazzoni group, 2013). The plants were grown at a local farm in Kent, UK, using commercial cultivation techniques (peat-bags). Eight strawberry plants were within each peat-bag. Plants used for trials were at the fruiting stage and kept in a glasshouse for 5 days until commencement of experiments. On procuring plants, a slow release fertilizer (Osmocote^{TM} 10g/peat bag) was applied. The peat bags were irrigated daily with 1.5 litres of water/ bag.

4.2.2. Light and temperature scheme

Once plants were relocated to the growth cabinets, strawberries were subjected to twelve hour temperature cycles of 20°C and 28°C, 16 hours light and 8 hours dark (Figure 4.1).

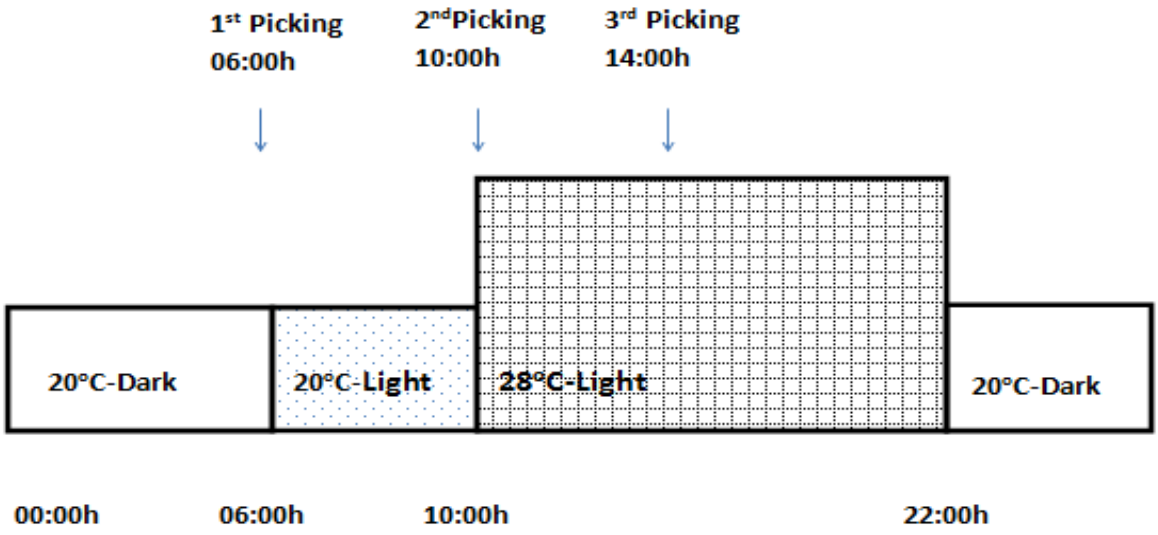

Figure 4.1: 24h cycle of light and temperature as well as picking regime in growth cabinets.

The growth cabinets (Model: LT1201L/RH, LEECS, UK) were modified by additional lighting provided by fitting fluorescent lamps (Warm White, 18W. Philips Fluorescent, Master TL-D Super 80 G13). Plants were placed 20 cm below the light source. Photon flux density was approximately 80 $\mu mol\ m^{-2}\ s^{-1}$ at the leaf surface as measured by a light meter (Young and Gibson 1987). Relative humidity was 65% (±5%). Data loggers (Tinytag plus, GEMINI, UK) were placed amongst plants to observe temperature and relative humidity. Temperature data were recorded to also observe for any effects of lighting on the temperature of the growth cabinets.

The total duration of the experiment was four weeks. Three harvests were made. For each harvest, three daily pickings of strawberries occurred. First picking at 06:00 at the end of the dark period and when the fruits were at 20°C; second picking at 10:00 where the effect of irradiation for 4h was studied; third picking at 14:00, four hours after the increase in temperature to 28°C (Figure 4.1). In total 144 fruits were used (8 plants * 2 cabinets * 3treatments * 3 weeks).

4.2.3. Light measurements

A light meter (Young and Gibson, 1987) was used to measure light levels inside the growth cabinets.

4.2.4. Firmness

One fruit per plant at each harvest was analysed for tissue firmness. Fruits were cut in halves. A texture analyser (TAXT plus, Stable Microsystems Ltd, UK) was used to measure firmness of one half using an 8mm probe, at the fruit shoulder. The test speed was 10 mm/sec. The value of the first peak (matching surface firmness) was recorded..

4.2.5. TSS

An electronic refractometer was used to measure TSS as described in chapter three (3.2.3).

4.2.6. Sugars-Acids

For quantification of organic acid and sugars the HPLC method described in chapter three was followed (3.2.7 and 3.2.8).

4.2.7. Total anthocyanins

Amount of total anthocyanins was measured according to the Folin – Ciocalteau method described in chapter three (3.2.9).

4.2.8. Colour

For determination of colour parameters *L**, *a**, *b**, Chroma and °Hue a Minolta colorimeter was used as described in chapter three (3.2.3).

4.2.9. Statistical methods

Results were analyzed using Tuckeys' (HSD) using software package R statistics 2.10.

4.3. RESULTS AND DISCUSSION

Strawberry fruits of both cultivars used in the experiments showed a significant decreasing trend in firmness over sampling weeks with fruits being softer at the final harvest week and firmer in the first harvest week, according to Tukey's HSD (Figure 4.2 and 4.3). Significant differences between treatments were observed for Elsinore fruits with fruits being softer at the 28°C – light treatment and firmer in the 20°C - dark, according to Tukey's HSD. Fruits harvested at the 20°C – light treatment were not different compared to the other two treatments according to Tukey's HSD. Strawberry fruits harvested from Capri plants again had lower firmness when harvested under the 28°C-Light treatment, however the firmest fruits were harvested under the 20°C-Light scheme, but the differences were not significant. Treatments were found to have a significant effect only on cv. Elsinore fruits.

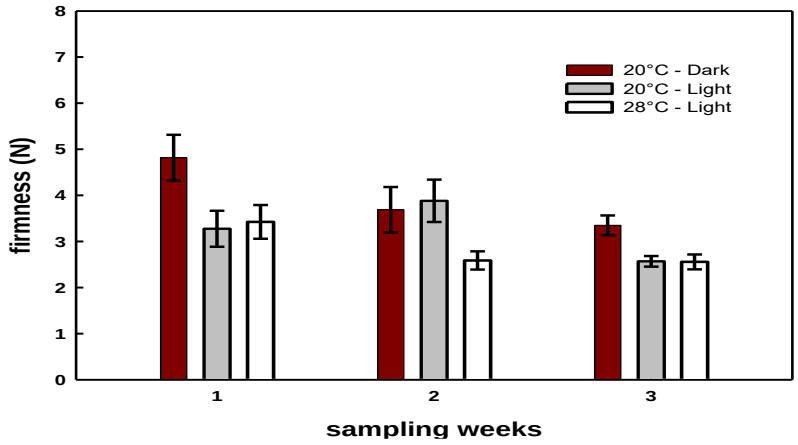

Figure 4.2: Postharvest firmness of fruits of cv. 'Elsinore® at the time of picking measured in response to three different treatments, over three sampling weeks (n=14). Treatment differences were significant $p<0.001$. Week differences were significant $p<0.005$ ±se .

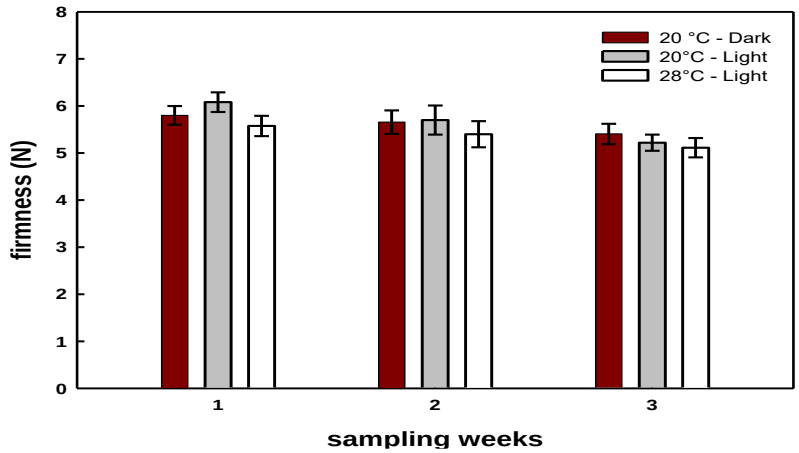

Figure 4.3: Postharvest firmness of fruits of cv. 'Capri® at the time of picking measured in response to three different treatments, over three sampling weeks. n=14. Treatment differences were not significant. Week differences were significant $p<0.05$ ±se.

The experiment provides an indication that there is an effect on firmness of strawberries when temperature levels are increased four hours before harvesting. The fact that higher growing temperature plays an important role on strawberry softening was observed earlier in the study (Chapter three) when fruits were found to respond to increased temperature levels by reducing their firmness. Similar observations were also recorded by others (Rose *et al*., 1934; Correia *et al*., 2011). However there was no indication of the critical period that negatively impacts strawberry firmness. Furthermore, this experiment indicated there may be both long (a few weeks before harvesting) and short-term (a few hours before harvesting) effects of increased temperature on strawberry firmness. For both cultivars in growth cabinets, a decreasing trend of firmness over sampling weeks was observed. However, more research is needed to verify findings since this could be an effect of alteration of irrigation (Hoppula and Salo, 2006) and fertilization conditions (Singh *et al*., 2007; Correia *et al*., 2011) after the movement of plants from farms to the growth cabinets. The action of enzymes related to strawberry softening may also play a role.

Table 4.1: Strawberry colour parameters, total anthocyanins (mg/10g Dry Weight) of strawberry fruits of cvs. Elsinore and Capri, harvested under three different treatments (1: 20°C-Dark, 2: 20°C- Light & 3: 28°C-Light).

variety	week	treatment	*L**	*a**	*b**	Chroma	°hue	total anthocyanins (mg/10g D.W.)
Elsinore	1	1	43.45	37.46	32.30	49.63	50.35	37.72
		2	42.74	38.10	29.83	48.46	58.46	38.97
		3	42.97	36.77	32.11	48.93	49.41	37.51
	2	1	42.26	37.54	32.12	49.57	50.90	37.71
		2	42.24	35.17	30.13	46.50	52.78	32.64
		3	42.59	35.27	29.87	46.33	52.16	31.73
	3	1	37.49	31.80	22.39	38.99	69.13	25.70
		2	41.66	34.11	26.77	43.52	60.03	31.41
		3	37.62	33.91	23.59	41.35	69.58	27.95
Capri	1	1	39.16	38.77	24.72	46.05	78.97	43.27
		2	37.99	37.12	23.53	43.97	78.63	48.39
		3	37.00	37.90	22.70	44.21	84.65	41.29
	2	1	36.12	36.51	21.84	42.59	85.16	36.14
		2	35.20	36.96	22.45	43.31	83.53	45.64
		3	36.85	38.37	24.35	45.50	79.22	28.91
	3	1	35.24	36.47	24.11	43.83	76.06	28.33
		2	34.01	35.63	21.02	41.44	87.91	35.42
		3	35.41	34.96	22.48	41.75	79.66	33.03
Elsinore								
	1		43.05a	37.44a	31.41a	49.00a	52.74a	38.07a
	2		42.36a	35.99a	30.71a	47.47a	51.95a	34.03b
	3		38.92b	33.28b	24.25b	41.29b	66.25b	28.35c
p value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		1	41.06a	35.60a	28.93a	46.06a	56.80a	33.71a
		2	42.21a	35.79a	28.91a	46.16a	57.09a	34.34a
		3	41.06a	35.32a	28.53a	45.54a	57.05a	32.39a
p value			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Capri								
	1		38.05a	37.93a	23.65a	44.75a	80.75a	44.3a
	2		36.06b	37.28a	22.88a	43.80ab	82.63a	36.9b
	3		34.89b	35.69b	22.54a	42.34b	81.21a	32.3c
p value			<0.001	<0.001	n.s.	n.s.	n.s.	<0.001
		1	36.84a	37.25a	23.56a	44.18a	80.06a	35.9a
		2	35.73a	36.57a	22.33a	42.90a	81.17a	43.2b
		3	36.42a	37.08a	23.18a	43.82a	83.36a	34.4a
p value			n.s.	n.s.	n.s.	n.s.	n.s.	<0.001

A negative effect of sampling week was identified for *a**, *b**, *L**, and Chroma values for cv. Elsinore, for Hue° a positive effect of sampling week was identified (Table 4.1). For cv. Capri similar trends, to those of cv. Elsinore, were noticed for *a**, *L** and Chroma. The amount of total anthocyanins was significantly reduced through harvesting weeks for both cultivars. This fact is in accordance with colour measurements where the *a** value was reduced (less red fruits)

(Nunes *et al.*, 1995). This observation could probably be explained since strawberries were moved from ambient conditions to growth cabinet environment where the light was reduced, resulting in lower amount of total anthocyanins (Tsormpatsidis *et al.*, 2011; Ordidge *et al.*, 2012).

Table 4.2: Strawberry sugar concentrations, total sugars (mg/g Dry Weight) and TSS (°brix) of strawberry fruits of cvs. Elsinore and Capri, harvested under three different treatments (1: 20°C-Dark, 2: 20°C- Light & 3: 28°C-Light).

variety	week	treatment	total sugars (mg/g D.W)	sucrose (mg/g D.W)	fructose (mg/g D.W)	glucose (mg/g D.W)	TSS °Brix
Elsinore	1	1	319.3	57.5	159.5	102.3	7.7
		2	282.1	53.9	140.9	87.4	5.8
		3	277.4	62.4	129.1	86.0	6.1
	2	1	277.8	57.8	132.6	87.5	5.7
		2	292.9	56.0	141.6	95.4	5. 7
		3	285.7	54.8	140.4	90.5	5.9
	3	1	264.8	53.7	127.3	83.8	5.6
		2	279.7	50.4	138.4	90.9	5.8
		3	266.6	43.2	135.2	88.2	5.5
Capri	1	1	393.2	102.7	176.5	114.0	6.4
		2	379.9	93.7	174.4	111.8	6.6
		3	370.0	79.1	172.8	118.3	6.5
	2	1	356.5	88.5	161.2	106.9	6.3
		2	353.3	95.2	156.5	101.7	6.2
		3	315.2	77.5	144.2	93.6	6.7
	3	1	304.3	70.6	131.3	102.4	6.8
		2	341.5	84.2	155.2	101.7	6.4
		3	347.0	87.3	155.7	104.1	6.2
Elsinore							
	1		292.9a	57.9a	143.2a	91.9a	6.5a
	2		285.5ab	56.1ab	138.2a	91.1a	5.6b
	3		270.4b	49.0b	133.6a	87.6a	5.8b
p value			<0.05	<0.05	n.s.	n.s.	n.s.
		1	287.3a	56.3a	139.8a	91.2a	6.3a
		2	284.9a	53.4a	140.3a	91.2a	5.8a
		3	276.6a	53.5a	134.9a	88.2a	5.8a
p value			n.s.	n.s.	n.s.	n.s.	n.s.
Capri							
	1		381.1a	91.8a	174.5a	114.7a	6.4a
	2		341.7b	87.0a	154.0b	100.7b	6.4a
	3		330.8b	80.7a	147.4b	102.7b	6.5a
p value			<0.001	n.s.	<0.001	n.s.	n.s.
		1	351.4a	87.2a	156.3a	107.8a	6.5a
		2	358.1a	91.0a	162.0a	105.1a	6.4a
		3	344.1a	81.3a	157.5a	105.3a	6.4a
p value			n.s.	n.s.	n.s.	n.s.	n.s.

Total sugars followed a decreasing trend over sampling weeks (Table 4.2) however there were significant differences for cv. Elsinore only for total amount and sucrose. Cv. Capri was found to have reduced levels of total sugars over sampling weeks. Fruits had the highest content of sugars in the first week and the lowest on the third sampling week. The main sugar for both cvs. Elsinore and Capri fruits was fructose, and sucrose appeared to have lower concentrations when compared to other two sugars. Cv. Capri fruits had higher amount of sugars compared to fruits produced from Elsinore plants. Plants were moved from field to artificial growing conditions where they faced increased temperature and reduced irradiation levels compared to those in the growing farms. The reduction in total sugars could be attributed to that fact (Wang and Camp, 2000). Generally fruits showed low levels of TSS when measured in °Brix scale compared to the standard commercial acceptability criteria. The levels that are considered acceptable are above seven-eight and for exceptional quality fruits supermarkets accept fruits of above ten. Again, because the harvested fruits were grown in growth cabinets condition the low levels of TSS were expected since levels of light in the cabinets can not match the levels of ambient light.

Total acids (Table 4.3) were change over harvesting weeks and the fruits sampled at week one had the lowest amount of total acids both for cvs. Elsinore and Capri. Fruits harvested from cv. Elsinore plants had lower levels of total acids. The main acid found was citric followed by malic and ascorbic at both cultivars. Cv. Elsinore was found to have reduced levels of total acids compared to cv. Capri. During the experiment a reduction in total acid concentration occurred over sampling weeks, possibly attributable to increased temperatures and reduced irradiation levels. Strawberry acids can be affected by changes in environmental conditions as irradiation (Atkinson *et al*., 2006), temperature (Wang and Camp, 2000), genotype and maturity stage (Sturm *et al*., 2003; Kafkas *et al*., 2007).

Table 4.3: Strawberry total acids (mg/g Dry Weight), weight (g) and dry matter % of strawberry fruits of cvs. Elsinore and Capri, harvested under three different treatments (1: 20°C-Dark, 2: 20°C- Light and 3: 28°C-Light).

variety	week	treatment	total acids (mg/g D.W)	malic (mg/g D.W)	citric (mg/g D.W)	ascorbic (mg/g D.W)	weight (g)	dry matter %
Elsinore	1	1	121.6	21.8	96.1	3.7	12.5	9.3
		2	122.3	23.0	94.3	5.0	8.0	8.9
		3	114.5	21.3	88.9	4. 2	10.5	9.5
	2	1	134.1	31.8	95.9	6.4	11.7	8.9
		2	135.5	25.3	105.0	5.2	9.0	9.0
		3	148.2	23.4	120.1	4.7	10.0	9.4
	3	1	135.8	27.2	102.8	5.8	8.3	8.9
		2	123.2	27.2	90.5	5.6	8.7	9.2
		3	156.0	35.1	114.0	6.9	9.8	9.1
Capri	1	1	144.2	31.3	106.2	6.7	13.3	8.6
		2	147.5	30.4	110.7	6.4	13.4	8.7
		3	140.8	25.3	109.1	6.3	14.0	8.1
	2	1	149.4	28.6	113.1	7.7	11.8	9.1
		2	142.0	28.1	106.3	7.6	12.2	9.4
		3	146.7	26.7	112.3	7.8	12.2	9.0
	3	1	159.7	36.6	114.0	9.1	10.7	9.8
		2	159.0	37.7	111.0	10.2	11.0	9.7
		3	157.3	38.5	108.8	10.0	11.5	9.1
Elsinore								
	1		119.4a	22.1a	93.1a	4.2a	10.3a	9.2a
	2		139.2b	26.8b	107.0b	5.4b	10.2a	9.1a
	3		138.3b	29.8c	102.4ab	6.1b	8.9a	9.0a
p value			<0.001	<0.001	<0.01	<0.001		
		1	130.4a	26.9a	98.3a	5.2a	10.8a	9.0a
		2	127.0a	25.2a	96.6a	5.3a	8.6b	9.0a
		3	139.5a	26.6a	107.7a	5.2a	10.0ab	9.3b
p value			<0.05	n.s.	<0.05	n.s.	n.s.	<0.001
Capri								
	1		144.2a	29.0a	108.7a	6.4a	13.6a	8.5a
	2		146.1a	27.8a	110.6a	7.7b	12.1ab	9.2b
	3		158.6b	37.6b	111.3a	9.7c	11.1b	9.5b
p value			<0.001	<0.001		<0.001	<0.01	<0.001
		1	151.1a	32.2a	111.1a	7.8a	11.94a	9.1ab
		2	149.5a	32.1a	109.3a	8.1a	12.18a	9.2a
		3	148.3a	30.2a	110.1a	8.0a	12.58a	8.7b
p value			n.s.	n.s.	n.s.	n.s.	<0.05	<0.001

Since strawberries are perishable fruits with limited shelf-life further understanding of their physiology could provide an indication about handling strategies that could be adopted in order to increase postharvest life. Firmness is related to increased ability of plants to withstand transport stress and consequently dry bruise (Nunes and Emond, 2003), as was shown in chapter three. It is known that increased temperature levels immediately after harvest can increase postharvest losses and deteriorate quality of strawberries (Nunes *et al*., 1995). It is also known

that increased preharvest temperatures are connected with loss of firmness (Rose *et al*., 1934), in this experiment indication was provided that increased temperature before harvest can reduce strawberry fruit firmness.

4.4. CONCLUSION

- The experiment provided evidence that there is both a short and long term effect of increased preharvest temperature on fruits resulting in reduced firmness of strawberries.

  Long term effects on fruits were identified. Strawberries became less red and had lower anthocyanin and sugar levels and more acids.

- Useful future work would include research on the physiological mechanisms involved in strawberry softening such as enzymatic action and water-plant relations.

Chapter 5 : PEL ACTIVITY IN STRAWBERRY FRUITS WITH CONTRASTING FIRMNESS

5.1. INTRODUCTION

Enzymatic action of PEL is regarded as an important mechanism for strawberry fruit softening. The role of PEL on softening of crops and more specifically strawberries has been studied in the past (Medina-Escobar *et al.*, 1997; Jiménez-Bermúdez *et al.*, 2002; Benitez-Burraco *et al.*, 2003; Figueroa *et al.*, 2008; Youssef *et al.*, 2009; Severo *et al.*, 2011; Wang *et al.*, 2014). PEL is known to contribute to strawberry softening by disassembly of the cell wall and more specifically by cleavage of polygalacturonic acid resulting in production of oligogalacturonates (Marin-Rodriguez *et al.*, 2002). Downregulation of PEL gene FaplC was found to produce firmer fruits without affecting colour and accumulation of sugars (Youssef *et al.*, 2009).

The aim of this chapter is to further understand how PEL action is affecting fruit firmness. In this chapter a modification of a spectrophotometric method for PEL activity *in vitro* is described. The method modification was necessary for assessing the action of PEL and further understand biochemical mechanisms contributing to loss of firmness in strawberry fruits.

In previous chapters it was observed that softer fruits were produced when higher temperatures during the harvesting season were recorded. Since it is likely that softer fruits have increased action of PEL the hypothesis that softer fruits will show increased levels of PEL enzymatic activity was tested. A study on strawberry fruits harvested from farms during early and late season in Huelva, Spain with contrasting firmness was undertaken.

Firmness is an important quality characteristic of strawberry fruits since it is connected to consumer acceptability. Strawberries are appreciated not only for their delicate taste, aroma and nutritional value but also for their juicy texture. Furthermore, softer strawberry varieties have been found to be more susceptible to bruise development and they are not preferred since they are more sensitive to transport stress, as it was also proposed in previous chapters. Further understanding of the role of PEL on strawberry softening could provide useful information to breeders in order to produce varieties that are going to be more desirable by consumers and retailers.

5.2 MATERIALS AND METHODS

5.2.1. Plant material and commercial enzyme extract

Strawberry fruits (cv. Candonga) were used for the PEL activity analyses. Fruits were grown on two commercial farms, SP1 and SP2, at the Spanish province of Huelva and sent to Rodanto Ltd packhouse in Kent. Fruits were sampled and after weighing, fruit halves were frozen in liquid nitrogen and kept in a -80˚C freezer. Fruits with known contrasting values of firmness (as determined in 3.2.4) were chosen for analysis. Firmness was tested with a penetrometer (Bishop Instruments Ltd) and an 8 mm probe, as described in 3.2.4.

PEL extract (Prozomix Ltd, Northumberland, UK) from a soil bacterium, *Cellvibrio japonicus* soil, was used to assess the enzymatic activity methods. PEL was supplied in a 3.2 M solution of ammonium sulphate and kept in a fridge at 4˚C. Activity of PEL specified by the manufacturer was 6195 U/ml and specific activity 583.4 U/mg.

5.2.2. PEL extraction from strawberry tissue

Two methods for the extraction of PEL were tested in this study (Marín-Rodríguez *et al.*, 2003; Payasi and Sanwal, 2003). They had both been used on bananas, however only one (Marín-Rodríguez *et al.*, 2003) proved effective in this study. All consumables, unless otherwise stated, were supplied by Sigma-Aldrich Ltd, UK.

I) PEL extraction from strawberry tissue described by Marin-Rodriguez

The first adopted method for the extraction of PEL from banana tissue (Marín-Rodríguez *et al.*, 2003) is described. Strawberry fruits obtained by the packhouse of Rodanto Ltd (Kent, UK) and grown in Spanish farms, were used for the experiment. Strawberry lyophilised powder (0.05 g) kept at -80 ˚C was homogenized with 1ml extraction buffer (0.5 M mannitol, 0.05 M sodium phosphate pH 7 and 2% mercaptoethanol) and 0.01g polyvinylpyrrolidone (PVP) 44000. The sample was then microcentrifuged at 12000 rpm for 20 minutes. 50 µl of the supernatant were then added to a Micro Bio-Spin column (BioRad) prepared with 0.5 ml buffer (Sephadex G-50 7% w/v, in 10 mM Tris-HCl, pH 7, 1mM EDTA and 100 mM sodium chloride). The sample was centrifuged for 2 minutes at 1100 rpm to remove residual alkaloids and potential polyphenol oxidase substrates, through gel filtration properties of Sephadex. The supernatant was delivered to a 3ml Eppendorf containing 0.5 ml of Tris-HCl buffer pH7. Sephadex G-50 was used effectively in banana extraction. Since PEL of banana and strawberry are of similar size around

40 KDa the same Sephadex grade was used. The extract was used for immediate analysis (Pilatzke-Wunderlich and Nessler, 2001).

II) PEL extraction from strawberry tissue described by Payasi

The second method was described by Payasi *et al.* (2003). Strawberry lyophilised powder stored at -80 ˚C was homogenized in a stock solution. The grinding medium consisted of 0.02 M Na-Pi buffer, pH 7.0, 0.02 M cysteine–HCl and 1% (v/v) Triton X-100. Muslin was used to strain the supernatant. 1.5 ml of the homogenate was centrifuged at 15,000 g for 30 min. $(NH_4)_2SO_4$ was added. The solution was stirred until it reached saturation and was stored at 4 ˚C for 4 h. Afterwards it was centrifuged at 15,000 revs/min for half hour. 0.5 ml of the suspension were mixed with 0.5 ml 0.02 M Na-Pi buffer, pH 7.0 and placed in Visking tubes, which were dialyzed in cold against 1.5 l of the same buffer overnight. The suspension constituted the $(NH4)_2SO_4$ fraction. However no PEL activity was detected when the adapted method (see next section) for PEL activity at 235 nm was used (Figure 5.1). A possible explanation for failing to detect any activity with the above method could be that Visking tube was too big in pore size, an observation that resulted in enzyme leaking through the pores to the dialysis medium. However, further research will be required to verify this assumption.

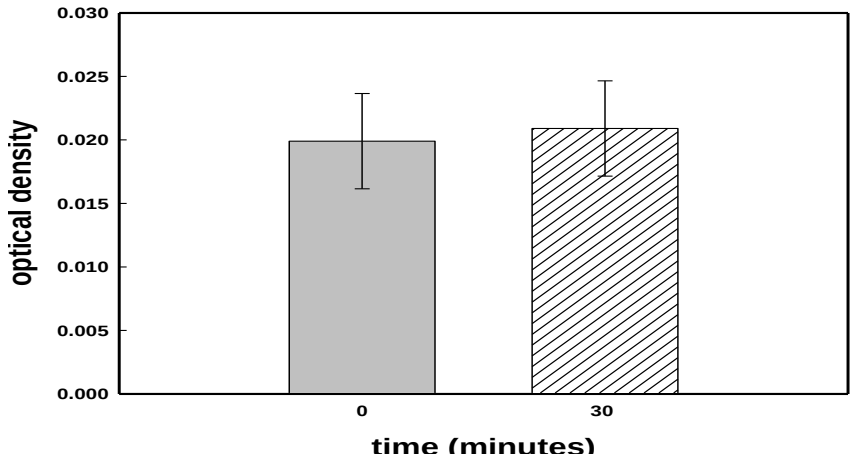

Figure 5.1 : OD at 235 nm over 30 minutes following dialysis extraction method. No change in OD between 0 and 30 minutes indicate no enzyme activity (n=9) ±se.

5.2.3. Determination of PEL activity

I) Collmer's method

A widely used method for determination of PEL activity is based on the spectrophotometric detection of 4,5 unsaturated reaction products deriving from polygalacturonates at 235nm (Jayani *et al.,* 2005). According to Collmer *et al.* (1988), the above method should be used with consideration when referring to plant extracts owing to interfering compounds and it should be ensured that enzyme extraction minimizes the effect of interfering compounds. Determination of PEL activity in fruits using a spectrophotometric method at 235 nm was used for mango (Chourasia *et al.*,2006), oranges (Lei *et al.*, 2010) and banana (Marín-Rodríguez *et al.*, 2003). All previous mentioned methods are based on the method of Collmer *et al.* (1988), which is described below:

Formation of 4,5 unsaturated products is recorded with a spectrophotometer at 235nm (Cecil, CE 9200). A substrate solution consisting of 60 mM Tris-HCl, pH 8.5, 0.6 mM calcium chloride and 0.24 % (w/v) polygalacturonic acid (PGA) is prepared. Tris-HCl acts as a buffer solution to control pH during the enzymatic assay. Calcium chloride provides the necessary Ca^{+2} as a co-factor and PGA is the substrate that PEL will act upon. Temperature should be kept constant during the reaction. Optical Density (OD) is recorded at a predefined time interval.

II) Modified method for measuring PEL activity at 235 nm

An adapted method (Marín-Rodríguez *et al.*, 2003) for the recording of PEL activity used on banana fruits was modified for in the present study for strawberries. A substrate solution consisting of 50 mM Tris-HCl, pH 8.5, 0.01 mM calcium chloride and 5.2 mg/100 ml polygalacturonic acid (PGA) was prepared. It was observed that decreased concentration of PGA improved sensitivity of the method, since it reduced its interference on spectrophotometer readings. PGA was added to the stock solution and pH adjusted 8.5 with addition of NaOH or HCl on the day of analysis, in order to always be freshly prepared.

The spectrophotometer (Cecil, CE 9200) was auto zeroed at 235 nm using distilled water as a blank. To a 1cm light path UV cuvette of 1.5 ml, 0.75 ml of substrate solution was added. The cuvettes were left to equilibrate at 30 ˚C for 20 minutes in a water bath. (Grant, Aqua plus) 100 µl of enzyme extract were added and a spectrophotometric reading was recorded. Cuvettes were placed back in the water bath for a further 30 minutes and a second reading was recorded. Blanks consisted of stock solution without strawberry PEL extract were used and read after 30 minutes. These readings were subtracted from the extract readings.

III) Optimisation of the method for determination of PEL

The initial attempts in assessing PEL activity were based on a method used to determine PEL from banana tissue (Marín-Rodríguez *et al.*, 2003), which was based on Collmer's method. Results (Figure 5.2) did not show any sign of progress for the reaction after ten minutes. Furthermore after three hours the absorbance of blanks was becoming unstable and therefore no further measurements took place above that time interval. The decision to 'zero' the spectrophotometer using distilled water and not PGA buffer solution was made since the absorbance of the above solution should be tested and its contribution to final activity should be evaluated. The main problem arising from that approach was the fact that initial absorption of the PGA solution was above 1. Assuming that the expected change in OD for enzyme activity could be below 0.01 (Marín-Rodríguez *et al.*, 2003) the fact that initial absorption was that high could hide any possible PEL activity generated by strawberry fruits and decrease the sensitivity of the method. Furthermore, it was noticed that PGA buffer solution did not show constant absorbance during incubation at 30˚C. The absorbance of the PGA blank was reduced from 0.3 to 0.27 after three hours of incubation. For the above reasons no definite conclusion could be drawn. Thus, a modification of the method was developed.

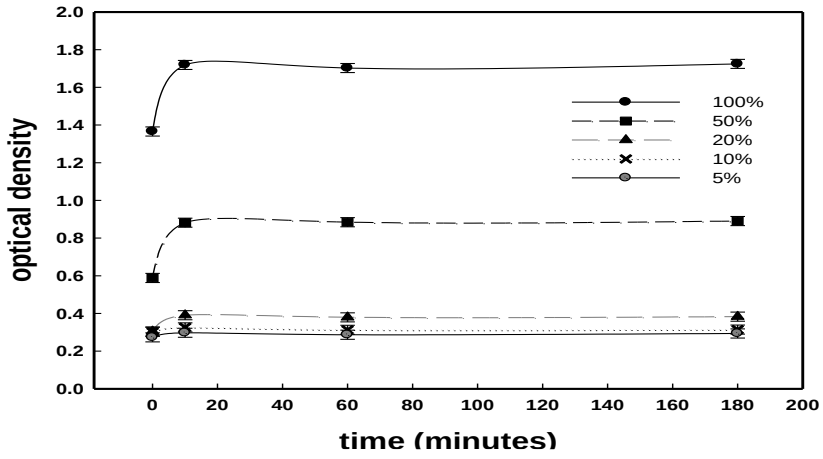

Figure 5.2: OD at 235 nm of strawberry extracts at different concentrations tested by Collmers' method. 100% is equal to 100 µl of strawberry enzyme extract ±se (cv Candonga).

The method was modified in order to reduce initial absorbance and increase its sensitivity. By reducing the amount of PGA added to buffer the initial absorbance was decreased and PEL activity was easier to detect. Furthermore, equilibration of blanks and buffer solution at 30 ˚C

for 20 minutes in water bath before initial measurement reduced variation and range of blank absorption. If there was not an initial 20 minute period for blanks to be kept at 30 ˚C, the absorbance of blanks could be increased up to 0.02. It was noticed that blank provided stable measurements after 20 minutes of being kept in the water bath.

In a subsequent experiment, commercial PEL was used to test the efficiency of the enzyme assay PEL sample in serial dilutions was tested over a period of 24 h with data recording points at 5 minutes, 10 minutes, 20 minutes, 40 minutes, 1 hour, 1.5 hour, 3 hours, 18 hours and 24 hours. The PEL extract concentrations that were used were 100%, 50%, 20% and 10% and buffer (blank). Results indicated that for the three highest concentrations, the reaction was complete or almost complete within 24h (Figure 5.3).

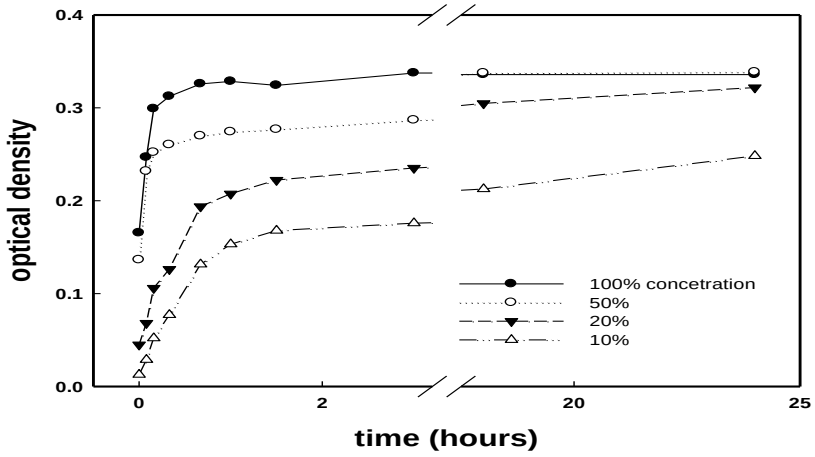

Figure 5.3: Logarithmic presentation of OD at 235nm of PEL from *Cellvibrio japonicus* at different concentrations over selected time intervals during 24h ±se.

Since the scope of the experiment was to test if fruits with contrasting firmness would also show different levels of PEL enzymatic activity it was not necessary to wait until the reaction was over and it was decided that a duration of 30 minutes would be an adequate time for the reaction to take place and have results to allow conclusions about enzymatic activity to be drawn.

Several tests and calibrations took place in order to identify possible limiting factors for the efficacy of the reaction and the accuracy of the method. Commercial PEL was also used at different concentrations to test the absorbance at 30 minutes. In figure 5.4 the relationship between concentration of PEL and activity is presented.

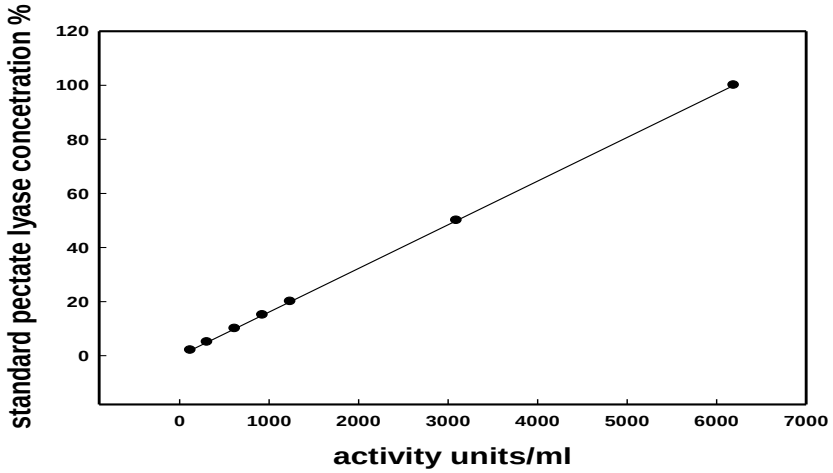

Figure 5.4: Relationship between concentration of PEL from *Cellvibrio japonicus* and enzymatic activity ±se.

A test with serial dilutions of pure PEL was used to test the efficiency of the method (Figure 5.5).

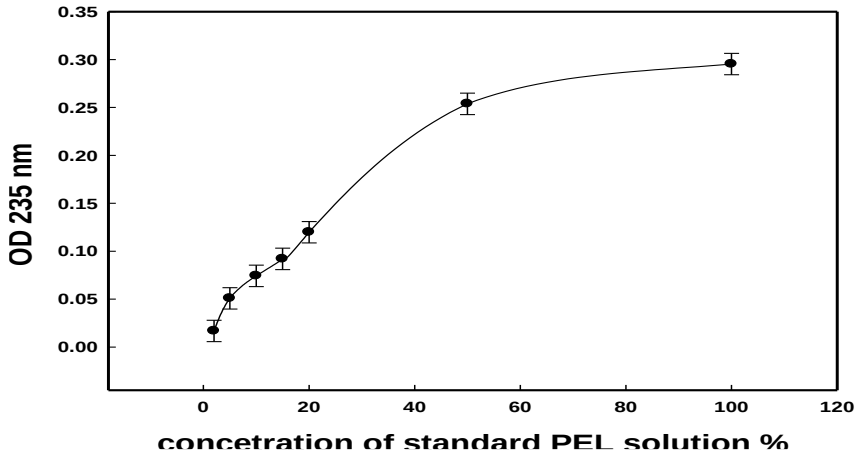

Figure 5.5: OD change 235 nm over 30 minutes of standard PEL from *Cellvibrio japonicus* at concentrations 100%, 50%, 20%, 15%, 10%, 5% and 2% ±se.

A calibration of the modified method with a range of PGA concentrations was made in order to verify that the presence of PGA at levels close to that used in the modified strawberry fruit method did not have a limiting effect on enzymatic activity. PEL standard extract was used at a concentration of 10% since absorption at that level was similar to expected results from strawberry PEL (Figure 5.6).

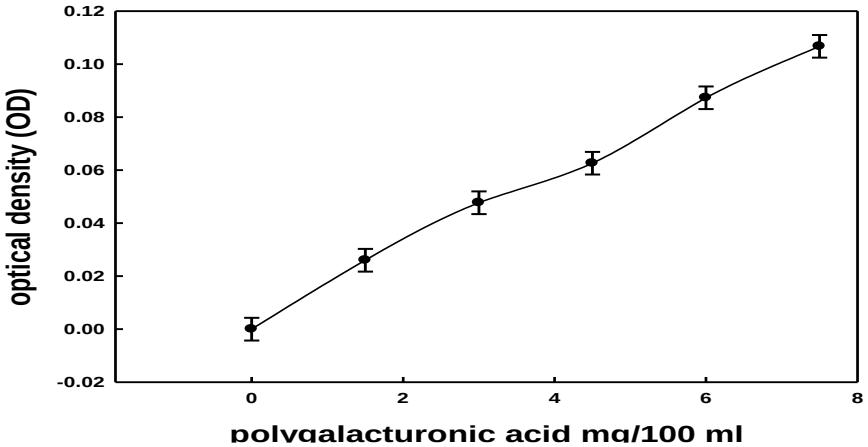

Figure 5.6: OD change at 235nm over 30 minutes of PEL from *Cellvibrio japonicus* at 10% concentration at varying levels of PGA, 0, 1.5, 3, 4.5, 6 & 7.5 mg/100 ml buffer solution ±se.

Initial tests with strawberry extracts showed that range of results was 0.01 - 0.1 OD at 235 nm over 30 minutes.

5.3. RESULTS AND DISCUSSION

5.3.1. Enzymatic activity of strawberry fruits with contrasting firmness

Growing conditions are presented at Table 5.1.

Table 5.1: Growing conditions 3 weeks before harvest day at SP1 and SP2 strawberry farms as recorded by local weather stations

Farm **Harvest day**	**SP1** **28-03-2010**	**SP1** **25-05-2010**	**SP2** **28-02-2011**	**SP2** **27-04-2011**
Average temperature (°C)	11.4	15.8	12.8	18.2
Maximum temperature (°C)	16.8	21.0	18.6	23.9
Minimum temperature (°C)	6.9	10.9	8.2	12.9
Solar irradiation MJ/m2 /day	15.3	26.2	12.6	20.8
Average relative humidity %	70.2	64.9	80.9	88.1
Maximum relative humidity %	88.5	82.8	96.3	93.6
Minimum relative humidity %	44.6	27.5	55.3	45.8
Average precipitation mm/day	2.3	1.4	2.1	0.4
Vapour pressure deficit (KPa)	0.4	0.6	0.3	0.3
Ozone μg/m3	41.4	77.8	65.7	79.0

Fruits from the SP2 site harvested on 28th February 2011 had an average firmness of 10.6 N and were firmer when compared to those harvested on the 27th of April 2011 from the same farm. The average firmness of the second soft fruit group was 7.8 N.

Results showed that firmer fruits on the first sampling week had higher PEL levels. Significantly ($p<0.01$) decreased OD was observed at the second sampling week where fruits were softer (Figure 5.7).

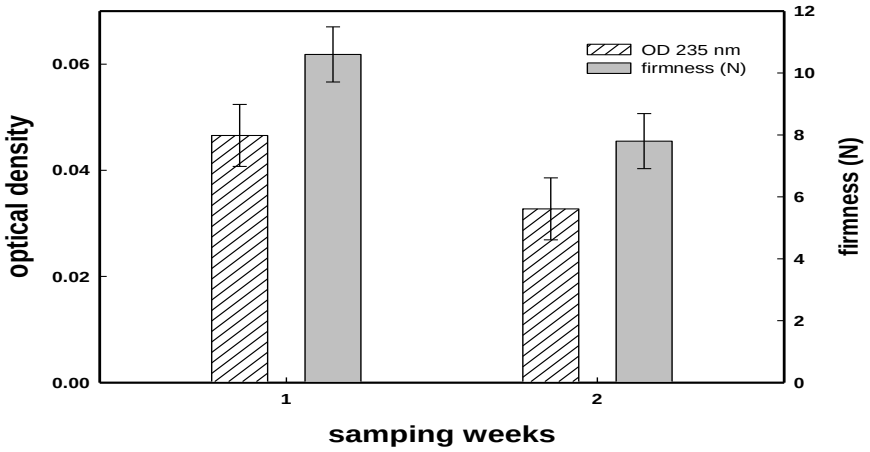

Figure 5.7: OD after 30 minutes at 235 nm of PEL from strawberry fruits (n=54) in week 1 (March 2011) and week 2 (April 2011) against fruit firmness ±se (cv Candonga)..

Fruits harvest from SP1 on 28th March and 25th May had average firmness levels of 8.2 N and 4.1 N, respectively. On this occasion, nine fruits per week were tested and again an increased (p=0.06) OD at 235 nm was observed when fruits where firmer at the first sampling week (Figure 5.8).

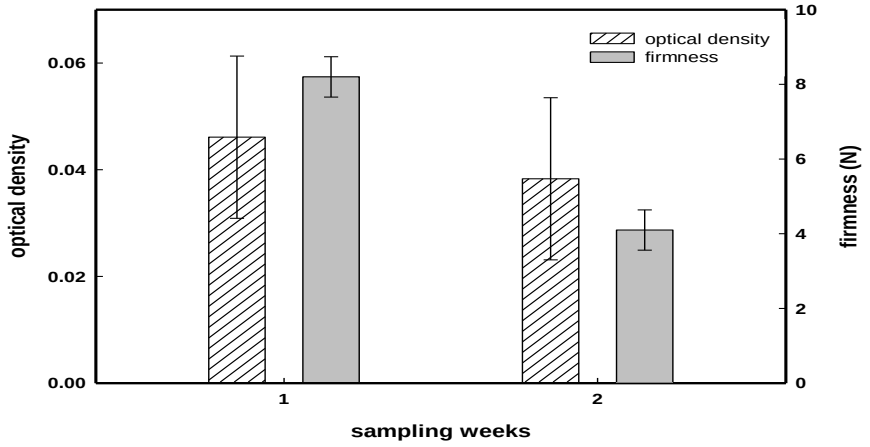

Figure 5.8: Optical density at 235 nm from PEL extracts from strawberry fruits (n=18) in week 1 (March 2010) and week 2 (May 2010) in relation to firmness (Newtons) in SP1 ±se (cv Candonga).

However there was no clear trend between enzymatic activity and firmness between fruits of the same week at SP2 (Figure 5.9) and SP1 (Figure 5.10).

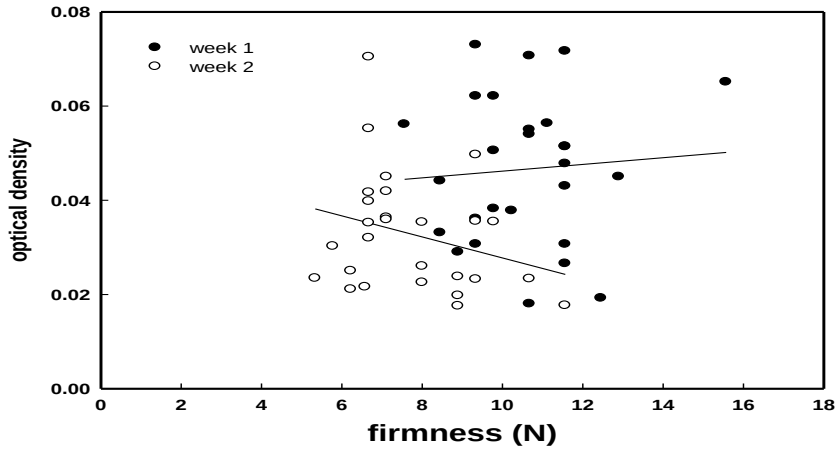

Figure 5.9: Firmness (N) of strawberry fruits harvested in week 1 (February 2011) and week 2 (April 2011) from SP2 against OD at 235 nm, indicating no clear trend between firmness and PEL activity across fruits harvested on the same week ±se (cv Candonga)..

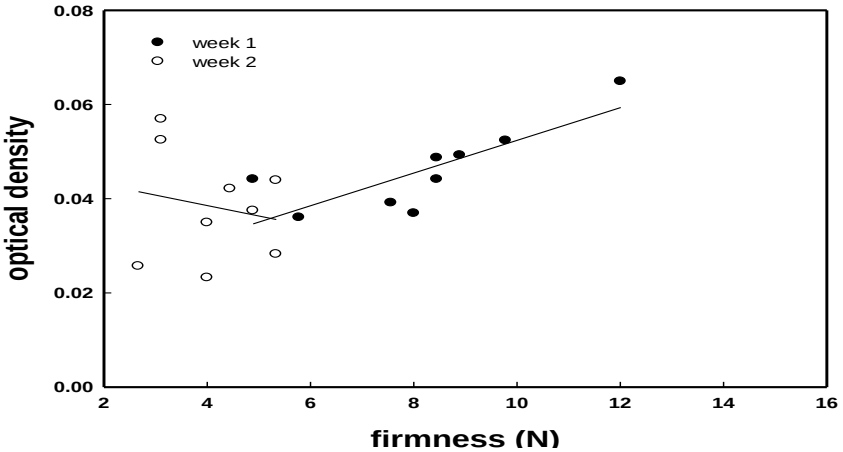

Figure 5.10: Firmness (N) of strawberry fruits harvested in week 1 • (March 2010) and week 2 (May 2010) from SP1 site against OD at 235 nm, indicating no clear trend between firmness and PEL activity across fruits harvested on the same week ±se (cv Candonga)..

The hypothesis that softness of strawberry fruits has a positive relationship with increased PEL enzymatic activity was not verified during the experiments. On the contrary there was a positive relationship between enhanced PEL activity and firmer fruits, when samples of different harvesting times were compared. In addition, there was no clear evidence for a positive or negative relationship between PEL enzymatic activity and firmness (as measured using a penetrometer) between fruits harvested at the same time. However, the absence of indications that increased PEL activity is related to strawberry softening should not be drawn as a conclusion of this experiment. The relationship between activity of pectolytic enzymes and disassembly of plant cell walls that consequently leads to firmness loss is well documented not only in

strawberry (Benitez-Burraco, 2003; Youssef *et al.*, 2009; Severo *et al.*, 2011), but in other fruits (Marin-Rodriguez, 2002a; Chourasia *et al.*, 2006).

There are several reasons that could explain the results above. Variance of fruit physiological age could be an explanation. Strawberry fruits were harvested in early and late season. First sampling date for SP1 and SP2 was on the 30th and 2nd of March respectively, while second sampling week was 27th of May for SP1 and 27th of April SP2. There was an almost two month interval between first and second sampling week in both cases. Weather during early season in Spain is not favourable for strawberry development and maturation whilst during late spring higher temperature and irradiation levels, boost strawberry plant development and fruit maturation (Palencia *et al.*, 2013). Fruits can require forty days to reach commercially acceptable ripeness during early season harvests in winter, whilst three weeks might be enough from flowering to fully mature fruit in the summer (F. Gonzalez pers. com. 2011; Palencia *et al.*, 2013). Since these fruits were grown in commercial farms and they were harvested when they were fulfilling minimum market requirements, it could be safe to assume that fruits harvested on the second sampling week in both farms actually spent less time on the plant from flowering to picking day. This fact could lead to the acceptance that fruits of different weeks were actually at different physiological stage despite the fact that they had been harvested as fully ripen, since they satisfied supermarkets main criteria (minimum amount in TTS and pink to red colour). There is evidence that pel1expression related to cell wall disassembly is affected by physiological stage of fruit since increased expression is observed at later stages of development (Youssef *et al.*, 2009). Since fruits of the second harvesting could be at an earlier stage of development, plC transcription could be reduced (Severo *et al.*, 2011), and therefore enzymatic activity could be found in lower levels compared to fruits of first week.

PEL activity is affected by many factors including pH, temperature (Jayani *et al.*, 2005; Yuan *et al.*, 2012) and presence of auxin, since increased level of the hormone regulate PEL activity (Manning, 1998). The fact that softer fruits were related to decreased PEL activity in the lab under certain conditions of temperature and pH shouldn't necessary be in agreement with the activity of PEL in the field where conditions were different. At the second sampling week for both sites ambient temperature was higher (Table 5.1) and this fact could have a positive effect on enzymatic activity. Increased preharvest temperatures in the field could have actually increased the activity of the enzyme despite the fact that PEL could have been present at lower levels, resulting in softer fruits on the second sampling week. Furthermore, since this study did not monitor the activity of PEL throughout the growing stages of fruits it could also be assumed

that the activity of PEL, of fruits sampled on the second week could have been increased at earlier stages and when the fruits got softer the enzymatic activity decreased. Since the fruits which were sampled at the first week were firm it could be assumed that actually fruits required increased PEL activity in order to become softer. Similar observations were noticed for banana where the pick of PEL activity occurred prior to fully ripe stage (Payasi and Sanwal, 2003). The results at SP1 site –where no statistical differences were noticed for PEL activity on the different sampling weeks– could be attributed to lower number of replications or other biochemical mechanisms that contribute to fruit softening as it is discussed below.

Softening of strawberries is related to pectolytic enzymatic activity. PEL is an enzyme related to strawberry softening, however, it is not the only enzyme that contributes to cell wall disassembly. During the experiment only PEL activity was assessed, enzymatic action of other enzymes that could possibly be related to fruit softening was not evaluated. Enzymes that are thought to contribute to strawberry softening are pectin methylesterases (PMEs) that are mainly active in early ripening stages and prepare plant cell wall for the catalytic action of PGs and PELs (Draye and Van Cutsem, 2008). Their action mechanism is de-esterification of pectin. This results in a formation of a neutral polymer, a necessary preparatory action for PGs and PELs in order to further disassembly pectin (Wolf *et al.*, 2009). PGs were also related to softening of strawberries since their decreased activity was related to firmer fruits (Lefever *et al.*, 2004). Other enzymes connected to strawberry are B-galactosidase since they are responsible for loss of galactan a structural component of pectin and hemicelluloses (Trainotti *et al.*, 2001). Another component of pectin is arabinan and its structure can by modified by the enzyme α-l-arabinofuranosidase which was found to be expressed in higher levels in mature fruits, and is thought to play a role in fruit softening (Carpita and Gibeaut, 1993; Rosli *et al.*, 2009). Endo-β-1,4-glucanases (EGases) can also act on xyloglucan, cellulose and glucomannan at plant cell wall (Harpster *et al.*, 1998; Brummell and Harpster, 2001). β-xyloxidase action can also be responsible for plant cell well disassembly by catalyzing cleavage of xylooligosaccharides (Martínez *et al.*, 2004; Bustamante *et al.*, 2006) . Breaking down of mannans by exo-manasses (Bourgault *et al.*, 2001) as well as that of xyloglucan by Xyloglucan endotransglucosylase (XTH) are processes related to strawberry loss of firmness (Fry, 2004). Furthermore, cell wall loosening could take place under the action of expansins, proteins that cleave hydrogen bonds of cellulose microfibrils resulting in cell wall expansion (Harrison *et al.*,2001). All the above mentioned enzymes act in a parallel and coordinated way resulting in softening of strawberry fruits. As it was mentioned before, expansin proteins, exomanasses PGs, B-galactosidase decompose structurally PELs substrate resulting in

exposure of pectate side-chains for the action of PELs. PELs are not the only enzymes responsible for strawberry softening.

Non enzymatic processes can result in strawberry loss of firmness such as cell loss of turgor (Shackel *et al.,* 1991). Presence of hydroxyl groups (Schopfer, 2001) and apoplastic active oxygen (Gomez *et al.,* 1995) can also result in pectin solubilisation. Loss of strawberry firmness could also be related to presence of calcium chelators (Brady, 1987).

In addition nutritional status of plants plays an important role on firmness , presence of adequate levels of calcium, boron which are both part of pectin is crucial for firmness. Primary cell wall of type I, the type of cell wall found in strawberries consists of pectins by 30%. Calcium presence helps cell wall maintain its structure and loss of calcium can result in loss of firmness (Wang *et al.*, 2014).

There are also several explanations about mismatch of enzymatic activity *in vivo* and *in vitro*. Since the presence of co-factors affect enzymatic activity a possible change in availability of a co-factor or spatial separation, between enzyme and cofactor could have an effect on enzymatic activity (Bourquin *et al.*, 2002).

Further work examining a number of these factors above would be necessary for further understanding. Quantification of protein as well as methods for determination of expression of PEL levels could improve understanding of PEL action under different climatic conditions and developmental stages of fruit. Since PEL activity contributes to loss of firmness in strawberry fruits, further exploration of different aspects of that enzyme could be beneficial for breeders, growers, retailers and consumers. Improvement of varieties that will be able to be withstand transport stress by being firmer as well as maintaining high nutritional value and flavour characteristics could be important in terms of reduction of postharvest waste as well as providing a healthier diet in affordable price for consumers.

Main findings - future work

- Hypothesis that strawberry fruits with lower firmness would show increased PEL activity was rejected

The above results could possibly be attributed to environmental effects (such as growing temperature). Research testing the hypothesis that PEL activity and subsequent softening of fruits was influenced by ambient temperature.

Chapter 6 : ASSESSING THE SUSCEPTIBILITY TO DRY BRUISE INJURY OF STRAWBERRIES AT DIFFERENT HARVEST DATES DURING THE GROWING SEASON

6.1. INTRODUCTION

Imported strawberry fruits have to travel a considerable distance to reach the final consumer. Time from harvesting to final destination can vary from a few hours, if fruits are sent by plane, to several days when they are sent by lorries. The most common way of sending fruits is by land using refrigerated containers, where the temperature is kept at about 4°C. A 'farm to fork' cold chain is used in order to maintain marketable quality characteristics of the fruits and prevent postharvest deterioration. Strawberries are perishable products and have greater postharvest waste (around 8%) compared to other fruits and require efficient temperature management to maintain good quality (Mena *et al.*, 2014). It is has been reported that a delay of six hours in imposing fruits in the cold chain can increase water loss by 50%, increase the incidence of shriveling and decrease firmness (Nunes *et al.*, 1995).

Pickers are trained to avoid excessive contact with fruits and it is common practice to harvest fruits by picking them from the stem in order to avoid unnecessary contact, since the force applied in picking is considered an extremely important factor in bruise development (Martinez-Romero *et al.*, 2004; Ferreira *et al.*, 2008). The detrimental effects of contact and application of force on quality of all soft fruits and more specifically strawberries are known by growers and have been described in the literature (Fischer *et al.*, 1992). Generally, the amount of energy applied on strawberries is a good predictor for bruising of strawberries (Holt and Schoorl, 1982). Strawberry bruise can by caused by several types of applied force such as impact, vibration (Fischer *et al.*, 1992) and compression, with the latter having the most detrimental effects causing cell breakage (Ferreira *et al.*, 2008; Ruiz-Altisent and Guillermo P. Moreda, 2011). Growers and breeders are aiming to produce varieties with increased firmness, since they are able to cope better with transport stress and postharvest handling. Generally varieties producing softer fruits are not considered suitable for exports since they are more susceptible to postharvest deterioration such as development of dry bruise. In previous chapters results were presented that related increased amount of dry bruised fruits with decreased firmness of strawberries, possibly caused by increased preharvest temperatures. Postharvest deterioration due to dry bruise is said to be caused by a combination of factors such as inappropriate harvesting handling that applies excessive force on fruits, delay in postharvest cooling and poor management of cold stores,

transport stress caused by vibration, compression and impact during shipping as well as forces between individual fruits, and fruits and packaging (Nunes *et al.*, 2003; Ferreira and Sargent, 2009). Furthermore, genotype (Ferreira *et al.*, 2008), cultural practices (irrigation and fertilization) as well as preharvest factors which have an indirect effect on firmness of fruits may affect the development of dry bruise (Opara and Pathare, 2014). Since dry bruise is considered a quality characteristic not desirable by consumers, supermarkets' common practice is to reject the whole load of strawberries which have dry bruise in excess of 20% (the percentage varies between supermarkets and availability of fruits in the market). A common practice from growers in order to reduce the amount of dry bruise of strawberries is to order them in single layers within the punnets. This practice, apart from resulting in better presentation of fruits, it is also effective in preventing excessive contact between fruits and reduces the force applied by the weight of the upper fruits on the fruits of the lower part of the punnet. In addition, a plastic bubble film is used to absorb vibration during transportation and postharvest handling of strawberries (Rodanto pers. comm.).

It is clear that the amount of energy and type of force as well as preharvest and postharvest conditions play a significant role in development of dry bruise. At present experiment the hypothesis that softer fruits are more susceptible to development of bruising when exposed to compression was tested and findings were discussed. Decreased firmness of strawberry fruits is related to increased temperature levels before harvest. Firmness varied through harvest season -as it was mentioned in previous chapters- and amount of dry bruised fruits increased as temperatures reached at higher levels from winter to summer. The experiment also tested the possible relationship between increased temperature, lower firmness and increased incidence of dry bruise on strawberry fruits.

6.2. MATERIALS AND METHODS

6.2.1. Plant material

Strawberry plants of cv. Candonga grown at commercial farms in the Huelva province of Spain were used for this study. Fruits were picked at consecutive weeks during the harvest period. They were placed in a cold store at the harvesting farms then shipped according to standard retail practices, to a packhouse based in Kent, England. Fruits were shipped by refrigerated lorries and temperature was kept at 4°C during transportation. At the packhouse in Kent the storage temperature for fruit was also 4°C. After sampling, fruits were transferred to NRI.

6.2.2. Compression method

Fruits sampled as described in paragraph 3.2.2.3 were used for the compression method. Fruits upon their arrival at NRI were stored for three hours at 3 °C before being used for the compression at room temperature. After the compression which typically lasted 5 minutes they were put back to the incubator at 3 °CFor exposing fruits to compression a TA-XT plus (Stablemicrosystem Ltd, UK) a texture analyzer was used. A cylinder of 50mm diameter was adjusted on the Texture analyzer Fruits were subjected to a constant compression of 10 Newtons over 10 sec.

6.2.3. Dry bruise evaluation

Fruits exposed to artificial bruising were stored at 4 °C for two days in an NRI cold chamber. Afterwards the maximum width and length (cm) of dry bruise of the area that was exposed to artificial bruise were recorded by a ruler. Dry bruising that developed on other areas of the fruit was not recorded during evaluation. Depth in mm was also recorded.

Figure 6.1: Texture analyser TA-XT plus, equipped with 50mm diameter cylinder used for exposing fruits to artificial bruising.

6.2.4. Statistical analysis

Pearson's' correlations were used to analyze data. R statistics 2.10. Twenty seven fruits per sampling week were analyzed.

6.3.RESULTS AND DISCUSSION

At both sites increased dry bruised areas of fruits were observed when higher temperatures prior to harvest took place during the growth period (Figures 6.2 and 6.3). Increased temperatures can lead to fruits with decreased firmness as discussed at chapter three. Decreased firmness of fruits was also related to increased dry bruised area of strawberry fruits. Growth temperature 2 days, 7 days, 14 days and 21 days prior harvesting were recorded and there was significant correlation between them. The highest correlation, in chapter three, was observed between firmness and temperature 14 days prior harvesting, therefore, relationships between firmness, temperature and dry bruised area of fruits, in this chapter, where based on average temperatures 14 days before harvesting. At SP1 site the highest dry bruised area of strawberry fruits was noticed during the second half of the period compared to the first when increased mean temperatures (above 17 °C) were observed. On the fourth sampling week (day 100) temperatures were also high, but dry bruise incidence was low (Figure 6.2). The depth of dry bruise varied through season and no clear trend was noticed. At SP2 site again over the second part of the period the increased dry bruised areas were noticed compared to the first half. However, despite the fact that the highest temperatures were noticed over the last two weeks, the highest dry bruise area was on the fifth and sixth week (Figure 6.3). Again, there was no clear trend for depth of dry bruise through the season.

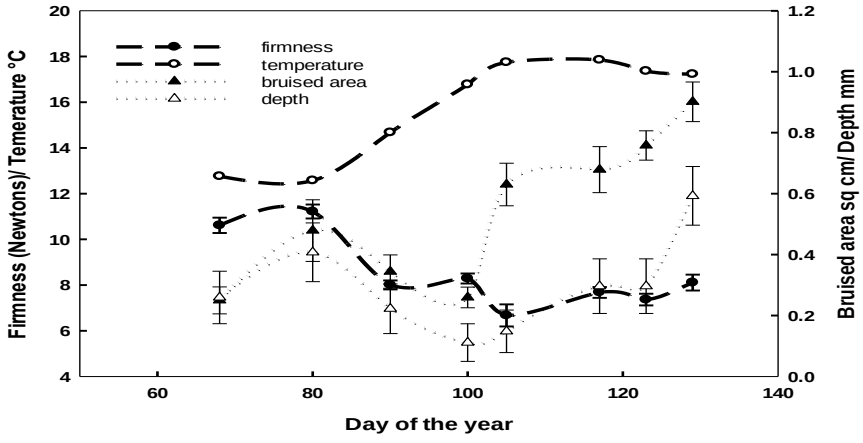

Figure 6.1: Dry bruised area (sq. cm), depth of dry bruise (mm) and firmness of fruits (Newton) in relation to average temperature 14 days prior harvesting in SP1 site (cv Candonga).

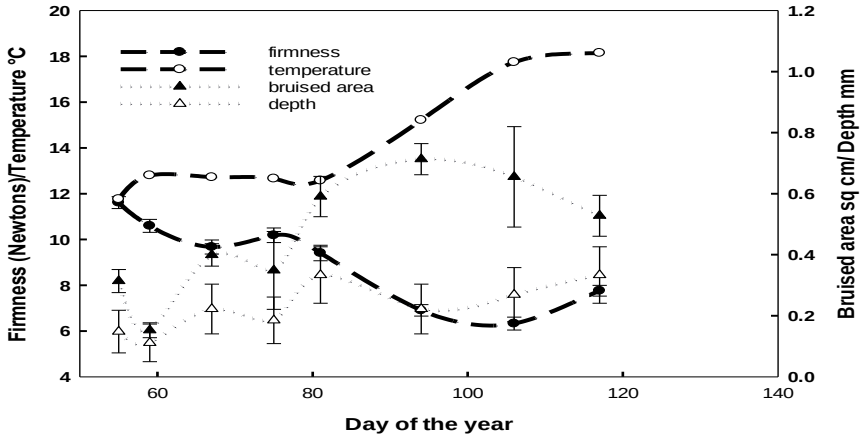

Figure 6.3: Dry bruised area (sq. cm), depth of dry bruise (mm) and firmness of fruits (Newton) in relation to average temperature 14 days prior harvesting at SP2 site.

At both sites there was a negative relationship between firmness and dry bruised area. At the SP2 farm this relationship was more intense and this was also highlighted by Pearson's correlations where this was above 0.8 (Table 6.2). A similar observation was also observed for SP1, but to a lower degree (-0.48) (Table 6.1). The negative association between firmness and dry bruised area of strawberry fruits is also shown in figures 6.4 and 6.5. The r^2 value for SP2 was 0.7, however the r^2 value for SP1 was 0.2.

Table 6.1: Pearson's correlations at SP1 farm (cv Candonga).

Variables	bruised area cm^2	depth mm	firmness (Newton)	average temperature °C 2 weeks prior harvest
bruised area cm^2	**1**	0.634	-0.480	0.558
depth mm	0.634	**1**	0.269	-0.112
firmness (Newton)	-0.480	0.269	**1**	**-0.916**
average temperature °C 2 weeks prior harvest	0.558	-0.112	**-0.916**	**1**

Table 6.2: Pearson's correlations at SP2 farm (cv Candonga)..

Variables	bruised area cm^2	depth mm	firmness (Newton)	average temperature °C 2 weeks prior harvest
bruised area cm^2	**1**	**0.741**	**-0.852**	0.645
depth mm	**0.741**	**1**	-0.600	0.576
firmness (Newton)	**-0.852**	-0.600	**1**	**-0.873**
average temperature °C 2 weeks prior harvest	0.645	0.576	**-0.873**	**1**

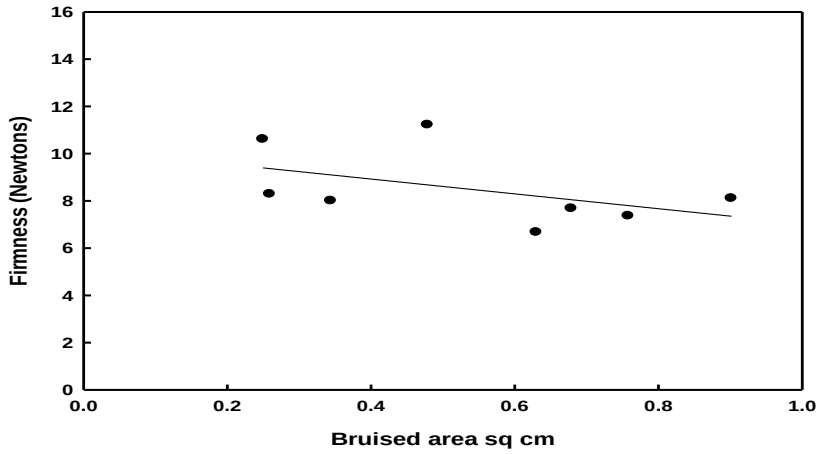

Figure 6.2: Relationship between firmness of fruits (Newton) and dry bruised are of intact strawberry fruits, from SP1, imposed to artificial bruising procedure (r^2=0.2) ±se (cv Candonga)..

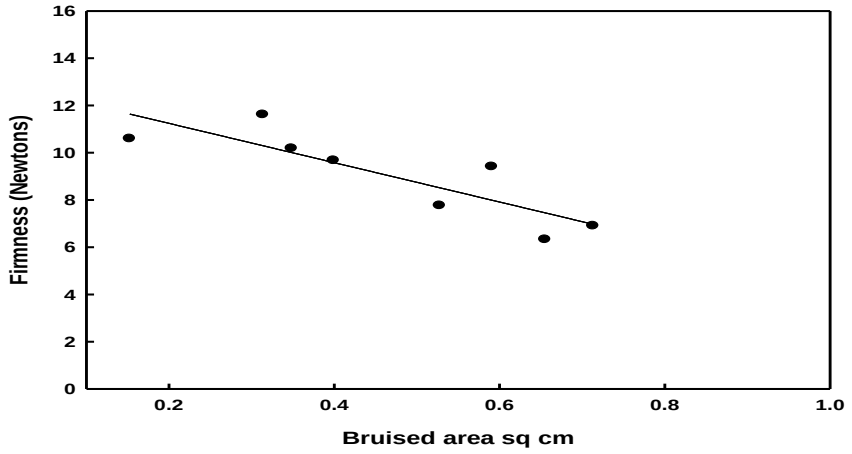

Figure 6.3: Relationship between firmness of fruits (Newton) and dry bruised are of intact strawberry fruits, from SP2, imposed to artificial bruising procedure (r^2=0.7) ±se (cv Candonga)..

Generally fruits had increased dry bruised area when exposed to additional force by TA-XT texture analyzer as the season progressed and increased temperatures led to decreased firmness. There was no clear trend for the depth of dry bruise. This observation comes in agreement with the results that were recorded on the environmental effects on postharvest quality of strawberries chapter (Chapter 3), where fruits were found to have increased amounts of dry bruise towards the end of the season. The evidence of the positive effect of increased temperature and reduced firmness on dry bruise development was stronger at the SP2 farm. It should be mentioned that temperature and firmness are not the only parameters that could affect the formation of bruise on fruits. Time of harvesting and/or delay in postharvest cooling (Nunes *et al.*, 1995) could have detrimental effects on quality of strawberries. If fruits were picked at the warmest period of the

day and failed to be introduced into the cold chain they could endure additional stress. This could be in the form of increased water losses which could have negative effects on turgor and firmness (Vicente *et al.*, 2007). Other parameters of interest is the position of the fruit in the punnets and packaging scheme, since they could affect the type of force applied on fruits (Martinez-Romero *et al.*, 2004; Ferreira *et al.*, 2008), efficiency of the cold chain, experience of the picker, and postharvest and preharvest practices (Opara and Pathare, 2014). With this experiment only compression force was applied on fruits that had already been exposed to substantial transport stress since they had already been transported for almost two days. More information would be acquired from future work looking at intact fruits imposed to different type of forces. Further understanding of the mechanism of dry bruise development has to offer important information on growers, breeders and retailers.

Dry bruise is a very common defect of strawberry fruits which decreases acceptance by consumers. Many varieties are not consider appropriate for commercial launch since they are soft and they cannot handle transport stress, which leads to dry bruise. If further research could provide solution in a way that dry bruise could be reduced then that would be beneficial for growers, retailers and consumers. Postharvest waste could be reduced and consumers could enjoy varieties that have excellent quality characteristics, but under current practices they are not launched because they are soft. Since dry bruise is related to firmness as well as transport stress, an efficient way of reducing injury would be to reduce transport stress apart from producing cultivars with increased firmness.

Since strawberries travel great distances in our days from farms to final consumer they are exposed to excessive amount of transport stress. This causes excessive amount of dry bruising, a mechanical damage which is caused among other factors by prolonged transportation. Bruising is one of the most significant mechanical quality deterioration due to three types of transport stress: impact, vibration, and compression. Bruising depends on factors such as firmness, cultivar, maturity stage, postharvest handling and shape of fruit. Generally fruits with small sphericity values are more susceptible to bruise development (Aliasgarian *et al.*, 2015).

Chapter 7 : SENSORY EVALUATION AND UK CONSUMER ACCEPTABILITY OF 'CANDONGA' STRAWBERRY FRUITS GROWN IN SPAIN AND HARVESTED AT DIFFERENT TIMES DURING THE GROWING SEASON

7.1. INTRODUCTION

One of the challenges that growers and agronomists face is the variability of strawberry quality through the harvesting season (Samykanno, 2012). A fact that has an impact on consumer acceptability of fruits (Vicente *et al.*, 2014). It is important to evaluate seasonal changes in fruit quality and moreover understand the way consumer acceptance is influenced by individual quality characteristics (Kafkas *et al*., 2007; Jouquand *et al.*, 2008; Schwieterman *et al*., 2014). Strawberry quality characteristics can be directly or indirectly affected by environmental conditions. Formation of phenolics, sugars and acids can be affected by the quantity and quality of solar irradiation (Atkinson *et al.*, 2006; Kawanobu *et al.* 2011; Tsormpatsidis *et al.*, 2011), temperature (Wang and Camp 2000; Mackenzie *et al*. 2011), humidity (Choi *et al.*, 1997; Lieten, 2000) and ozone (Keutgen *et al.*, 2005; Keutgen and Pawelzik, 2008). In addition, it is known that strawberry fruit quality is affected by the physiological stage of the plant e.g. size of primary fruit is much bigger when compared to the fruits of the following trusses (Darrow, 1966) and accumulation of soluble solids can be affected by crop load (Correia *et al.*, 2011). There are also interactions between environment and physiological stage of the plant; an example is the effect of increased irradiation that leads to increased formation of phenolic compounds (Tsormpatsidis *et al.*, 2011) and can also lead to earlier maturation and harvesting (Palencia *et al.*, 2008; Schwieterman *et al.*, 2014). However, because of earlier ripening and harvesting, fruits spend less time on the plant, a fact that is related to decreased formation of phenolics (Andrianjka-Camps *et al.*, 2012).

Fruit quality attributes that are related to increased consumer acceptability, such as sugars and anthocyanins, can be affected by many factors such as environmental conditions (Samykanno, 2012), cultivar (Crespo *et al*., 2010), pre- and postharvest agricultural practices (Nunes and Emond, 2003; Terry et al., 2007) and the physiological state of the fruit (Correia *et al*., 2011). All the above parameters can change during the harvesting period resulting in fruits with completely different quality profiles that impact consumer acceptance (Vicente *et al*., 2014). The aim of this chapter was to evaluate the way that consumers perceive quality of strawberries harvested at different times over the harvesting season and relate their acceptance to preharvest

growing conditions wherever possible. Findings of this chapter could help growers and retailers understand shortcomings within their production and how these affect consumer perception.

7.2. MATERIALS AND METHODS

7.2.1. Plant material

Strawberry fruits of cv. Candoga were grown at a commercial farm near the town of Moguer in the Huelva province of Spain. Fruits were harvested and shipped to the UK following standard commercial practices. On arrival to the UK, fruits were stored at a local packhouse in Kent. Samples were collected and taken to the Natural Resources Institute (NRI), University of Greenwich. They were exposed to ambient temperatures (approximately 18 °C) for less than thirty minutes during transportation. Fruits were kept at 4 °C overnight and the sensory evaluation and consumer acceptability studies undertaken the following day. Samples were collected on five occasions during the harvesting season (5th, 13th and 26th March, 24th April and 3rd May 2012) in order to monitor potential changes in the quality of fruits exposed to variable environmental conditions and the acceptance by consumers.

7.2.2. Sensory evaluation panel

A sensory panel consisting of fifteen panelists was formed. All participants were members of staff and students at NRI. The panel was drawn from ten female and five male participants with ages between eighteen and seventy years. Thirteen out of fifteen panelists were Europeans. All the participants declared that they were consuming and buying strawberries regularly (at least once every two months).

An initial discussion and training took place and panelists contributed to formulation of the evaluation sheet and the fruit characteristics that would be assessed (Table 7.1) All participants were notified that they could abort the procedure anytime should they wish to do so and an information sheet was supplied to them.

Fruits were sensory evaluated at five different harvesting weeks over the harvesting period. At each sampling week, panelists were called to evaluate fruits according to predetermined characteristics. An instructions' document (Appendix C) was given to panelists at the beginning of each session in order to remind them of the procedure that they should follow. Four fruits at each evaluation session were presented to each panelist in a white plastic dish. Four fruit samples were labeled with randomly formatted three digit numbers. A glass of water was given to panelists before tasting of each fruit and they were asked not to consume anything that could

interfere with their taste and aroma perception before the consumption of fruits. All panelists were assessing fruits in the same room. They were separated from each other by booths. At the end of each session evaluation documents were signed and delivered.

Table 7.1: Sensory evaluation form as developed by panellists

Category	Variable assessed	Lower end	Higher end
Appearance	Size	Very small	Very big
	Red colour	Not red	Very red
	White colour	Not white	Very white
	Uniformity of colour	Not uniform	Very uniform
	Shiny surface	Not shiny	Very shiny
	Bruising	Not bruised	Very bruised
	Irregular shape	Not (normal shape)	Very (irregular shape)
Odour	Strong strawberry aroma	Not strong	Very strong
Texture	Firm (hand)	Not firm	Very firm
	Firm (mouth)	Not firm	Very firm
	Juicy texture	Not juicy	Very juicy
	Internal flesh firmness	Not firm	Very firm
Taste	Sweet taste	Not sweet	Very sweet
	Acidic taste	Not acidic	Very acidic
	Strong fruity taste	Not (weak taste)	Very (strong taste)
	Fermented/off flavour	Not fermented	Very fermented
	Ripeness	Not ripe	Very ripe

7.2.3. Consumer acceptability study

A consumer acceptability study was performed before the sensory evaluation. The consumer acceptability study took place at University of Greenwich and 30 randomly chosen participants were selected at each sampling week session. The participants were called to evaluate 3 fruits based on a 9 point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) for taste, odour (panelists decided to use odour to describe strong strawberry aroma) appearance and overall acceptability. Strawberries were tested over five weeks through the harvest season. All evaluation spaces were separated with booths and participants were provided

with a cup of water before testing. It was made clear that participants should have tasted strawberries before and they were asked to sign a declaration form that they had no known problems related to strawberry consumption.

7.2.4. Statistical analysis

PCA, ANOVA, Agglomerative hierarchical clustering and Tukey's HSD were performed with R statistics and XL STAT statistical software packages(Bechoff *et al.*, 2014).

7.3.RESULTS AND DISCUSSION

7.3.1. Consumer acceptability testing

All fruits were acceptable to consumers since they selected scores above five for all evaluated aspects (overall, taste and appearance). The overall acceptance of strawberries did not differ significantly (Table 7.2). However, significant differences were observed in terms of taste and appearance acceptance over the weeks. The sampling week with the highest acceptance for taste was the first week followed by week four. Both of them were not significantly different according to Tukey's honest significant differences (HSD). The second sampling week received the lowest score in terms of taste acceptance and was significantly different from all the rest. The third and fourth weeks were in the middle of acceptance range with respect to taste criterion, belonging to the same group. Appearance scored highest in the second week, and the week with the lowest score was week five. All the other weeks received similar scores and did not vary significantly. Despite the fact that in overall acceptance there were no significant differences between weeks, variation was noticed though for individual characteristics (taste and appearance).

Table 7.2: Mean acceptance scores. Different letters indicate different groups as determined by Tukey's HSD (cv Candonga).

Sampling week	Taste (1-9)	Appearance (1-9)	Overall (1-9)
1 05/03/2012	8.2±1.0c	7.3±1.3ab	7.5±1.1a
213/03/2012	6.9±1.5b	7.9±1.4b	7.4±1.2a
3 26/03/2012	6.1±1.2a	7.4±1.5ab	7.4±1.1a
4 24/04/2012	7.8±1.1c	7.3±1.5ab	7.3±1.2a
5 03/05/2012	7.0±1.7b	6.8±1.4a	7.0±1.7a

7.3.2. Testing and verification of consistency of sensory evaluation panellists

In order to verify that panelists behaved in a similar way, that their responses and evaluation followed a consistent pattern, a hierarchical clustering was performed (Figure 7.1). Panelists were classified into three main clusters.

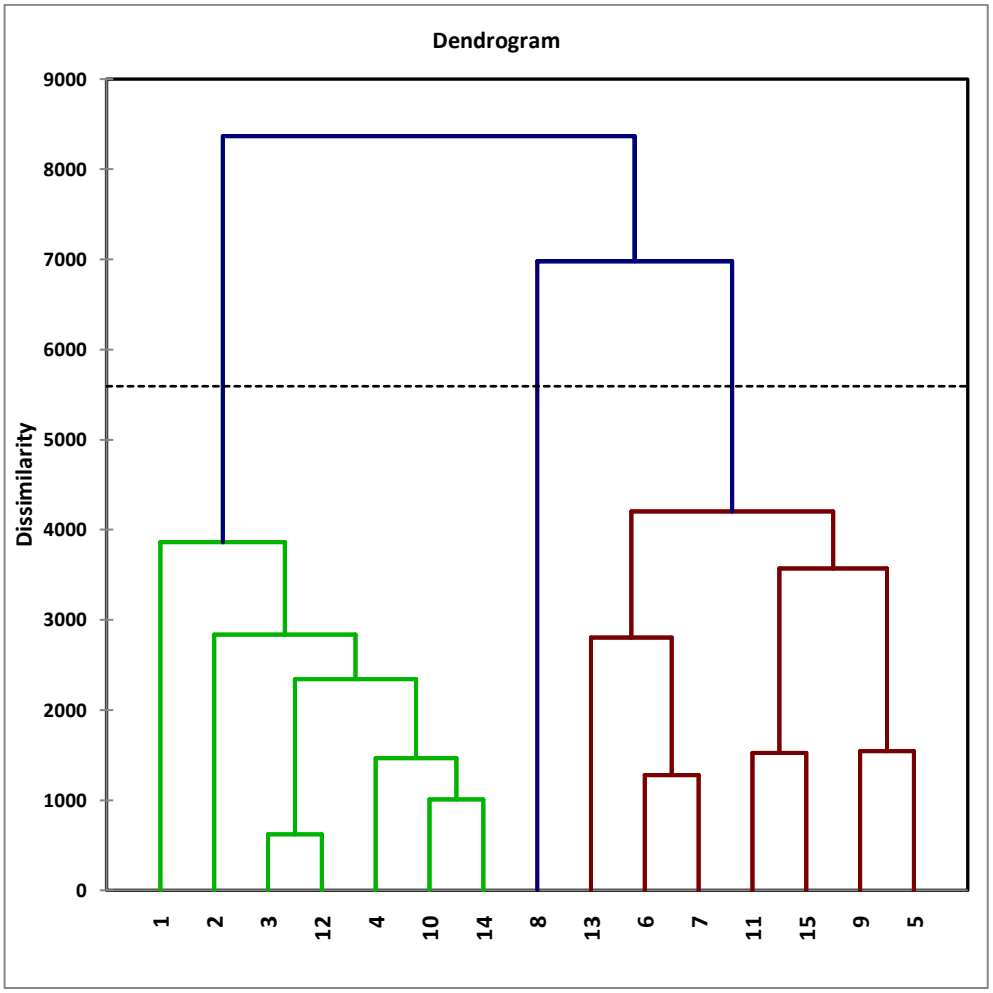

Figure 7.1: Agglomerative hierarchical clustering dendrogram for dividing panellists into groups of similar perception of strawberry quality characteristics (n=15).

The first and third cluster consisted of seven panelists. The second cluster consisted only of one panelist. Furthermore, by application of PCA (Figure 7.2) the same panelist was found at the border of the diagram. Therefore it was decided to exclude that panelist from the analysis of the results and all associated evaluations from this were removed before proceeding into further analysis.

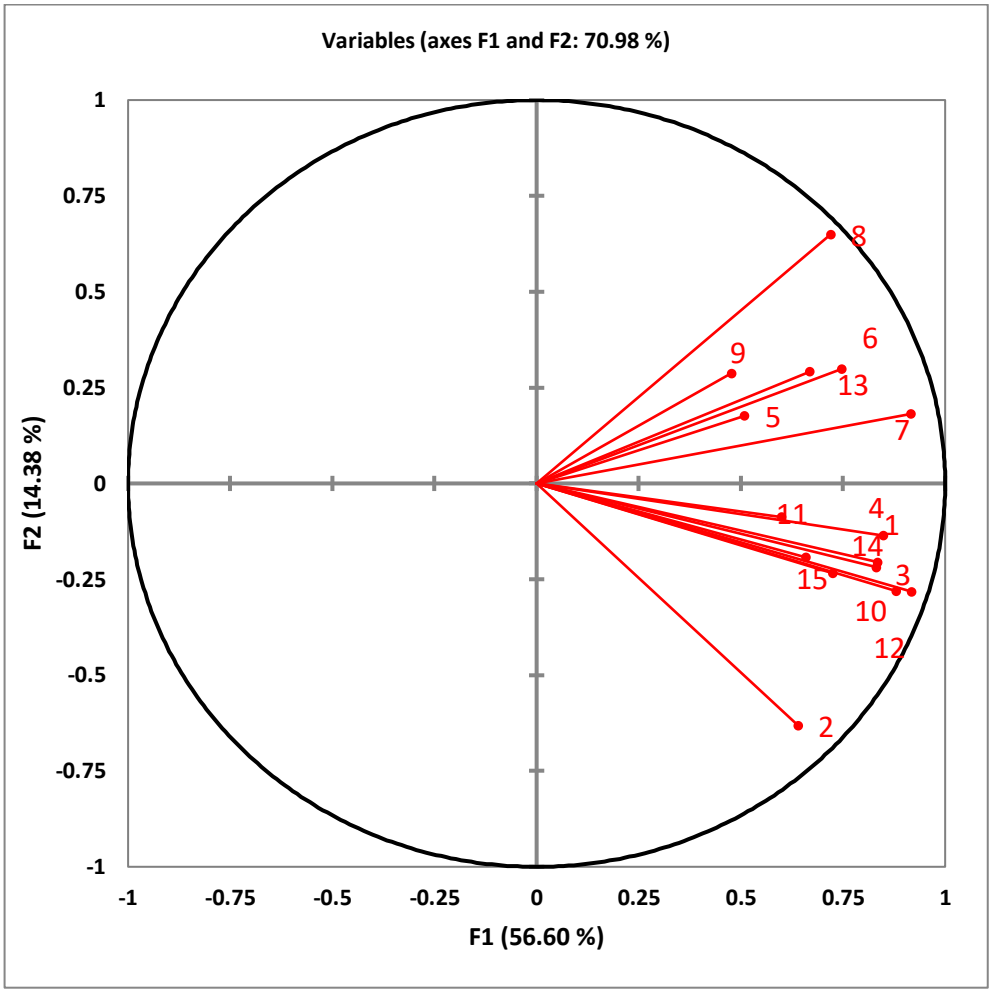

Figure 7.2: Principal component analysis over panellists and their perception of strawberry quality characteristics (n=15).

A hierarchical clustering (Figure 7.3) and PCA (Figure 7.4) was again performed after the removal of the panelist that was identified as an outlier. This was done in order to verify that the remaining panelists were evaluating fruits in a similar way. All panelists were shown to have positive loadings on the horizontal axis and the total percentage of the variability of parameters that could be explained by the first two principal components exceeded 70% with the first variable explaining almost 60%. Therefore this is an indication of the panelists evaluating fruits in a similar way.

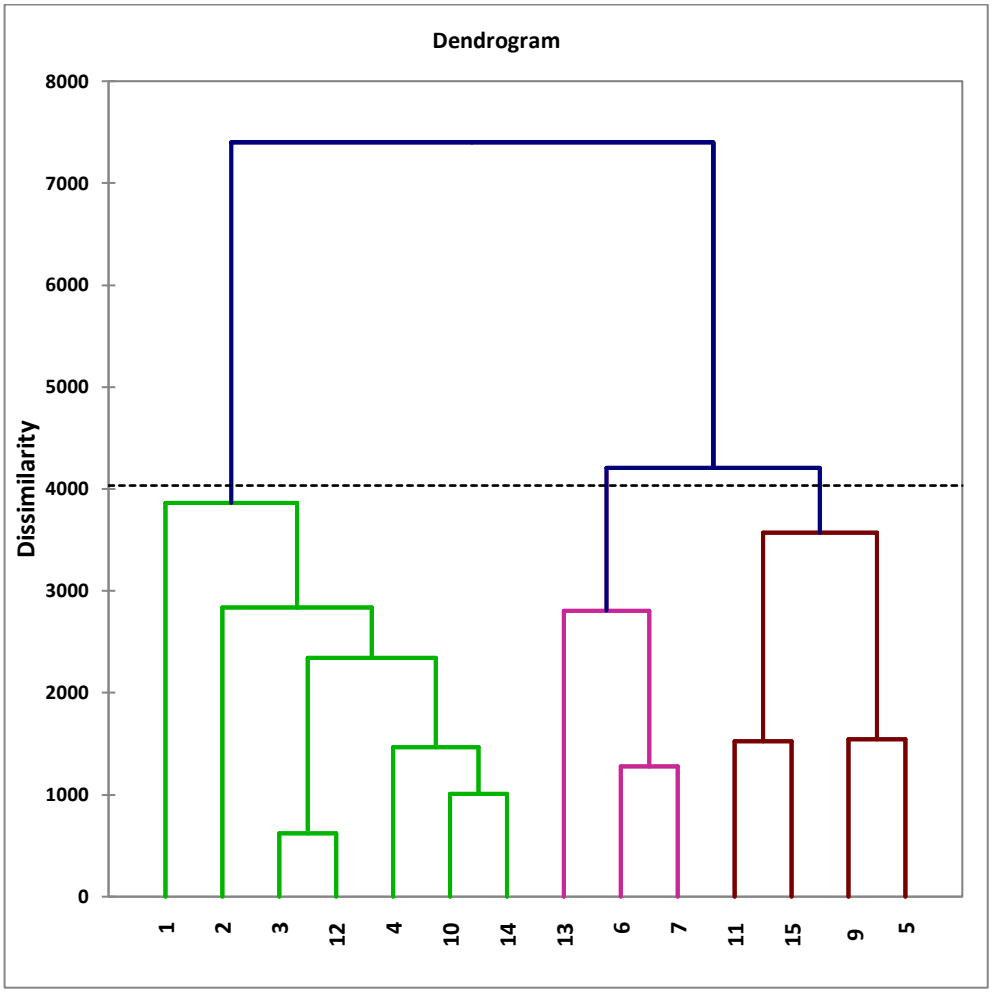

Figure 7.3: Agglomerative hierarchical clustering dendrogram for dividing panellists into groups of similar perception of strawberry quality characteristics (n=14).

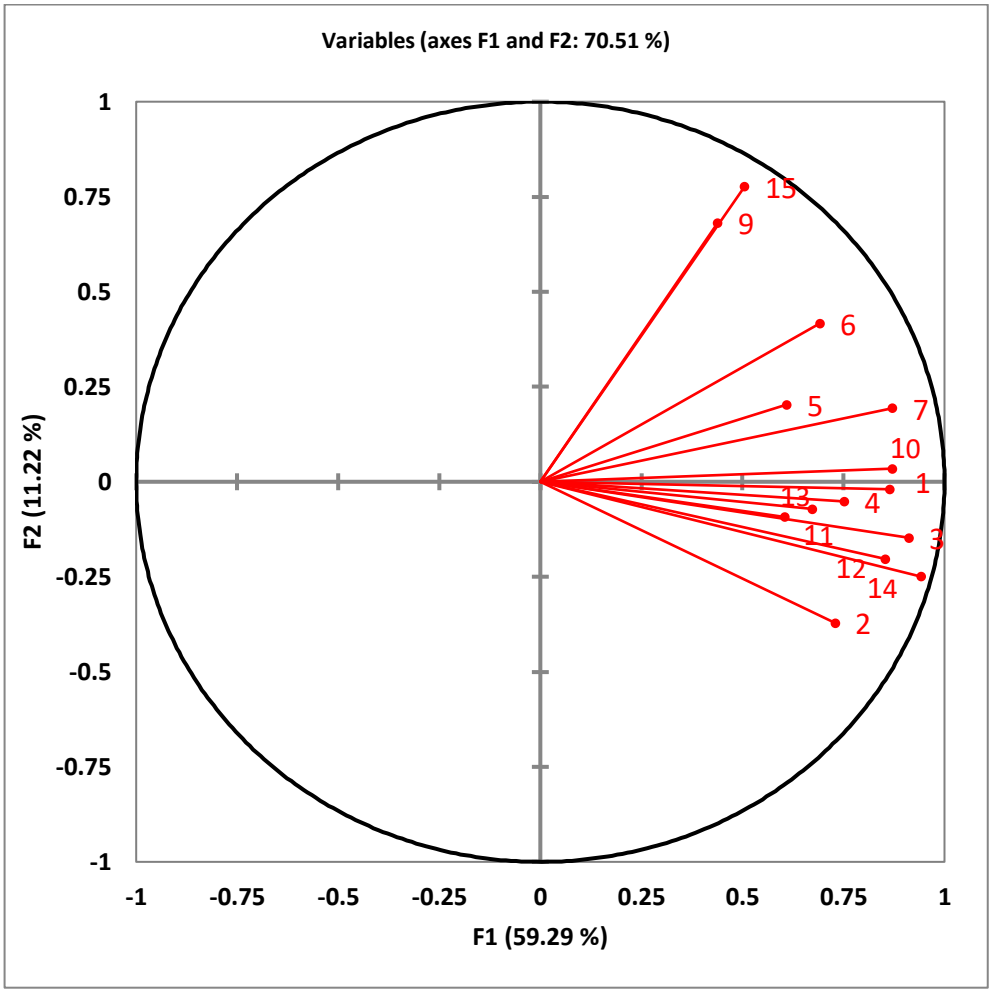

Figure 7.4: Principal component analysis over panellists and their perception of strawberry quality characteristics (n=14).

7.3.3. Perception of colour and relationship with total anthocyanins and colorimetric measurements

A correlation matrix is calculated relating instrumental measurements with the perception of sensory evaluation panel (Table 7.6). In this study it was observed that there was a relationship between consumers' perception of red colour and amount of total anthocyanins, however, at no significant level (Table 7.6). Although red colour perception was positively correlated to total anthocyanins, it was negatively correlated to all colorimetric parameters and more specifically the *a** value, an indication of redness of fruits. *a** mean values had a narrow range between 36.6 and 38.8 (Table 7.4). In literature, the amount of total anthocyanins is not always related to fruit ripeness, and also not considered a good way of predicting ripeness (Ordidge *et al.*, 2012). White colour perception by panelists was positively related to *a** values, at no significant level. Colour of strawberries is affected by several factors such as temperature (Wang and Camp 2000), irradiation (Tsormpatsidis *et al.*, 2011), ozone and early harvesting (Andrianjka-Camps *et al.*,

2012; Palencia, 2013) having quite often contradicting effects. Increased temperatures can decrease formation of ellagic acid - a compound related to red colour of strawberries (Wang and Zheng, 2001) - however can also decrease the amount of time between flowering and harvesting (Andrianjka-Camps *et al.*, 2012; Palencia, 2013) resulting in lower levels of anthocyanin levels. Furthermore, increased UV radiation levels can enhance formation of anthocyanins (Tsormpatsidis *et al.*, 2011) and increased temperature could be related to increased bruise through transport stress (Nunes *et al.*, 2003). Relating consumer acceptance, sensory evaluation and instrumental analysis for colour evaluation of strawberry fruit is a quite complex procedure and from the findings no clear pattern could be identified.

7.3.4. Size weight and appearance

There was a significant positive relationship between perception of size and weight of strawberry fruits (Table 7.6 and figure 7.5). The second week where the fruits were bigger in size compared to the following weeks, panelists gave higher scores for appearance. However, fruits of the first week, despite the fact that they were the largest, received average scores. This fact could be explained since fruits of first week had increased % of dry bruise on fruits compared to fruits of second week. As far as dry bruise is concerned, sensory panelists were not able to discriminate between the different amounts of dry bruised fruits between different sampling weeks (Table 7.3). Fruits of the last week received the lowest score – with regards to appearance- since they were the smallest and had the highest levels of dry bruise (Table 7.4). Panelists gave the highest score for irregular shape fruits during the last sampling week (Table 7.3). This could be an additional reason explaining the low score of appearance that was noticed in the last week (Table 7.2). The gradual reduction of strawberry size as identified by panelists could possibly be attributed to differences in size between primary and the following fruit waves (Darrow, 1966). Then increased percentage of dry bruised fruits could be a result of increased preharvest temperatures (Table 7.8) (Nunes *et al.*, 2003).

7.3.5. Perception of taste

Sweet taste as perceived by panelists was positively related to total sugars and soluble solids and negatively related to total acids (Table 7.6). Furthermore, there was a significant negative association between perception of acidic taste and perception of sweet taste. Ripeness was also related to sweet taste. Panelists perceived sweet fruits as more ripe. Ripeness was also positively correlated at a significant level to strong fruity taste. A positive correlation of ripeness was noticed with the amount of soluble solids. Generally it could be said that fruits that were

perceived as more sweet, ripe, with stronger fruity taste were related to higher levels of soluble solids, total sugars and lower levels of acids, a trend that comes in agreement with others when cv Camarosa was tested (Roussos *et al.*, 2009). However, more ripe fruits were also more likely to be perceived as having an off flavour. Furthermore, consumers gave higher rating to taste acceptability in weeks where fruits were sweeter and had lower acids (Table 7.2). The third week received the lowest score in terms of taste acceptance. At the third week the lowest amount of total soluble solids was noticed. The second week received the second lowest score in consumer acceptance for taste and it was also classified as the week with the most unripe fruits, less sweet and most intense acidic taste. On the same week the second lowest total soluble solid content was recorded and the lowest total sugar and sweetness index values (Table 7.5). Furthermore, total acids were quite high receiving the second highest value. Despite the fact that first week had the highest content of total acids it was not perceived as the most intense in terms of acidic taste and also received the highest score in consumer acceptance for taste. This could probably be explained since the highest amount of soluble solids, total sugars and sweetness index was observed that week, with sweet taste perception receiving the second highest score from panelists. Panelists related good quality of strawberry fruits with increased levels of sugars (Schwieterman *et al.*, 2014) and reduced levels of acids, an observation that comes in agreement with others (Vicente *et al.*, 2014). Formation of sugars, acids and soluble solids is affected by many factors including developmental stage and interval between flowering and harvesting (Montero et al. 1996; Kafkas *et al*. 2007), temperature (Wang and Camp, 2000) and amount of solar irradiation (Atkinson *et al.*, 2006).

7.3.6. Perception of firmness

Fruit firmness as recorded by penetrometer measurements and perception of firmness by taste panel were correlated. Strawberry firmness when tested by panelists' hands was correlated positively with mouth perception of firmness as well as penetrometer measurements (Table 7.6 and 7.5). The only exception was internal flesh firmness that showed lower levels of correlation with the above parameters. Generally panelists were able to discriminate between softer and firmer fruits. No clear trend between consumer acceptance and firmness could be identified. Reduction of firmness through season could possibly be attributed to higher temperatures prior to harvesting, as season progresses (Table 7.). Similar observations between firmness of fruits and environmental temperature was also noticed in other studies (Correia *et al.*, 2011).

Table 7.3: Mean weekly values of quality attributes as recorded by panellists. Different letters indicate different groups as determined by Tukey's HSD ±se (cv Candonga)..

Descriptor	texture				Taste				
	Hand firmness	Mouth firmness	Juicy texture	Internal flesh firmness	Sweet taste	Acidic taste	Strong fruity taste	Fermented / off flavour	Ripeness
Week 1	63.7±14.5a	64.4±15.6b	73.3±22.3a	51.0±21.3a	51.9±20.8b	40.3±20.1ab	50.0±22.8b	20.1±22.4a	63.4±22.1b
Week 2	64.6±14.0a	66.4±19.3b	52.1±18.8a	60.0±21.3a	34.2±18.1a	48.9±19.6b	29.5±17.2a	20.7±20.6a	39.8±19.9a
Week 3	55.1±16.2a	57.3±20.4ab	62.7±20.6a	51.2±22.9a	44.2±21.4ab	42.0±24.5ab	38.2±19.1ab	12.9±18.4a	52.3±15.9ab
Week 4	55.6±20.6a	57.5±20.3ab	53.1±20.8a	58.6±22.1a	42.1±23.1ab	47.3±20.6ab	41.8±21.3ab	13.2±13.9a	46.5±21.5a
Week 5	57.1±18.3a	49.0±24.4a	55.1±21.3a	52.8±21.7a	54.4±24.3b	35.1±22.2a	43.7±23.9b	25.0±25.7a	52.2±20.2ab
Week	0.018	<0.001	0.20	0.18	0.001	0.026	0.0012	0.047	0.001

Descriptor	Appearance						
	Size	Red colour	White colour	Uniformity of colour	Shiny surface	Bruising	Irregular shape
Week 1	57.8±2.8c	61.3±3.3a	38.4±4.0a	50.6±3.9ab	58.4±3.5ab	31.0±3.6a	26.8±3.1a
Week 2	54.9±3.4bc	55.9±3.9a	38.9±4.0a	48.4±3.9ab	59.7±3.0ab	35.3±3.8a	29.4±3.7a
Week 3	49.0±3.9bc	57.8±4.0a	16.9±3.7b	63.5±4.1b	61.1±4.2ab	30.7±4.1a	29.4±4.3a
Week 4	42.4±3.4ab	57.9±2.8a	38.0±3.6a	43.6±3.6a	49.2±3.2a	31.6±2.6a	30.1±3.9a
Week 5	41.8±3.2a	50.0±3.6a	28.7±3.6ab	46.2±4.3a	66.0±2.8b	36.2±3.7a	40.9±5.0a
Week	0.001	0.14	0.001	0.016	0.01	0.73	0.11

Table 7.4: Mean weekly values of instrumental analyses related to appearance of fruits. Different letters indicate different groups as determined by Tukey's HSD ±se. Instrumental analysis data was also included in chapter 3 (cv Candonga).

	*L**	*a**	*b**	chroma	hue	weight	% dry bruise	Anthocyanins (mg/100g fresh weight)
Week 1	37.3±2.1a	37.1±1.5b	23.8±3.5a	44.2±2.9b	32.5±3.3a	32.3±7.1a	12.0±6.5ab	34.7±4.8a
Week 2	37.6±2.6ab	38.7±1.1a	27.0±3.8bc	47.3±2.7a	34.7±3.6a	29.4±7.6ab	5.4±7.2a	35.6±9.0a
Week 3	38.4±3.0ab	36.5±1.8b	25.5±4.4ab	44.7±3.5bc	34.6±4.1a	24.5±6.3b	13.2±10.ab	34.5±6.1a
Week 4	41.5±4.6c	36.6±3.3b	29.6±4.9c	47.3±3.1a	38.8±6.2b	17.1±3.4c	9.0±10.1a	33.2±6.6a
Week 5	39.9±3.3bc	37.8±1.5ab	27.0±4.4bc	46.6±3.4ac	35.3±3.8a	18.7±8.3c	23.9±15.8b	32.8±6.9a

Table 7.5: Mean weekly values of instrumental analyses related to texture and flavour of fruits. Different letters indicate different groups as determined by Tukey's HSD ±se. Instrumental analysis data was also included in chapter 3 (cv Candonga).

	TSS	total sugars (mg/g of fresh weight)	sweetness index	Total acids (mg/g of fresh weight)	% dry matter	Firmness
Week 1	10.3±1.5c	55.2±7.6b	89.8±12.3b	14.5±1.1a	8.9±0.9a	13.0±2.4c
Week 2	8.5±1.2ab	45.7±18.3a	73.3±30.6a	13.9±1.5a	8.6±1.1a	10.6±2.1a
Week 3	7.6±0.9a	49.7±4.1ab	80.5±6.4ab	13.1±1.4a	8.9±1.2a	10.1±2.0a
Week 4	9.0±1.2b	52.1±4.1ab	84.6±6.4ab	8.8±.08b	8.3±1.0a	10.1±2.3a
Week 5	8.8±1.3b	49.7±5.3ab	79.7±8.6ab	9.5±2.4b	8.5±0.9a	7.6±2.5b

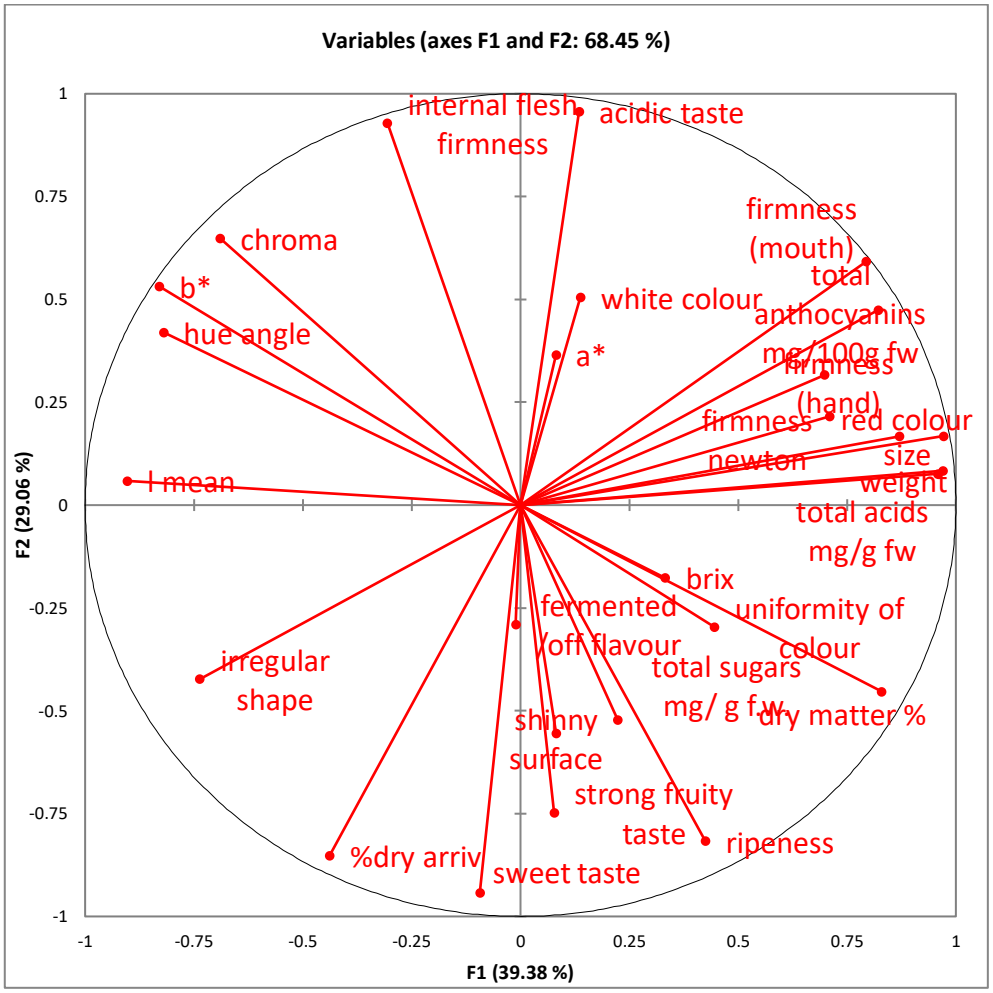

Figure 7.5: Principal component analysis over sensory panel quality attributes and instrumental analyses (cv Candonga).

Table 7.6: Pearson correlation values between sensory attributes and instrumental analyses of strawberry fruits. The correlations were calculated on the basis of five sampling weeks. Significant correlations are shown in bold.

Variables	size	colour	colour	of colour	surface	shape	(hand)	(mouth)	firmness	taste	taste	taste	flavour	ripeness	weight	brix	Hue angle	chroma	bmean	amean	l mean	matter %	(Newton)	mg/ g fw	mg/g fw	mg/100g fw	bruise
size	**1.00**	0.64	0.29	0.30	0.07	-0.71	0.83	0.87	-0.11	-0.23	0.25	-0.07	0.10	0.25	**0.99**	0.34	-0.78	-0.51	-0.73	0.29	**-0.92**	0.71	0.84	0.08	**0.96**	0.89	-0.53
red colour	0.64	**1.00**	0.23	0.29	-0.60	**-0.96**	0.28	0.73	-0.08	-0.24	0.46	0.20	-0.60	0.33	0.56	0.37	-0.23	-0.51	-0.37	-0.46	-0.35	0.44	**0.93**	0.52	0.55	0.54	-0.68
white colour	0.29	0.23	**1.00**	-0.80	-0.48	-0.25	0.65	0.52	0.58	-0.22	0.43	0.06	0.29	-0.14	0.24	0.72	0.10	0.40	0.22	0.40	0.01	-0.39	0.41	0.16	0.03	0.16	-0.461
uniformity of colour	0.30	0.29	-0.80	**1.00**	0.33	-0.32	-0.21	0.08	-0.58	-0.03	-0.13	-0.12	-0.43	0.25	0.32	-0.53	-0.45	-0.69	-0.57	-0.36	-0.48	0.77	0.17	-0.077	0.53	0.40	0.00
shinny surface	0.07	-0.60	-0.48	0.33	**1.00**	0.57	0.12	-0.34	-0.51	0.41	-0.69	-0.05	0.67	0.19	0.19	-0.27	-0.61	-0.27	-0.51	0.45	-0.46	0.44	-0.39	-0.34	0.25	0.03	0.652
irregular shape	-0.71	**-0.96**	-0.25	-0.32	0.57	**1.00**	-0.38	-0.85	-0.10	0.48	-0.64	0.07	0.59	-0.11	-0.63	-0.21	0.22	0.38	0.31	0.27	0.44	-0.42	**-0.90**	-0.26	-0.64	-0.71	0.83
firmness (hand)	0.83	0.28	0.65	-0.21	0.12	-0.38	**1.00**	0.78	0.21	-0.23	0.25	-0.14	0.50	0.02	0.83	0.50	-0.61	-0.07	-0.45	0.68	-0.74	0.31	0.60	-0.10	0.71	0.72	-0.43
firmness (mouth)	0.87	0.73	0.52	0.08	-0.34	-0.85	0.78	**1.00**	0.33	-0.59	0.68	-0.29	-0.16	-0.10	0.82	0.29	-0.38	-0.15	-0.33	0.26	-0.65	0.35	0.84	-0.04	0.77	0.89	-0.86
internal flesh firmness	-0.11	-0.08	0.58	-0.58	-0.51	-0.10	0.21	0.33	**1.00**	-0.79	0.81	-0.67	-0.09	-0.87	-0.18	-0.12	0.60	0.88	0.74	0.45	0.31	-0.73	-0.11	-0.54	-0.23	0.17	-0.62
sweet taste	-0.23	-0.24	-0.22	-0.03	0.41	0.48	-0.23	-0.59	-0.79	**1.00**	**-0.93**	0.87	0.41	0.82	-0.15	0.43	-0.31	-0.49	-0.39	-0.30	0.08	0.24	-0.15	0.64	-0.22	-0.60	0.85
acidic taste	0.25	0.46	0.43	-0.13	-0.69	-0.64	0.25	0.68	0.81	**-0.93**	**1.00**	-0.64	-0.52	-0.67	0.15	-0.15	0.39	0.45	0.43	0.12	0.02	-0.31	0.35	-0.34	0.16	0.53	-0.95
strong fruity taste	-0.07	0.20	0.06	-0.12	-0.05	0.07	-0.14	-0.29	-0.67	0.87	-0.64	**1.00**	0.12	**0.90**	-0.05	0.70	-0.21	-0.55	-0.36	-0.53	0.12	0.22	0.23	**0.93**	-0.16	-0.50	0.50
fermented /off flavour	0.10	-0.60	0.29	-0.43	0.67	0.59	0.50	-0.16	-0.09	0.41	-0.52	0.12	**1.00**	0.11	0.18	0.33	-0.43	0.13	-0.23	0.74	-0.28	0.00	-0.25	-0.17	0.054	-0.09	0.50
ripeness	0.25	0.33	-0.14	0.25	0.19	-0.11	0.02	-0.10	-0.87	0.82	-0.67	**0.90**	0.11	**1.00**	0.30	0.57	-0.57	-0.85	-0.72	-0.47	-0.28	0.62	0.41	0.84	0.24	-0.16	0.43

Table 7.6: Pearson correlation values between sensory attributes and instrumental analyses of strawberry fruits. The correlations were calculated on the basis of five sampling weeks. Significant correlations are shown in bold.

	weight	brix	hue angle	chroma	bmean	amean	l mean	dry matter %	firmness (Newton)	sugars mg/ g fw	acids mg/g fw	anthocyanins mg/100g fw	% dry bruise
weight	**0.99**	0.56	0.24	0.32	0.19	-0.63	0.83	0.82	-0.18	-0.15	0.15	-0.05	0.18	0.30	**1.00**	0.33	-0.84	-0.54	-0.79	0.32	**-0.95**	0.76	0.79	0.06	**0.97**	0.87	-0.43
brix	0.34	0.37	0.72	-0.53	-0.27	-0.21	0.50	0.29	-0.12	0.43	-0.15	0.70	0.33	0.57	0.33	**1.00**	-0.26	-0.22	-0.27	-0.01	-0.12	0.05	0.56	0.73	0.11	-0.06	-0.02
hue angle	-0.78	-0.23	0.10	-0.45	-0.61	0.22	-0.61	-0.38	0.60	-0.31	0.39	-0.21	-0.43	-0.57	-0.84	-0.26	**1.00**	0.74	**0.96**	-0.26	**0.92**	**-0.89**	-0.50	-0.14	-0.847	-0.56	-0.11
chroma	-0.51	-0.51	0.40	-0.69	-0.27	0.38	-0.07	-0.15	0.88	-0.49	0.45	-0.55	0.13	-0.85	-0.54	-0.22	0.74	**1.00**	**0.89**	0.46	0.58	**-0.92**	-0.54	-0.57	-0.59	-0.25	-0.17
bmean	-0.73	-0.37	0.22	-0.57	-0.51	0.31	-0.45	-0.33	0.74	-0.39	0.43	-0.36	-0.23	-0.72	-0.79	-0.27	**0.96**	**0.89**	**1.00**	0.00	0.85	**-0.96**	-0.55	-0.32	-0.80	-0.48	-0.11
amean	0.29	-0.46	0.40	-0.36	0.45	0.27	0.68	0.26	0.45	-0.30	0.12	-0.53	0.74	-0.47	0.32	-0.01	-0.26	0.46	0.00	**1.00**	-0.39	-0.13	-0.16	-0.67	0.26	0.38	-0.09
l mean	**-0.92**	-0.35	0.01	-0.48	-0.46	0.44	-0.74	-0.65	0.31	0.08	0.02	0.12	-0.28	-0.28	**-0.95**	-0.12	**0.92**	0.58	0.85	-0.39	**1.00**	-0.84	-0.59	0.099	**-0.97**	-0.83	0.24
dry matter %	0.71	0.44	-0.39	0.77	0.44	-0.42	0.31	0.35	-0.73	0.24	-0.31	0.22	0.00	0.62	0.76	0.05	**-0.89**	**-0.92**	**-0.96**	-0.13	-0.84	**1.00**	0.55	0.24	0.83	0.55	0.03
firmness (Newton)	0.84	**0.92**	0.41	0.17	-0.39	**-0.90**	0.60	0.84	-0.11	-0.15	0.35	0.23	-0.25	0.41	0.79	0.56	-0.49	-0.54	-0.55	-0.16	-0.59	0.55	**1.00**	0.49	0.72	0.65	-0.63
sugars mg/ g fw	0.07	0.51	0.16	-0.07	-0.34	-0.26	-0.10	-0.04	-0.54	0.64	-0.34	**0.93**	-0.17	0.84	0.06	0.73	-0.14	-0.57	-0.32	-0.67	0.10	0.23	0.49	**1.00**	-0.05	-0.32	0.17
acids mg/g fw	**0.96**	0.55	0.03	0.53	0.25	-0.64	0.71	0.77	-0.23	-0.22	0.16	-0.16	0.05	0.24	**0.97**	0.11	-0.84	-0.59	-0.80	0.26	**-0.97**	0.83	0.72	-0.05	**1.00**	0.91	-0.43
anthocyanins mg/100g fw	**0.89**	0.53	0.16	0.39	0.03	-0.71	0.72	**0.89**	0.17	-0.60	0.53	-0.50	-0.09	-0.16	0.87	-0.06	-0.56	-0.25	-0.48	0.38	-0.83	0.55	0.65	-0.32	0.91	**1.00**	-0.712
% dry bruise	-0.53	-0.68	-0.46	0.00	0.65	0.83	-0.43	-0.86	-0.62	0.85	**-0.95**	0.50	0.50	0.43	-0.43	-0.02	-0.11	-0.17	-0.13	-0.08	0.23	0.03	-0.63	0.17	-0.43	-0.71	**1.00**

Table 7.7: Average values for the time interval, 3 weeks prior harvest up to harvesting point, of environmental parameters as recorded by weather and environmental stations at Moguer, Huelva.

Week	Temperature °C	Irradiation MJ/sq meter/ day	Relative Humidity %	Vapour Pressure Deficit (kPa)	Ozone (µg/m³)
1	8.63	16.13	68.60	0.35	57.06
2	10.55	17.14	72.93	0.34	58.13
3	12.14	19.65	70.07	0.42	62.97
4	14.05	19.71	72.31	0.44	68.08
5	14.88	22.00	69.98	0.51	71.63

7.4. CONCLUSION

The sensory evaluation was restricted to the final year of the research, in order to evaluate strawberry quality changes and perception by consumer throughout the harvesting season, where environmental variation existed. The environmental variables patterns followed a certain model, lower temperatures, reduced ozone, irradiation levels and VPD values in winter increasing progressively towards summer. Therefore, sampling of five weeks, two in early season, one in the middle and two at the end of the season was considered adequate amount of replications to capture changes over the season. The quality of fruits was found to be variable through the harvest season and consumers were able to identify these variations in part. There was large variability between visual and organoleptic quality traits that could be attributed to the differences in the scale that panelists score the quality attributes, however they were all evaluating in a similar way despite that fact that the scores were different. Furthermore the variability across consumers could be attributed to the fact that they were not evaluating the same fruit, a fact that was known prior conducting the taste panel and it was decided to proceed by using increased number of panelists (ten) and fruits/week/panelist (four). The variability between visual and organoleptic characteristics throughout harvesting season could be attributed to the fact that individual quality characteristics are affected in different ways by environmental factors as described in chapter three. Many researchers have recorded the differences in quality attributes of strawberry fruits through harvest season (MacKenzie and Chandler, 2011; Whitaker *et al.*, 2011; Vicente *et al.*, 2014). Strawberry fruits with decreased content of soluble solids and total sugars were found to be less acceptable by consumers, an observation which comes in agreement with findings of present study, since when levels of carbohydrates measured by

HPLC, were lower fruits scored lower in terms of consumer acceptance. Generally, fruits with increased levels of sugars, sweetness index, sugar/acid ratio and reduced levels of acids are preferred by consumers (Jouquand *et al.*, 2008; Roussos *et al.*, 2009). The ability to understand the way that consumer acceptance changes in accordance to quality parameters, as well as relating instrumental measurements and human perception through sensory evaluation, could be proved a useful tool for growers, retailers and breeders. Adopting varieties that could adapt more easily to variable environmental conditions could result to increased acceptance and elevated consumption of strawberries, promoting a healthier diet and reducing postharvest waste.

Chapter 8 : GENERAL DISCUSSION

8.1. DISCUSSION

The main aim of the study was to investigate potential effects of environmental variability on postharvest quality of strawberries. Strawberries have a quite lengthy harvesting period. In Spain, harvesting can start around late December and continue until early May. During this almost half year harvesting season the quality profile of strawberries harvested from the same plants grown in the same fields can be extremely variable. The research presented in this thesis verified this. The variability of strawberry quality is a major concern for producers, importers and retailers. It can result in development of problems, increased waste, consumer complaints, additional running costs regrading of fruit and disposal of waste and possibly reduced sales and decreased profit at all levels of the supply chain.

Postharvest quality of strawberries is affected by many factors including genotype, cultural practices and pre- and postharvest environmental conditions (Wang and Camp, 2000; Atkinson *et al.*, 2006; Tsormpatsidis *et al.*, 2011). The focus of this study was to monitor, in detail, changes of preharvest environmental conditions and the effect on selected quality attributes of fruits. Chapter three describes the methodology followed in order to record key environmental conditions (temperature, VPD, ozone and solar irradiation) and the impact of these environmental factors on postharvest quality of strawberries. Chapter three was the initial and lengthiest part of the experimental work since the recording of fruit quality spanned over three seasons, two countries and three growing sites.

Initial results of the first year's observations revealed that firmness of fruits was reduced when an increase in levels of preharvest temperature were recorded, a fact that was in agreement with others (Correia *et al.*, 2011). However there was no clear indication about the critical period over which temperature had an effect on reducing fruit firmness since general linear models showed that temperature had an effect over a long period (three weeks prior harvesting), mid-range (one week prior harvesting) and short-term (two days prior harvesting). The above observation triggered the need for further investigation of the phenomenon, and defined the course of further research and focus of the study. The research was focused on the evaluation of the physiological mechanisms contributing to softening of strawberry fruits and the implications that these mechanisms could have on fruit quality.

As far as it concerns accumulation of sugars and acids, the concentration of individual sugars was variable over years. For the first year 2010 where the mean temperature was slightly lower compared to 2011 at both Spanish sites sucrose levels were increased and fructose levels were decreased compared to 2011 (results shown in appendix figures b1-b18). this finding comes in agreement with others were it was found that in strawberry leaves sucrose contents decrease as temperature increases (Wang and Camp, 2000) and on the contrary fructose levels increase. Research conducted on Californian strawberries on several varieties showed that sugars decrease as temperature is being increased in the range of 25-40°C (Schwieterman *et al.*, 2014; MacKenzie and Chandler, 2011). However, this finding does not come in agreement with the observations at Spanish sites since at all locations there was a decline in sugars in the middle of the season and higher levels of sugars were observed in the initial and the final part of harvesting season. This could be an effect of crop load since in research conducted in Portugal on Candonga variety, the same as the one grown in the Spanish farms SP1 and SP2 the higher crop load was found to appear in the middle of the growing season which collides with low sugars. A fact that should be taken in to account is that in the research conducted in California the growth period is much shorter (December-March) is shorter compared to the growing season in Spain (January-early June), therefore there is no indication of the tested varies behaviour at longer harvesting periods. An additional consideration that should be taken in to account is the breakdown of sucrose into fructose and glucose under higher temperature and the translocation of sugars form photosynthetic tissue to roots and fruits. It was observed that at day/night (18/12°C) scheme (Wang and Camp, 2000) root development is increased compared to vegetative development and the accumulation of sugars is increased at roots compared to higher temperature conditions (25/22°C).

For acids at SP1 site malic and citric where higher at 2010 compared to 2011 where the mean temperature was increased compared to 2010. At SP2 site the levels of malic and citric where similar for 2010 and 2011, however there was a noticeable decrease in malic acid in 2012 which was the coldest year of all since the temperature was lower it could be expected to have decreased rate of photosynthesis and respiration, a fact that could have a negative effect of transformation of malates to citric acid.

Despite the fact that there were not measurements on the amount of primary and secondary fruits harvested over season, it is known that primary fruits exhibit better quality characteristics as far as it concerns size, sugars and colouration. However, it should be noticed that primary fruits in California and Spain are harvested in winter where the average time interval between flowering

and harvesting is about 40 days. In the spring and summer where temperature is higher the time interval between anthesis and harvesting could drop to 20 days. This fact could have an effect on accumulation of sugars and acids as well as accumulation of anthocyanins and firmness as discussed.

When fruits were subjected to treatments with different temperature and light levels, the hypotheses that strawberry fruits respond to increased preharvest temperature at short (four hours) and long term (one to four weeks) were tested. It was shown that the two tested varieties responded differently. The softer variety Elsinore was responsive both to short and long term temperature changes and the firmer variety Capri proved to be responsive only to long term increased temperature. There were also indications that increased of temperature and not solar irradiation are responsible for loss of firmness of strawberry fruits.

The fact that more information was acquired supporting the hypothesis provided an indication that higher temperature have a negative effect on firmness of strawberries triggered the need for further research on the physiological and biochemical mechanisms of the phenomenon. One of the mechanisms that has been proposed to contribute to fruit softening is the enzymatic breaking down of cell walls (Fry, 2004). PEL is one of the enzymes that is responsible for depolymerisation of polygalacturonates of the cell wall of strawberries (Benitez-Burraco, 2003; Figueroa *et al.*, 2008; Wang *et al.*, 2014). The hypothesis that softer fruits should have increased activity of PEL was tested. Fruits harvested at two different sampling weeks were compared. The, softer fruits, harvested at the second sampling week, at which point preharvest temperature was higher, were found to have reduced activity of PEL . The initial hypothesis was therefore not supported leaving space for speculation and further work about the causes of the observation. Since the action of PEL is not the sole mechanism contributing to loss of fruit firmness, the action of other enzymes (Draye and Van Cutsem, 2008; Nardi *et al.*, 2013) as well non enzymatic processes (e.g. hydrolysis) and loss of cell turgor should be monitored in parallel in order to have a more clear image (Fry, 2004). Furthermore, activity of PEL was measured in vitro at a constant temperature and it is likely that adjusting measurements in order to be closer to environmental condition and the fruit environment (pH value) could provide more information about the causes of softening and the role of PEL. However a problem that this approach could have is the reduction of sensitivity of the modified method. In addition, the research in this chapter monitored the activity of PEL at one time point only, that of fully mature fruit. An experiment that could monitor the activity of PEL over the ripening period, using more sampling points, could provide additional information about the activity of PEL over a broader

time spectrum (Marin-Rodriguez, 2002). This approach could be useful if it was assumed that activity of PEL was higher in softer fruits prior harvesting and afterwards was decreased.

The amount of dry bruise that was recorded showed an increasing trend towards the end of the season where increased temperature and production of softer fruits took place. This was the case mainly for fruits produced in Spain and it was consistently observed over the three years. The amount of bruised fruits obtained from the UK production site, despite the fact that fruits were softer, was much lower compared to the Spanish fruit. This could be an effect of increased transport stress that Spanish fruits were subjected to. The fact that softer fruits are more susceptible to bruise was referred to the literature and was further tested as described within the artificial bruise chapter six where it was shown that firmer fruits are more likely to withstand transport stress (Ruiz-Altisent and Guillermo P. Moreda, 2011; Opara and Pathare, 2014).

The interaction between higher temperatures, softness and transport stress was assessed in the sixth chapter where fruits sampled through harvesting season were subjected to additional pressure. The hypothesis was that softer fruits would show enhanced levels of dry bruise compared to firmer fruit, harvested at the part of season where preharvest temperature was lower. It was shown that there was positive relationship between increased preharvest temperature, softness of fruits and levels of dry bruise on strawberry fruits. The negative effect of increased temperature on strawberry quality, especially at postharvest level is well known. It has been stated that any delay in cooling of strawberry can have a significant negative effect on their quality. Farmers, packers and retailers understand the importance of temperature and amount of applied force applied on fruits. Common harvesting strategies include minimal impact of pickers on fruit, rapid field heat removal and subsequently application of the cold chain until the fruit reach the final consumer. In order to minimize transport stress fruits are packed in punnets where impact absorbent pads or bubble wraps are placed. Dry bruise is a very common defect of fruits and it is not considered as severe as wet bruise in terms of consumer acceptance. Shipments of strawberries can be rejected even when few fruits with wet bruise and/or moulds are detected. On the contrary, dry bruise can be quite severe before fruits are rejected. Because it is such a common deformity and it is not very easy to prevent it could be a case that retailers have actually accepted the idea that is not a major concern. However, the impact that dry bruise has on consumer acceptance should be evaluated in a more detailed way. It is not clear how consumers would react given the choice between strawberries with excessive amount of dry bruise and strawberries that have low presence of the deformity. It is also not known how important the competitive advantage of growers producing fruits with reduced amount of dry bruise would be.

Proposed strategies for reducing dry bruise would be to invest in the development of innovative packaging that would reduce transport stress, or investigate the possibility of extending the application of the cold chain. At the moment the cold chain is applied from farm to fork and to be more precise from packhouse to consumer (Rodanto per. comm.). Extending the cold chain closer to harvesting and as close to the plant as possible by adopting a plant to fork cold chain approach could have benefits in reducing amount of dry bruise on berries.

Current practices for extending ability of fruits to withstand transport stress and reduce amount of dry bruise include introduction of firmer varieties. Breeders are focused on producing strawberry fruit with increased tolerance to transport stress and therefore the ability to reach distant markets. During the selection process, varieties with excellent taste and aroma quality characteristics are often excluded from commercial launch due to their reduced firmness (S&A perpers. comm.). In the third chapter (3.3.1.1) it was shown that firmness of fruits can vary up to three fold during the harvest season. It is probable that by introducing firmer varieties the dry bruise problem could be addressed during the warmer part of harvesting season when it is more prominent. However, if firmer varieties respond in a similar way as cv. Candonga, it is possible that they would be too firm for consumers to accept. Further work should be carried out in order to investigate these hypotheses. The seventh chapter described the perception of firmness. Panellists were able to distinguish between softer and firmer fruits, however no clear preference was shown. It should be noticed that firmness did not receive extreme values and it is not clear how panellists would behave if fruits were extremely hard.

Panellists identified sugars and sweetness as a main attribute for strawberry quality; a finding that agrees with literature (Schwieterman *et al.*, 2014; Vicente *et al.*, 2014). In the third chapter (3.3.2.1) sugars generally showed a double peak at each season, over the three years of observations. The models failed to describe accurately the way that environmental factors contributed to accumulation of sugars in fruits. Work carried out on cv. Candonga fruits showed a similar pattern (Correia *et al.*, 2011). However, these results did not agree with others (MacKenzie and Chandler, 2011). It is proposed that a model that includes measurements of crop load (number of fruits /number of total leaves) could improve the prediction power of the models.

Total anthocyanins had a variable profile through harvesting season. Despite the fact that favourable conditions for increased accumulation of anthocyanins were noticed towards the end of the season when there was increased temperature and solar irradiation, anthocyanins levels did not generally increase during that part of the season (Tsormpatsidis *et al.*, 2011). Incorporation of the period between flowering and harvesting into the existing models could potentially

improve their accuracy. It is known that fruits which spend more time on the plant have increased levels of anthocyanins (Andrianjka-Camps *et al.*, 2012). Acids also had a mixed profile through seasons and harvesting period and the models were not able to explain sufficiently their amount. Incorporation of additional physiological factors to the models may improve their predicting ability.

There is an extensive literature describing effects of preharvest environmental factors on quality of strawberry fruits, however, in many cases only the effect of individual factors is tested and less frequently an approach where all environmental factors change simultaneously is studied. One of the main challenges that was faced during the initial experimental chapter was that of the plethora of factors contributing to quality of fruits. The fact that in most cases the factors studied were also changing over the harvest period following similar patterns added complexity to the research and made it more difficult to extract outcomes confidently. Furthermore, the fact that cultural practices, commercial strategies and physiological measurements were not taken during the full growth period resulted in a lack of some crucial information that could lead to improved models and better understanding of the mechanisms affecting postharvest quality. However, due to the scale of the research and the limited human resources the idea of having additional physiological field measurements was not feasible. This area could be part of proposed future work.

Postharvest quality of strawberries is a complex bunch of characteristics, defined by numerous factors. Decisions and management at the preharvest and postharvest stages will influence the quality of the product reaching the final consumer and the level of satisfaction (Kidd, 2010). Selection of site, cultivar, fertilization and irrigation scheme, harvesting method, storage, transportation, postharvest applications and retail display are some of the stages that are crucial for determination of postharvest quality of strawberries (Terry *et al.*, 2007; Crespo *et al.*, 2010; Mena *et al.*, 2014). This study focused on environmental variability and its effect on strawberry quality. The aims of the research were to further understand the complicated interactions between environment and individual quality characteristics, as well as to provide - if possible – a useful decision making tool for growers and retailers based on information provided by local weather stations and/or data loggers placed in the farms. The proposed tool could enable the strawberry sector to identify the effect of key environmental factors and enhance quality of strawberries, increase profitability through identification of appropriate markets, and minimize impact of strawberry cultivation on the environment. Minimizing environmental impact of

horticulture could be achieved both by managing resources in a more efficient way and in parallel decrease postharvest losses.

Strawberry production is an intensive cultivation and requires a vast amount of natural resources. In recent years strawberry cultivation has been criticized for the extensive use of water, agrochemicals and plastic for construction of polytunnels. Strawberry production is quite expensive, the capital investment for a 40 ha farm is estimated at £175K for a ten year growing period (Berrygardens, 2011) and the hourly cost of a packhouse is about £5K/hour. It is quite clear that profitability of growers can be increased not only by increasing production, but equally by improving quality and minimizing postharvest problems that can lead to rejection of shipments and high volumes of waste. It is estimated that at the supply chain level, strawberry waste can be up to 8.5%. This amount does not take in to account the waste produced at the consumer level (Mena *et al.*, 2014). The research conducted could potentially contribute to waste reduction, adaptation to environmental variability of strawberry sector and help towards a more sustainable management of the strawberry supply chain.

8.2. FUTURE WORK

- Conduct research on effect of crop load on fruit quality parameters (size, sugars, acids, colour, anthocyanins, firmness)
- Run trials with independent effects of environmental conditions (light, temperature, ozone, VPD)
- Examine packaging/handling strategies that could reduce transport stress and bruising
- Exploring the effect of other candidate enzymes on strawberry fruit softening, using molecular biology methods
- Using advanced statistical methods to model strawberry quality in relationship to environmental parameters

The data acquired through the three year research mainly from the Spanish growing sites were subjected to statistical analysis by the use of general linear models. Complex biological systems, such as agricultural production systems, involve numerous processes taking place simultaneously at different scales of biological organisation. The final product is the result of interactions between the biotic and abiotic environment and the genetic background of the organism (Hammer *et al.*, 2002; Gallego *et al.*, 2011). The implication of environmental conditions, energy input, genetic material and cultural practises form a complex background of relationships that

affects the quality and quantity of the produced crop (Khoshnevisan *et al.*, 2014). One of the techniques used for the development of increased accuracy models is artificial neural networks (ANN). Neural networks (NN) is a form of artificial intelligence. NN models can identify and explore relationships between many factors and parameters affecting crops. One of the advantages of NN compared to regression models is that they can identify non linear relationships. It is anticipated that use of NN with the addition of inputs related to physiological data such as crop load and time interval between flowering and harvesting could potentially improve the prediction power of the models produced.

Exploring further the biochemical and physiological mechanisms of strawberry softening in relation to environmental conditions as well as definitions of threshold for firmness for consumer acceptability could help growers and breeders produce varieties of strawberry fruits that could adapt to a changing environment. Furthermore, the development of varieties that could withstand environmental stresses and equally provide consumers with exceptional quality of fruits could be beneficial for growers, retailers and consumers. Increasing the profit of growers and retailers by minimizing waste and improving quality could also be beneficial for consumers and the environment since better quality of fruits could contribute towards a healthier diet and in parallel reduce environmental impact of strawberry production by decreasing postharvest waste.

Additional future work could focus on finding alternative packaging, harvesting and postharvest management methods of strawberry fruits. In this research it was noticed that dry bruise development is related to fruit firmness, preharvest temperatures and transport stress. Methods that reduce the amount of applied force on strawberry fruits through all stages of the supply chain could improve quality and reduce postharvest waste.

Overall the strawberry is one of the most popular fruits and its consumption is known to be greatly beneficial for human health. Finding the balance between optimum quality and production as well as minimum environmental impact is an extremely difficult process affected by many factors. This research aimed to provide further information and to enhance our understanding of strawberry quality aspects in relation to existing environmental conditions and possibly increase awareness of plant behaviour in a predicted variable environment.

References

Aguayo, E., Jansasithorn, R., Kader, A.A. (2006) Combined effects of 1-methylcyclopropene, calcium chloride dip, and/or atmospheric modification on quality changes in fresh-cut strawberries. *Postharvest Biology and Technology*. **40**(3), 269–278.

Aharoni, A., Keizer, L.C.P., Van Den Broeck, H.C., Blanco-portales, R., Bois, G., Smit, P., Vos, R., De Connell, C.H. (2002) Novel Insight into Vascular, Stress and Auxin-Dependent and -Independent Gene Expression Programs in Strawberry , a Non-Climacteric Fruit. *Plant Physiology*. **129**(3), 1019–1031.

Alkorta, I., Garb, C. (1998) Industrial applications of pectic enzymes : a review. *Process Biochemistry*. **33**(I), 21–28.

Almeida, J.R.M., D'Amico, E., Preuss, A., Carbone, F., de Vos, C.H.R., Deiml, B., Mourgues, F., Perrotta, G., Fischer, T.C., Bovy, A.G., Martens, S., Rosati, C. (2007) Characterization of major enzymes and genes involved in flavonoid and proanthocyanidin biosynthesis during fruit development in strawberry (Fragaria xananassa). *Archives of Biochemistry and Biophysics*. **465**(1), 61–71.

Andrianjka-Camps, Z.-N., Crespo, P., Ancay, A., Carlen, C. (2012) Strawberry 'Clery' fruit quality evolution during harvest. In *SHE 2012*. Leuven: ISHS.

Arcas, M.C., Botía, J.M., Ortuño, A.M., Del Río, J.A. (2000) UV irradiation Alters the Levels of Flavonoids Involved in the Defence Mechanism of Citrus aurantium Fruits against Penicillium digitatum. *European Journal of Plant Pathology*. **106**(7), 617–622.

Arias, L.J.M. (2006) United States Plant Patent Candonga-Sabrosa.

Atkinson, C.J., Dodds, P.A.A., Ford, Y.Y., Le Mière, J., Taylor, J.M., Blake, P.S., Paul, N. (2006) Effects of cultivar, fruit number and reflected photosynthetically active radiation on Fragaria x ananassa productivity and fruit ellagic acid and ascorbic acid concentrations. *Annals of Botany*. **97**(3), 429–41.

Atta-Aly, M.A., Brecht, J.K., Huber, D.J. (2000) Ethylene feedback mechanisms in tomato and strawberry fruit tissues in relation to fruit ripening and climacteric patterns. *Postharvest Biology and Technology*. **20**(2), 151–162.

Barry, C.S., Giovannoni, J.J. (2007) Ethylene and Fruit Ripening. *Journal of Plant Growth Regulation*. **26**(2), 143–159.

Bartling, S., Wegener, C., Olsen, O. (1995) Synergism between Erwinia pectate lyase isoenzymes that depolymerize both pectate and pectin. *Microbiology (Reading, England)*. **141**(4), 873–881.

Basson, C.E., Groenewald, J.-H., Kossmann, J., Cronjé, C., Bauer, R. (2010) Sugar and acid-related quality attributes and enzyme activities in strawberry fruits: Invertase is the main sucrose hydrolysing enzyme. *Food Chemistry*. **121**(4), 1156–1162.

Basu, S., Ghosh, A., Bera, A., Saha, M.N., Chattopadhyay, D., Chakrabarti, K. (2008) Thermodynamic characterization of a highly thermoactive extracellular pectate lyase from a new isolate Bacillus pumilus DKS1. *Bioresource Technology*. **99**(17), 8088–8094.

Benitez-Burraco, A. (2003) Cloning and characterization of two ripening-related strawberry (Fragariaxananassa cv. Chandler) pectate lyase genes. *Journal of Experimental Botany*. **54**(383), 633–645.

Benitez-Burraco, A., Blanco-Portales, R., Redondo-Nevado, J., Bellido, M.L., Moyano, E., Caballero, J.L., Muñoz-Blanco, J. (2003) Cloning and characterization of two ripening-related strawberry (Fragariaxananassa cv. Chandler) pectate lyase genes. *Journal of Experimental Botany*. **54**(383), 633–645.

Besford, R.T., Ludwig, L.J., Withers, A.C. (1990) The greenhouse effect: acclimation of tomato plants growing in high CO2, photosynthesis and ribulose-1, 5-bisphosphate carboxylase protein. *Journal of Experimental Botany*. **41**(8), 925–931.

Bianco, L., Lopez, L., Scalone, A.G., Di Carli, M., Desiderio, A., Benvenuto, E., Perrotta, G. (2009) Strawberry proteome characterization and its regulation during fruit ripening and in different genotypes. *Journal of Proteomics*. **72**(4), 586–607.

Blanke, M.M., Cooke, D.T. (2004) Effects of flooding and drought on stomatal activity, transpiration, photosynthesis, water potential and water channel activity in strawberry stolons and leaves. *Plant Growth Regulation*. **42**(2), 153–160.

Bordonaba, J.G. (2010) *Towards a better understanding and new tools for soft fruit quality control*. Cranfield University.

Bordonaba, J.G., Terry, L.A. (2008) Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. *Journal of Agricultural and Food Chemistry*. **56**(16), 7422–7430.

Bourgault, R., Bewley, J.D., Alberici, A. (2001) Endo-β β -mannanase Activity in Tomato and Other Ripening Fruits. *Horticultural Science*. **36**(1), 72–75.

Bourquin, V., Nishikubo, N., Abe, H., Brumer, H., Denman, S., Eklund, M., Christiernin, M., Teeri, T.T., Sundberg, B., Mellerowicz, E.J. (2002) Xyloglucan endotransglycosylases have a function during the formation of secondary cell walls of vascular tissues. *The Plant Cell*. **14**(12), 3073–3088.

Brady, C. (1987) Fruit ripening. *Annual Review of Plant Physiology*.

Brummell, D. a, Harpster, M.H. (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology*. **47**(1-2), 311–40.

Brummell, D.A. (2006) Review : Cell wall disassembly in ripening fruit. *Functional Plant Biology*. **33**(2), 103–119.

Bustamante, C.A., Rosli, H.G., Añón, M.C., Civello, P.M., Martínez, G.A. (2006) β-Xylosidase in strawberry fruit: Isolation of a full-length gene and analysis of its expression and enzymatic activity in cultivars with contrasting firmness. *Plant Science*. **171**(4), 497–504.

Carlen, C., Potel, A.M., Ançay, A., Krüger, E., Mezzetti, B. (2009) Photosynthetic response of strawberry leaves to changing temperatures. *Acta Horticulturae*. (838), 73–76.

Carpita, N.C., Gibeaut, D.M. (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal : For Cell and Molecular Biology*. **3**(1), 1–30.

Casal, C., Vílchez, C., Forján, E., De La Morena, B.A. (2009) The absence of UV-radiation delays the strawberry ripening but increases the final productivity, not altering the main fruit nutritional properties. *Acta Horticulturae*. **842**, 159–162.

Castellvi, F., Perez, P., Stockle, C., Ibanez, M. (1997) Methods for estimating vapor pressure deficit at a regional scale depending on data availability. *Agricultural and Forest Meteorology*. **1923**(97), 243–252.

Chapman, R.F. (2009) The chemical inhibition of feeding by phytophagous insects: a review. *Bulletin of Entomological Research*. **64**(03), 339.

Charkowski, A., Blanco, C., Condemine, G., Van der Wolf, J.M. (2012) The Role of Secretion Systems and Small Molecules in Soft-Rot Enterobacteriaceae Pathogenicity.*Annual Review of Phutopathology*. **50**, 425–429.

Chatterjee, A.K., Buchanan, G.E., Behrens, M.K., Starr, M.P. (1979) Synthesis and excretion of polygalacturonic acid trans-eliminase in Erwinia, Yersinia, and Klebsiella species. *Canadian Journal of Microbiology*. **25**(1), 94–102.

Chesson, A., Codner, T.L.R.C. (1978) The Maceration of Vegetable Tissue by a Strain of Bacillus subtilis. *Journal of Applied Microbiology*. **44**(3), 347–364.

Choi, J.H., Chung, G.C., Suh, S.R. (1997) Effect of night humidity on the vegetative growth and the mineral composition of tomato and strawberry plants. *Scientia Horticulturae*. **70**(4), 293–299.

Chourasia, A., Sane, V.A., Nath, P. (2006) Differential expression of pectate lyase during ethylene-induced postharvest softening of mango (Mangifera indica var. Dashehari). *Physiologia Plantarum*. **128**(3), 546–555.

Collmer, A., Ried, J.L., Mount, M.S. (1988) Assay methods for pectic enzymes. In S. T. K. B. T.-M. in E. Willis A. Wood, ed. *Biomass Part B: Lignin, Pectin, and Chitin*. Academic Press, pp. 329–335.

Correia, P.J.J., Pestana, M., Martinez, F., Ribeiro, E., Gama, F., Saavedra, T., Palencia, P. (2011) Relationships between strawberry fruit quality attributes and crop load. *Scientia Horticulturae*. **130**(2), 398–403.

Crespo, P., Bordonaba, J.G., Terry, L.A., Carlen, C. (2010) Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. *Food Chemistry*. **122**(1), 16–24.

Darrow, G.M. (1966) The strawberry. History, breeding and physiology. *The strawberry. History, breeding and physiology.*

Davik, J., Bakken, A. (2006) Effects of genotype and environment on total anti-oxidant capacity and the content of sugars and acids in strawberries (Fragaria X ananassa Duch.). *Journal of Horticultural Science & Biotechnology*. **81**, 1057–1063.

Del Río, J. (2003) Enhancement of phenolic compounds in olive plants (Olea europaea L.) and their influence on resistance against Phytophthora sp. *Food Chemistry*. **83**(1), 75–78.

Diamanti, J., Capocasa, F., Mezzetti, B., Tulipani, S., Battino, M. (2008) The interaction of plant genotype and temperature conditions at ripening stage affects strawberry nutritional quality. In *Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838*. pp. 183–186.

Dominguez-Puigjaner, E., Llop, I., Vendrell, M., Prat, S. (1997) A cDNA Clone Highly Expressed in Ripe Banana Fruit Shows Homology to Pectate Lyases. *Plant Physiology*. **114**(3), 1071–1076.

Dotto, M.C., Martínez, G.A., Civello, P.M. (2006) Expression of expansin genes in strawberry varieties with contrasting fruit firmness. *Plant Physiology and Biochemistry : PPB / Société française de physiologie végétale*. **44**(5-6), 301–7.

Drake, B.G., Gonzalez-Meler, M.A., Long, S.P. (1997) More efficient plants: A Consequence of Rising Atmospheric CO2. *Annual Review of Plant Physiology and Plant Molecular Biology*. **48**, 609–639.

Draye, M., Van Cutsem, P. (2008) Pectin methylesterases induce an abrupt increase of acidic pectin during strawberry fruit ripening. *Journal of Plant Physiology*. **165**(11), 1152–60.

Drogoudi, P., Ashmore, M. (2000) Does elevated ozone have differing effects in flowering and deblossomed strawberry? *New Phytologist*, 561–569.

Drogoudi, P.D., Ashmore, M.R. (2001) 14C-allocation of flowering and deblossomed strawberry in response to elevated ozone. *New Phytologist*. **152**(3), 455–461.

Drori, N., Rollins, J., Dinoor, A., Pines, O., Prusky, D., Okon, Y. (2003) External pH and Nitrogen Source Affect Secretion of Pectate Lyase by Colletotrichum gloeosporioides External pH and Nitrogen Source Affect Secretion of Pectate Lyase by Colletotrichum gloeosporioides. *Applied and Environmental Microbiology*. **69**(6), 3258–3262.

Enoch, H.Z., Rylski, I., Spigelman, M. (1976) CO_2 enrichment of strawberry and cucumber plants grown in unheated greenhouses in Israel. *Scientia Horticulturae*. **5**(1), 33–41.

Fait, A., Hanhineva, K., Beleggia, R., Dai, N., Rogachev, I., Nikiforova, V.J., Fernie, A.R., Aharoni, A. (2008) Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. *Plant Physiology*. **148**(2), 730–50.

Felgines, C., Gonthier, M., Texier, O., Scalbert, A., Lamaison, J., Re, C. (2003) Human Nutrition and Metabolism Strawberry Anthocyanins Are Recovered in Urine as Glucuro- and Sulfoconjugates in Humans. *The Journal of Nutrition*. **133**(5), 1296–1301.

Ferreira, M., Sargent, S. (2009) Strawberry Bruising Sensitivity Depends on the Type of Force Applied, Cooling Method, and Pulp Temperature. *Horticultural Science*. **44**(7), 1953–1956.

Ferreira, M., Sargent, S., Brecht, J., Chandler, C. (2008) Strawberry fruit resistance to simulated handling. *Scientia Agricola*. **65**(5), 490–495.

Ferreyra, R.M., Viña, S.Z., Mugridge, A., Chaves, A.R. (2007) Growth and ripening season effects on antioxidant capacity of strawberry cultivar Selva. *Scientia Horticulturae*. **112**(1), 27–32.

Figueroa, C.R., Pimentel, P., Gaete-Eastman, C., Moya, M., Herrera, R., Caligari, P.D.S., Moya-León, M.A. (2008) Softening rate of the Chilean strawberry (Fragaria chiloensis) fruit reflects the expression of polygalacturonase and pectate lyase genes. *Postharvest Biology and Technology*. **49**(2), 210–220.

Fischer, D., Craig, W.L., Watada, A.E., Douglas, W., Ashby, B.H. (1992) Simulated in-transit vibration damage to packaged fresh market grapes and strawberries. *Applied Engineering in Agriculture*. **8**(3), 363–366.

Fowler, H., Ekström, M. (2009) Multimodel ensemble estimates of climate change impacts on UK seasonal precipitation extremes. *International Journal of Climatology*. **29**(3), 385–416.

Fry, S. (2004) Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. *New Phytologist*. **161**(3), 641–675.

Fuhrer, J. (2009) Ozone risk for crops and pastures in present and future climates. *Die Naturwissenschaften*. **96**(2), 173–94.

Gallego, P., Gago, J., Landín, M. (2011) Artificial neural networks technology to model and predict plant biology process. *Artificial Neural Networks-Methodological Advances and Biomedical Applications. Rijeka, Croatia: Intech Open Access Publisher*, 197–217.

Galletta, G.J., Himelrick, D.G. (1990) *Small fruit crop management.* Prentice-Hall, Inc.

García-Gago, J. a J., Posé, S., Muñoz-Blanco, J., Quesada, M. a, Mercado, J. a (2009) The polygalacturonase FaPG1 gene plays a key role in strawberry fruit softening. *Plant Signaling & Behavior*. **4**(8), 766–8.

Giampieri, F., Alvarez-Suarez, J.M., Mazzoni, L., Romandini, S., Bompadre, S., Diamanti, J., Capocasa, F., Mezzetti, B., Quiles, J.L., Ferreiro, M.S., Tulipani, S., Battino, M. (2013) The potential impact of strawberry on human health. *Natural Product Research*. **27**(4-5), 448–55.

Giné Bordonaba, J., Terry, L.A. (2010) Manipulating the taste-related composition of strawberry fruits (FragariaXananassa) from different cultivars using deficit irrigation. *Food Chemistry*. **122**(4), 1020–1026.

Giovannoni, J. (2001) Molecular biology of fruit maturation and ripening. *Annual Review of Plant Biology*. **25**(1), 725–749.

Giusti, M.M., Wrolstad, R.E. (2001) Characterization and measurement of anthocyanins by UVvisible spectroscopy. *Current Protocols in Food Analytical Chemistry*. **24**(3), 227–247.

Given, N.K., Venis, M.A., Gierson, D. (1988) Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta*. **174**(3), 402–406.

Gómez, L., Casano, L., Trippi, V. (1995) Effect of hydrogen peroxide on degradation of cell wall associated proteins in growing bean hypocotyls. *Plant and Cell Physiology*. **36**(7), 1259–1264.

Goulao, L., Oliveira, C. (2008) Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends in Food Science & Technology*. **19**(1), 4–25.

Gruda, N. (2005) Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. *Critical Reviews in Plant Sciences*. **24**(3), 227-247.

Hammer, G., Kropff, M., Sinclair, T., Porter, J. (2002) Future contributions of crop modelling—from heuristics and supporting decision making to understanding genetic regulation and aiding crop improvement. *European Journal of Agronomy*. **18**(1), 15–31.

Hancock, J., Finn, C., Luby, J. (2010) Reconstruction of the Strawberry, Fragaria× ananassa, Using Genotypes of F. virginiana and F. chiloensis. *Horticultural Science*. **45**(7), 1006–1013.

Hancock, J., Lavín, A., Retamales, J. (1999) Our southern strawberry heritage: Fragaria chiloensis of Chile. *Horticultural Science*. **34**(5), 1998–2000.

Harmens, H., Mills, G., Emberson, L.D., Ashmore, M.R. (2007) Implications of climate change for the stomatal flux of ozone: a case study for winter wheat. *Environmental Pollution*. **146**(3), 763–70.

Harpster, M.H., Brummell, D.A., Dunsmuir, P. (1998) Expression analysis of a ripening-specific, auxin-repressed endo-1, 4-beta-glucanase gene in strawberry. *Plant Physiology*. **118**(4), 1307–16.

Harrison, E.P., McQueen-Mason, S.J., Manning, K. (2001) Expression of six expansin genes in relation to extension activity in developing strawberry fruit. *Journal of Experimental Botany*. **52**(360), 1437–46.

Hassan, S., Shevchik, V.E., Robert, X., Hugouvieux-Cotte-Pattat, N. (2013) PelN is a new pectate lyase of Dickeya dadantii with unusual characteristics. *Journal of Bacteriology*. **195**(10), 2197–2206.

Heng Koh, T., Melton, L.D. (2002) Ripening-related changes in cell wall polysaccharides of strawberry cortical and pith tissues. *Postharvest Biology and Technology*. **26**(1), 23–33.

Herms, D.A., Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, 283–335.

Hertog, M., Boerrigter, H.A.M., Van den Boogaard, G., Tijskens, L.M.M., Van Schaik, A.C.R. (1999) Predicting keeping quality of strawberries (cv.Elsanta') packed under modified atmospheres: an integrated model approach. *Postharvest Biology and Technology*. **15**(1), 1–12.

Holcroft, D., Kader, A. (1999) Carbon Dioxide–induced Changes in Color and Anthocyanin Synthesis of Stored Strawberry Fruit. *Horticultural Science*. **34**(7), 1244–1248.

Holt, J.E., Schoorl, D. (1982) Strawberry bruising and energy dissipation. *Journal of Texture Studies*. **13**(3), 349–357.

Hoppula, K.I., Salo, T.J. (2006) Tensiometer-based irrigation scheduling in perennial strawberry cultivation. *Irrigation Science*. **25**(4), 401–409.

Houghton, J., Firor, J. (1995) Global warming: The complete briefing. *Nature*. **22**(3), 270–271.

Howell, T.A.T., Dusek, D.D.A. (1995) Comparison of vapor-pressure-deficit calculation methods-Southern High Plains. *Journal of Irrigation and Drainage Engineering*. **121**(2), 191–198.

Hugouvieux-Cotte-Pattat, N., Dominguez, H., Robert-Baudouy, J. (1992) Environmental conditions affect transcription of the pectinase genes of Erwinia chrysanthemi 3937. *Journal of Bacteriology*. **174**(23), 7807–7818.

Iannetta, P.P.M., Laarhoven, L.-J., Medina-Escobar, N., James, E.K., McManus, M.T., Davies, H. V., Harren, F.J.M. (2006) Ethylene and carbon dioxide production by developing strawberries show a correlative pattern that is indicative of ripening climacteric fruit. *Physiologia Plantarum*. **127**(2), 247–259.

Iglesias, A., Quiroga, S., Moneo, M., Garrote, L. (2011) From climate change impacts to the development of adaptation strategies: Challenges for agriculture in Europe. *Climatic Change*. **112**(1), 143–168.

Iglesias, A., Quiroga, S., Schlickenrieder, J. (2010) Climate change and agricultural adaptation: assessing management uncertainty for four crop types in Spain. *Climate Research*. **44**(1), 83–94.

INRA (2013) Elsinore. [online]. Available from: http://www.bordeaux.inra.fr/eustrawberrydb/individual/124 [Accessed October 21, 2013].

Jayani, R.S., Saxena, S., Gupta, R. (2005) Microbial pectinolytic enzymes: A review. *Process Biochemistry*. **40**(9), 2931–2944.

Jiménez-Bermúdez, S., Redondo-Nevado, J., Muñoz-Blanco, J., Caballero, J.L., López-Aranda, J.M., Valpuesta, V., Pliego-Alfaro, F., Quesada, M.A., Mercado, J.A. (2002) Manipulation of Strawberry Fruit Softening by Antisense Expression of a Pectate Lyase Gene. *Plant Physiology* . **128** (2), 751–759.

Jones, C.G., Hartley, S.E. (1999) A Protein Competition Model of Phenolic Allocation A protein competition model of phenolic allocation. *Oikos*. **86**(1), 27–44.

Joseph, J., Shukitt-Hale, B. (2008) 'Brainberries' and Aging. *Clinical Nutrition Insight*. **55**, 46.

Josuttis, M., Dietrich, H., Treutter, D., Will, F., Linnemannstöns, L., Krüger, E. (2010) Solar UVB Response of Bioactives in Strawberry (Fragaria × ananassa Duch. L.): A Comparison of Protected and Open-Field Cultivation. *Journal of Agricultural and Food Chemistry*. **58**(24), 12692–12702.

Jouquand, C., Plotto, A., Goodner, K. (2008) A sensory and chemical analysis of fresh strawberries over harvest dates and seasons reveals factors that affect eating quality. *Journal of the American Society for Horticultural Science*. **133**(6), 859–867.

Kader, A. (1991) Quality and its maintenance in relation to the postharvest physiology of strawberry. In *The strawberry into the 21st century.* Portland, OR: Timber Press, pp. 145–152.

Kafkas, E., Koşar, M., Paydaş, S., Kafkas, S., Başer, K.H.C. (2007) Quality characteristics of strawberry genotypes at different maturation stages. *Food Chemistry*. **100**(3), 1229–1236.

Kawanobu, S., Zushi, K., Wajima, T., Chikushi, J., Mori, T., Kondo, K., Matsuzoe, N. (2011) The effect of dark treatment on anthocyanin composition and content of strawberry fruit. *Journal of Food, Agriculture & Environment*. **9**(1), 325–328.

Kelemu, S., Collmer, A. (1993) Erwinia chrysanthemi EC16 Produces a Second Set of Plant-Inducible Pectate Lyase Isozymes. *Applied Environmental Microbiology*. **59**(6), 1756–1761.

Keutgen, A., Noga, G., Pawelzik, E. (2005) Cultivar-specific impairment of strawberry growth, photosynthesis, carbohydrate and nitrogen accumulation by ozone. *Environmental and Experimental Botany*. **53**(3), 271–280.

Keutgen, A.J., Pawelzik, E. (2008) Influence of pre-harvest ozone exposure on quality of strawberry fruit under simulated retail conditions. *Postharvest Biology and Technology*. **49**(1), 10–18.

Khoshnevisan, B., Rafiee, S., Mousazadeh, H. (2014) Application of multi-layer adaptive neuro-fuzzy inference system for estimation of greenhouse strawberry yield. *Measurement*. **47**, 903–910.

Kidd, P. (2010) *The Use of Sensing and ICT to Improve the Sustainability of International Food Production and Distribution Systems*.

Kim, J.F., Beer, S. V. (1998) HrpW of Erwinia amylovora, a New Harpin That Contains a Domain Homologous to Pectate Lyases of a Distinct Class. *Journal of Bacteriology*. **180**(19), 5203–5210.

Kobayasi, T., Kenzo, K., Yoshimatsu, T., Higaki, N., Atsushi, S., Tadahiro, O., Yuji, H., Susumu, I. (1999) Purification and properties of a low-molecular-weight, high alkaline pectate lyase from alkaliphilic strain of Bacillus. *Bioscience, Biotechnology & Biochemistry*. **63**(1), 65–72.

Koricheva, J., Nykänen, H., Gianoli, E. (2004) Meta-analysis of trade-offs among plant antiherbivore defenses: are plants jacks-of-all-trades, masters of all? *The American Naturalist*. **163**(4), E64–75.

Krüger, E., Josuttis, M., Dietrich, H. (2008) Effect of Pre-Harvest Temperature, Photoactive Radiation and Fruit Thinning on Strawberry Bioactive Compounds. In *Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838*. pp. 199–204.

Krüger, E., Schmidt, G., Rasim, S. (2000) Effect of irrigation on yield, fruit size and firmness of strawberry cv. Elsanta. In *IV International Strawberry Symposium 567*. pp. 471–474.

Kumar, S., Dey, P. (2011) Effects of different mulches and irrigation methods on root growth, nutrient uptake, water-use efficiency and yield of strawberry. *Scientia Horticulturae*. **127**(3), 318–324.

De la Fuente, J., Amaya, I., Castillejo, C., Sánchez-Sevilla, J.F., Quesada, M. a, Botella, M. a, Valpuesta, V. (2006) The strawberry gene FaGAST affects plant growth through inhibition of cell elongation. *Journal of Experimental Botany*. **57**(10), 2401–11.

Langner, J., Bergström, R., Foltescu, V. (2005) Impact of climate change on surface ozone and deposition of sulphur and nitrogen in Europe. *Atmospheric Environment*. **39**(6), 1129–1141.

Lattanzio, V., Cardinali, A., Linsalata, V. (2012) Plant phenolics: a biochemical and physiological perspective. *Recent Advances in Polyphenol Research*. **3**, 1–39.

Lattanzio, V., Cardinalli, A. (2006) Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry: Advances in research:*. **661**(2), 23–67.

Lee, S.K., Kader, A. a. (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*. **20**(3), 207–220.

Lefever, G., Vieuille, M., Delage, N., de Monteclerc, J., Bompeix, G. (2004) Characterization of Cell Wall Enzyme Activities, Pectin Composition, and Technological Criteria of Strawberry Cultivars (Fragaria×ananassa Duch). *Journal of Food Science*. **69**(4), 221–226.

Lei, Y., Liu, Y.-Z., Zeng, W.-F., Deng, X.-X. (2010) Physicochemical and molecular analysis of cell wall metabolism between two navel oranges (Citrus sinensis) with different mastication traits. *Journal of the Science of Food and Agriculture*. **90**(9), 1479–1484.

Leis, M. (2013) Capri patent.

Liao, C., Revear, L. (1999) Genetic and biochemical characterization of an exopolygalacturonase and a pectate lyase from Yersinia enterocolitica. *Canadian Journal of Microbiology*. **45**(5), 396–403.

Liao, C.H. (1991) Cloning of pectate lyase gene pel from Pseudomonas fluorescens and detection of sequences homologous to pel in Pseudomonas viridiflava and Pseudomonas putida. *Journal of Bacteriology*. **173**(14), 4386–93.

Lieten, P. (2000) The effect of humidity on the performance of greenhouse grown strawberry. In *IV International Strawberry Symposium 567*. pp. 479–482.

Lillo, C., Lea, U.S., Ruoff, P. (2008) Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. *Plant, Cell & Environment*. **31**(5), 587–601.

Link, H. (2000) Significance of flower and fruit thinning on fruit quality. *Plant Growth Regulation*. **31**(1-2), 17–26.

Lohani, S., Trivedi, P.K., Nath, P. (2004) Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: effect of 1-MCP, ABA and IAA. *Postharvest Biology and Technology*. **31**(2), 119–126.

De Lorenzo, G., Cervone, F., Hahn, M.G., Darvill, A., Albersheim, P. Bacterial endopectate lyase : evidence that plant cell wall pH prevents tissue maceration and increases the half-life of elicitor-active oligogalacturonides. *Physiological and Molecular Plant Pathology*. **39**(5), 335–344.

MacKenzie, S., Chandler, C. (2011) The role of temperature in the late-season decline in soluble solids content of strawberry fruit in a subtropical production system. *Horticultural Science*. **46**(July), 1562–1566.

Mahajan, S., Tuteja, N. (2005) Cold, salinity and drought stresses: an overview. *Archives of Biochemistry and Biophysics*. **444**(2), 139–58.

Makinen, K., Soderling, E. (1980) A quantitative study of mannitol, sorbitol, xylitol, and xylose in wild berries and commercial fruits. *Journal of Food Science*. **45**(2), 367–371.

Manninen, S., Siivonen, N. (2003) Differences in ozone response between two Finnish wild strawberry populations. *Environmental and Experimental Botany*. **49**, 29–39.

Manning, K. (1994) Changes in gene expression during strawberry fruit ripening and their regulation by auxin. *Planta*. **194**(1), 62–68.

Manning, K. (1998) Isolation of a set of ripening-related genes from strawberry : their identification and possible relationship to fruit quality traits. *Planta*. **255**(4), 622–631.

Manning, K. (1993) Soft fruit. In *Biochemistry of fruit ripening*. Springer, pp. 347–377.

Marin-Rodriguez, M.C. (2002) *Investigation of the role of pectate lyase in banana fruit softening*. Chatham, UK.: University of Greenwich.

Marin-Rodriguez, M.C., Orchard, J., Seymour, G.B. (2002) Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany*. **53**(377), 2115–2119.

Marín-Rodríguez, M.C., Smith, D.L., Manning, K., Orchard, J., Seymour, G.B. (2003) Pectate lyase gene expression and enzyme activity in ripening banana fruit. *Plant Molecular Biology*. **51**(6), 851–7.

Martínez, G.A., Chaves, A.R., Civello, P.M. (2004) Beta-Xylosidase Activity and Expression of a Beta-Xylosidase Gene During Strawberry Fruit Ripening. *Plant Physiology and Biochemistry : PPB / Société française de physiologie végétale*. **42**(2), 89–96.

Martinez-Romero, D., Serrano, M., Carbonell, A., Castillo, S., Riquelme, F., Valero, D. (2004) Mechanical Damage During Fruit Post-Harvest Handling: Technical and Physiological Implications. In R. Dris & S. M. Jain, eds. *Production Practices and Quality Assessment of Food Crops*. Springer Netherlands, pp. 233–252.

Matsuzoe, N., Kawanobu, S., Matsumoto, S., Kimura, H., Zushi, K. (2006) Effect of night temperature on sugar, amino acid, ascorbic acid, anthocyanin and ellagic acid in strawberry (Fragaria× ananassa Duch.) Fruit. *Journal of Society of High Technology in Africulture*. **18**(2), 115–122.

Mazzoni group (2013) Capri characteristics. [online]. Available from: http://www.strawberries.eu.com/capri_21.html.

Mazzoni group (2011) Elsinore characteristics. [online]. Available from: http://www.strawberries.eu.com/elsinore_10.html.

McKey, D. (1974) Adaptive patterns in alkaloid physiology. *American Naturalist*, 305–320.

Medina-Escobar, N., Cárdenas, J., Moyano, E., Caballero, J.L., Muñoz-Blanco, J. (1997) Cloning, molecular characterization and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants. *Plant Molecular Biology*. **34**(6), 867–77.

Medina-Suarez, R., Manning, K., Fletcher, J., Aked, J., Bird, C.R., Seymour, G.B., Medina-Suárez, R. (1997) Gene Expression in the Pulp of Ripening Bananas (Two-Dimensional Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of in Vitro Translation Products and cDNA Cloning of 25 Different Ripening-Related mRNAs). *Plant Physiology*. **115**(2), 453–461.

Meleux, F., Solmon, F., Giorgi, F. (2007) Increase in summer European ozone amounts due to climate change. *Atmospheric Environment*. **41**(35), 7577–7587.

Melkonyan, A., Wagner, P. (2013) Ozone and its projection in regard to climate change. *Atmospheric Environment*. **67**, 287–295.

Mena, C., Terry, L.. LA, Williams, A., Ellram, L. (2014) Causes of waste across multi-tier supply networks: Cases in the UK food sector. *International Journal of Production Economics*. **152**, 144–158.

Mercado, J., Trainotti, L., Jiménez-Bermúdez, L., Santiago-Doménech, N., Posé, S., Donolli, R., Barceló, M., Casadoro, G., Pliego-Alfaro, F., Quesada, M. a. (2010) Evaluation of the role of the endo-β-(1,4)-glucanase gene FaEG3 in strawberry fruit softening. *Postharvest Biology and Technology*. **55**(1), 8–14.

Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H. (2003) Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*. **51**(23), 6887–92.

Mira de Orduña, R. (2010) Climate change associated effects on grape and wine quality and production. *Food Research International*. **43**(7), 1844–1855.

Mirza, M. (2003) Climate change and extreme weather events: can developing countries adapt? *Climate Policy*. **3**(3), 233–248.

Mitchell, F., Mayer, G., Maxie, E., Coates, W. (1974) Cold storage effects on fresh market peaches, nectarines & plums estimating freezing points using low temperatures to delay internal breakdown. *California Agriculture*. **28**(10), 12–14.

Montero, T., Mollá, E. (1996) Quality attributes of strawberry during ripening. *Scientia Horticulturae*. **65**(4), 239–250.

Moore, J.N., Brown, G.R., Brown, E.D. (1970) Comparison of factors influencing fruit size in large-fruited and small-fruited clones of strawberry. *Journal of the American Society of Horticultural Science*. **95**, 827–831.

Moretti, C.L., Mattos, L.M., Calbo, A.G., Sargent, S.A. (2010) Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food Research International*. **43**(7), 1824–1832.

Mori, T., Sakurai, M., Sakuta, M. (2001) Effects of conditioned medium on activities of PAL, CHS, DAHP synthase (DS-Co and DS-Mn) and anthocyanin production in suspension cultures of Fragaria ananassa. *Plant Science : An International Journal of Experimental Plant Biology*. **160**(2), 355–360.

Morinaga, K., Imai, S., Yakushiji, H., Koshita, Y. (2003) Effects of fruit load on partitioning of 15N and 13C, respiration, and growth of grapevine roots at different fruit stages. *Horticultural Science*. **97**, 239–253.

Mukkun, L., Singh, Z. (2009) Methyl jasmonate plays a role in fruit ripening of 'Pajaro'strawberry through stimulation of ethylene biosynthesis. *Horticultural Science*. **123**(1), 5–10.

Nagel, C.W., Wilson, T.M. (1970) Pectic Acid Lyases of Bacillus polymyxa. *Applied Environmental Microbiology*. **20**(3), 374–383.

Nakajima, N., Ishihara, K., Tanabe, M., Matsubara, K., Matsuura, Y. (1999) Degradation of pectic substances by two pectate lyases from a human intestinal bacterium, Clostridium butyricum-beijerinckii group. *Journal of Bioscience and Bioengineering*. **88**(3), 331–3.

Nardi, C., Escudero, C., Villarreal, N., Martínez, G., Civello, P.M. (2013) The carbohydrate-binding module of Fragaria × ananassa expansin 2 (CBM-FaExp2) binds to cell wall polysaccharides and decreases cell wall enzyme activities 'in vitro'. *Journal of Plant Research*. **126**(1), 151–9.

Neocleous, D., Vasilakakis, M. (2007) Effects of NaCl stress on red raspberry (Rubus idaeus L. 'Autumn Bliss'). *Scientia Horticulturae*. **112**(3), 282–289.

Niatthijsen, J., Builtjes, P. (1997) Modelling cloud effects on ozone on a regional scale: a case study. *Atmospheric Environment*. **31**(19), 3227–3238.

Nitsch, J. (1955) Free Auxins and Free Tryptophane in the Strawberry. *Plant Physiology*. (15), 33–39.

Njuguna, W., Liston, A., Cronn, R., Ashman, T.-L., Bassil, N. (2013) Insights into phylogeny, sex function and age of Fragaria based on whole chloroplast genome sequencing. *Molecular Phylogenetics and Evolution*. **66**(1), 17–29.

Nunan, K., Davies, C., Robinson, S., Fincher, G. (2001) Expression patterns of cell wall-modifying enzymes during grape berry development. *Planta*. **214**(2), 257–264.

Nunes, M., Emond, J., Brecht, J. (2003) Quality of strawberries as affected by temperature abuse during ground, in-flight and retail handling operations. In *International Conference on Quality in Chains. An Integrated View on Fruit and Vegetable Quality*. pp. 239–246.

Nunes, M.C.N., Brecht, J.K., Morais, A.M.M.B., Sargent, S.A. (1995) Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. *Postharvest Biology and Technology*. **6**(1-2), 17–28.

Nunes, M.C.N., Emond, J.P., Rauth, M., Dea, S., Chau, K. V. (2009) Environmental conditions encountered during typical consumer retail display affect fruit and vegetable quality and waste. *Postharvest Biology and Technology*. **51**(2), 232–241.

Olesen, J.E., Bindi, M. (2002) Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy*. **16**(4), 239–262.

Olsen, K.M., Lea, U.S., Slimestad, R., Verheul, M., Lillo, C. (2008) Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. *Journal of Plant Physiology*. **165**(14), 1491–9.

Opara, U.L., Pathare, P.B. (2014) Bruise damage measurement and analysis of fresh horticultural produce—A review. *Postharvest Biology and Technology*. **91**, 9–24.

Opazo, M., Lizana, R., Pimentel, P. (2013) Changes in the mRNA abundance of FcXTH1 and FcXTH2 promoted by hormonal treatments of Fragaria chiloensis fruit. *Postharvest Biology and Technology*. **77**, 28–34.

Ordidge, M., García-Macías, P., Battey, N.H., Gordon, M.H., John, P., Lovegrove, J. a, Vysini, E., Wagstaffe, A., Hadley, P. (2012) Development of colour and firmness in strawberry crops is UV light sensitive, but colour is not a good predictor of several quality parameters. *Journal of the Science of Food and Agriculture*. **92**(8), 1597–604.

Osman, A., Dodd, P. (1994) Effects of Different Levels of Preharvest Shading on the Storage Quality of Strawberry (Fragaria x ananassa Duchesne) cv. Ostara-II. Chemical. *Pertanika*. **17**(1), 65–71.

Palencia, P., Martínez, F. (2013) Strawberry yield efficiency and its correlation with temperature and solar radiation. *Horticultura Brasileira*. **31**(1), 93–99.

Palencia, P., Martínez, F., Medina, J.J., Vàzquez, E., Flores, F., López-Medina, J. (2008) Effects of climate change on strawberry production. In *Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838*. pp. 51–54.

Parikka, P., Lemmetty, A. (2008) Plant diseases in changing northern climatic conditions. In *Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838*. pp. 123–128.

Parisi, A. V, Schouten, P., Downs, N.J., Turner, J. (2010) Solar UV exposures measured simultaneously to all arbitrarily oriented leaves on a plant. *Journal of Photochemistry and Photobiology. B, Biology*. **99**(2), 87–92.

Parry, M.L. (2007) *Climate Change 2007: impacts, adaptation and vulnerability: contribution of Working Group II to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Paull, R. (1999) Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biology and Technology*. **15**(3), 263–277.

Payasi, A., Mishra, N.N., Chaves, A.L.S., Singh, R. (2009) Biochemistry of fruit softening: an overview. *Physiology and Molecular Biology of Plants*. **15**(2), 103–113.

Payasi, A., Sanwal, G.. (2003) Pectate lyase activity during ripening of banana fruit. *Phytochemistry*. **63**(3), 243–248.

Perkins-Veazie, P., Huber, D.J. (1988) Growth and ripening of strawberry fruit under field conditions. In *Proceedings of the Annual Meeting of the Florida State Horticulture Society*.

Pilatzke-Wunderlich, I., Nessler, C. (2001) Expression and activity of cell-wall-degrading enzymes in the latex of opium poppy, Papaver somniferum L. *Plant Molecular Biology*. **45**(5), 567–576.

Porter, J.R., Semenov, M. a (2005) Crop responses to climatic variation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. **360**(1463), 2021–35.

Potel, A.M., Ançay, A., Carlen, C. (2006) Influence of Leaf/Fruit Ratio of Strawberry Plants on the Sensory Quality of Their Fruits. In *XXVII International Horticultural Congress-IHC2006: International Symposium on Advances in Environmental Control, Automation 761*. pp. 121–126.

Qin, Y., Teixeira da Silva, J.A., Zhang, L., Zhang, S. (2008) Transgenic strawberry: state of the art for improved traits. *Biotechnology Advances*. **26**(3), 219–32.

Redgwell, R., MacRae, E., Hallett, I., Fischer, M. (1997) In vivo and in vitro swelling of cell walls during fruit ripening. *Planta*. **64**, 162–173.

Reilly, J., Paltsev, S., Felzer, B., Wang, X. (2007) Global economic effects of changes in crops, pasture, and forests due to changing climate, carbon dioxide, and ozone. *Energy Policy*. **35**(11), 5370–5383.

Robert, F., Risser, G., Petel, G. (1999) Photoperiod and temperature effect on growth of strawberry plant (Fragaria Âananassa Duch.): development of a morphological test to assess the dormancy induction. *Scientia Horticulturae*. **82**, 217–226.

Rose, D.H., Haller, M.H., Harding, P.L. (1934) Relation of temperature of fruit to firmness in strawberries. In *Proceedings of American Society of Horticultural Sciience*. pp. 429–430.

Rosenzweig, C., Strzepek, K.M., Major, D.C., Iglesias, A., Yates, D.N., McCluskey, A., Hillel, D. (2004) Water resources for agriculture in a changing climate: international case studies. *Global Environmental Change*. **14**(4), 345–360.

Rosli, H.G., Civello, P.M., Martínez, G. a (2009) alpha-l-Arabinofuranosidase from strawberry fruit: cloning of three cDNAs, characterization of their expression and analysis of enzymatic activity in cultivars with contrasting firmness. *Plant physiology and biochemistry : PPB / Société Française de Physiologie Végétale*. **47**(4), 272–81.

Roussos, P. a., Denaxa, N.-K., Damvakaris, T. (2009) Strawberry fruit quality attributes after application of plant growth stimulating compounds. *Scientia Horticulturae*. **119**(2), 138–146.

Ruiz-Altisent, M., Guillermo P. Moreda (2011) Fruits, mechanical properties and bruise sesceptibility. In J. Gliński, J. Horabik, & J. Lipiec, eds. *Encyclopedia of Agrophysics*. Encyclopedia of Earth Sciences Series. Dordrecht: Springer Netherlands, pp. 318–321.

Salinari, F., Giosuè, S., Rossi, V., Tubiello, F.N., Rosenzweig, C., Gullino, M.L. (2007) Downy mildew outbreaks on grapevine under climate change: elaboration and application of an empiricalstatistical model*. *EPPO bulletin*. **37**(2), 317–326.

Sams, C. (1999) Preharvest factors affecting postharvest texture. *Postharvest Biology and Technology*. **15**(June 1998), 249–254.

Samykanno, K. (2012) *Environmental effects on flavour development in Australian-grown strawberry varieties*. RMIT University.

Schlosser, E. (1988) Preformed Chemical Barriers in Host-Parasite Incompatibility. *Experimental and Conceptual Plant Pathology*. **3**, 465.

Scholz, G., Quinton, J.N., Strauss, P. (2008) Soil erosion from sugar beet in Central Europe in response to climate change induced seasonal precipitation variations. *Catena*. **72**(1), 91–105.

Schopfer, P. (2001) Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: implications for the control of elongation growth. *The Plant Journal : For Cell and Molecular Biology*. **28**(6), 679–88.

Schouten, R. E., Kessler, D., Orcaray, L., & van Kooten, O. (2002). Predictability of keeping quality of strawberry batches. Postharvest Biology and Technology, **26**(1), 35–47.

Schubert, P., Golldack, J., Schwärzel, H., Lentzsch, P. (2008) Influence of soil temperature to the pathosystem strawberry-Verticillium. In *Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838.* pp. 139–144.

Schwieterman, M.L., Colquhoun, T.A., Jaworski, E.A., Bartoshuk, L.M., Gilbert, J.L., Tieman, D.M., Odabasi, A.Z., Moskowitz, H.R., Folta, K.M., Klee, H.J., Sims, C.A., Whitaker, V.M., Clark, D.G. (2014) Strawberry flavor: diverse chemical compositions, a seasonal influence, and effects on sensory perception. *PloS one*. **9**(2), e88446.

Severo, J., Tiecher, A., Chaves, F.C., Silva, J.A., Rombaldi, C.V. (2011) Gene transcript accumulation associated with physiological and chemical changes during developmental stages of strawberry cv. Camarosa. *Food Chemistry*. **126**(3), 995–1000.

Sexton, R., Palmer, J. (1997) Cellulase, Fruit Softening and Abscission in Red RaspberryRubus idaeusL. cv Glen Clova. *Annals of Botany*. **80**(3), 371–376.

Shackel, K.A., Greve, C., Labavitch, J.M., Ahmadi, H. (1991) Cell turgor changes associated with ripening in tomato pericarp tissue. *Plant Physiology*. **97**(2), 814–6.

Shewfelt, R.L. (1999) What is quality? *Postharvest Biology and Technology*. **15**(3), 197–200.

Da Silva, F.L., Escribano-Bailón, M.T., Pérez Alonso, J.J., Rivas-Gonzalo, J.C., Santos-Buelga, C., Silva, F. da (2007) Anthocyanin pigments in strawberry. *LWT - Food Science and Technology*. **40**(2), 374–382.

Singh, R., Sharma, R.R., Tyagi, S.K. (2007) Pre-harvest foliar application of calcium and boron influences physiological disorders, fruit yield and quality of strawberry (Fragaria×ananassa Duch.). *Scientia Horticulturae*. **112**(2), 215–220.

Sivakumar, M., Das, H., Brunini, O. (2005) Impacts of present and future climate variability and change on agriculture and forestry in the arid and semi-arid tropics. *Climatic Change*. **70**(1-2), 31–72.

Sommer, N.F. (1960) Fresh fruit temperatures and transit injury. In *Proceedings. American Society for Horticultural Science*. pp. 156–162.

Sønsteby, A., Heide, O.M. (2006) Dormancy relations and flowering of the strawberry cultivars Korona and Elsanta as influenced by photoperiod and temperature. *Scientia Horticulturae*. **110**(1), 57–67.

Starr, M.P., Moran, F. (1962) Eliminative split of pectic substances by phytopathogenic soft-rot bacteria. *Science*. **135**(3507), 920–921.

Sturm, K., Koron, D., Stampar, F. (2003) The composition of fruit of different strawberry varieties depending on maturity stage. *Food Chemistry*. **83**(3), 417–422.

Sunley, R.J., Atkinson, C.J., Jones, H.G. (2006) Chill unit models and recent changes in the occurrence of winter chill and spring frost in the United Kingdom. *Journal of Horticultural Science & Biotechnology*. **81**(6), 949–958.

Taniguchi, Y., Ono, A., Sawatani, M., Nanba, M., Kohno, K., Usui, M., Kurimoto, M., Matuhasi, T. (1995) Cry j I, a major allergen of Japanese cedar pollen, has pectate lyase enzyme activity. *Allergy*. **50**(1), 90–93.

Tarasova, N., Gorshkov, V. (2013) Potato signal molecules that activate pectate lyase synthesis in Pectobacterium atrosepticum SCRI1043. *World Journal of Microbiology and Biotechnology*. **29**(7), 1189–1196.

Teramura, A.H., Sullivan, J.H. (1994) Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynthesis Research*. **39**(3), 463–473.

Terry, L.A. (2011) *Health-promoting properties of fruits and vegetables*. CABI.

Terry, L.A., Chope, G.A., Bordonaba, J.G. (2007) Effect of water deficit irrigation and inoculation with Botrytis cinerea on strawberry (Fragaria x ananassa) fruit quality. *Journal of Agricultural and Food Chemistry*. **55**(26), 10812–10819.

Toivonen, P.M.A., Brummell, D.A. (2008) Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology*. **48**(1), 1–14.

Törrönen, R., Määttä, K. (2000) Bioactive substances and health benefits of strawberries. In *IV International Strawberry Symposium 567*. pp. 797–803.

Trainotti, L., Spinello, R., Piovan, A., Spolaore, S., Casadoro, G., Biologia, D., Padova, Á., Colombo, V.G., Padova, I.- (2001) b -Galactosidases with a lectin-like domain are expressed. *Journal of Experimental Botany*. **52**(361), 1635–1645.

Tsormpatsidis, E., Henbest, R.G.C., Davis, F.J., Battey, N.H., Hadley, P., Wagstaffe, a. (2008) UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. *Environmental and Experimental Botany*. **63**(1-3), 232–239.

Tsormpatsidis, E., Ordidge, M., Henbest, R.G.C., Wagstaffe, A., Battey, N.H., Hadley, P. (2011) Harvesting fruit of equivalent chronological age and fruit position shows individual effects of UV radiation on aspects of the strawberry ripening process. *Environmental and Experimental Botany*. **74**(0), 178–185.

Tuovinen, T. (2008) Risk of invasive arthropod pests related to climate change in the northernmost small fruit production area in EU. In *Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838*. pp. 151–154.

Turner, L.B., Mueller-Harvey, I., McAllan, A.B. (1993) Light-induced isomerization and dimerization of cinnamic acid derivatives in cell walls. *Phytochemistry*. **33**(4), 791–796.

United Nations (2013) Framework Convention on Climate Change. [online]. Available from: https://unfccc.int/2860.php.

Verbeyst, L., Oey, I., Van der Plancken, I., Hendrickx, M., Van Loey, A. (2010) Kinetic study on the thermal and pressure degradation of anthocyanins in strawberries. *Food Chemistry*. **123**(2), 269–274.

Vicente, A.R., Saladié, M., Rose, J.K., Labavitch, J.M. (2007) The linkage between cell wall metabolism and fruit softening: looking to the future. *Journal of the Science of Food and Agriculture*. **87**(8), 1435–1448.

Vicente, E., Varela, P., de Saldamando, L., Ares, G. (2014) Evaluation of the sensory characteristics of strawberry cultivars throughout the harvest season using projective mapping. *Journal of the Science of Food and Agriculture*. **94**(3), 591–9.

Wang, M.H., Kim, J.G., Ahn, S.E., Lee, A.Y., Bae, T.M., Kim, D.R., Hwang, Y.S. (2014) Potential role of pectate lyase and Ca(2+) in the increase in strawberry fruit firmness induced by short-term treatment with high-pressure CO2. *Journal of Food Science*. **79**(4), S685–92.

Wang, S.Y., Bunce, J.A., Maas, J.L. (2003) Elevated carbon dioxide increases contents of antioxidant compounds in field-grown strawberries. *Journal of Agricultural and Food Chemistry*. **51**(15), 4315–20.

Wang, S.Y., Camp, M.J. (2000) Temperatures after bloom affect plant growth and fruit quality of strawberry. *Scientia Horticulturae*. **85**(3), 183–199.

Wang, S.Y., Zheng, W. (2001) Effect of plant growth temperature on antioxidant capacity in strawberry. *Journal of Agricultural and Food Chemistry*. **49**(10), 4977–4982.

Warrick, R.A., Kenny, G.J., Harman, J.J. (2001) *The Effects of Climate Change and Variation in New Zealand An Assessment Using the CLIMPACTS System*.

Watson, R. (2002) Influence of harvest date and light integral on the development of strawberry flavour compounds. *Journal of Experimental Botany*. **53**(377), 2121–2129.

Whitaker, V.M., Hasing, T., Chandler, C.K., Plotto, A., Baldwin, E. (2011) Historical Trends in Strawberry Fruit Quality Revealed by a Trial of University of Florida Cultivars and Advanced Selections. *Horticultural Science*. **46**(4), 553–557.

Wilby, R.L., Orr, H.G., Hedger, M., Forrow, D., Blackmore, M. (2006) Risks posed by climate change to the delivery of Water Framework Directive objectives in the UK. *Environment International*. **32**(8), 1043–55.

Wills, R.B.H., Kim, G.H. (1995) Effect of ethylene on postharvest life of strawberries. *Postharvest Biology and Technology*. **6**(3-4), 249–255.

Win, R.A., Yamaguchi, J., Larabell, S.K., Ursin, V.M., Mccormick, S. (1990) Molecular and genetic characterization of two pollen-expressed genes that have sequence similarity to pectate lyases of the plant pathogen Erwinia. *Plant Molecular Biology*. **14**(1), 17–28.

Wolf, S., Mouille, G., Pelloux, J. (2009) Homogalacturonan methyl-esterification and plant development. *Molecular Plant*. **2**(5), 851–60.

Wu, Y., Qiu, X., Du, S., Erickson, L. (1996) PO149, A new member of pollen pectate lyase-like gene family from Alfa-Alfa. *Plant Molecular Biology*. **32**(6), 1037–1042.

Yakoby, N., Kobiler, I., Dinoor, A., Prusky, D., Yakoby, N.I.R. (2000) pH Regulation of Pectate Lyase Secretion Modulates the Attack of Colletotrichum gloeosporioides on Avocado Fruits pH Regulation of Pectate Lyase Secretion Modulates the Attack of Colletotrichum gloeosporioides on Avocado Fruits. *Applied and Environmental Microbiology*. **66**(3), 1026–1030.

Yoder, M.D., Keen, N.T., Jurnak, F., others (1993) New domain motif: the structure of pectate lyase C, a secreted plant virulence factor. *Science*. **260**(5113), 1503–1507.

Yokoyama, R., Nishitani, K. (2004) Genomic basis for cell-wall diversity in plants. A comparative approach to gene families in rice and Arabidopsis. *Plant & Cell Physiology*. **45**(9), 1111–21.

Young, S., David, C., Gibson, G. (1987) Light measurement for entomology in the field and laboratory. *Physiological Entomology*. **12**(4), 373–379.

Youssef, S.M., Jiménez-Bermúdez, S., Bellido, M.L., Martín-Pizarro, C., Barceló, M., Abdal-Aziz, S.A., Caballero, J.L., López-Aranda, J.M., Pliego-Alfaro, F., Muñoz, J., Quesada, M.A., Mercado, J.A. (2009) Fruit yield and quality of strawberry plants transformed with a fruit specific strawberry pectate lyase gene. *Scientia Horticulturae*. **119**(2), 120–125.

Yuan, P., Meng, K., Wang, Y., Luo, H., Shi, P., Huang, H., Tu, T., Yang, P., Yao, B. (2012) A Low-Temperature-Active Alkaline Pectate Lyase from Xanthomonas campestris ACCC 10048 with High Activity over a Wide pH Range. *Applied Biochemistry and Biotechnology*. **168**(6), 1489–1500.

Zhang, Y., Seeram, N.P., Lee, R., Feng, L., Heber, D. (2008) Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties. *Journal of Agricultural and Food Chemistry*. **56**(3), 670–675.

Zucker, M., Hankin, L. (1970) Regulation of pectate lyase synthesis in Pseudomonas fluorescens and Erwinia carotovora. *Journal of Bacteriology*. **104**(1), 13–8.

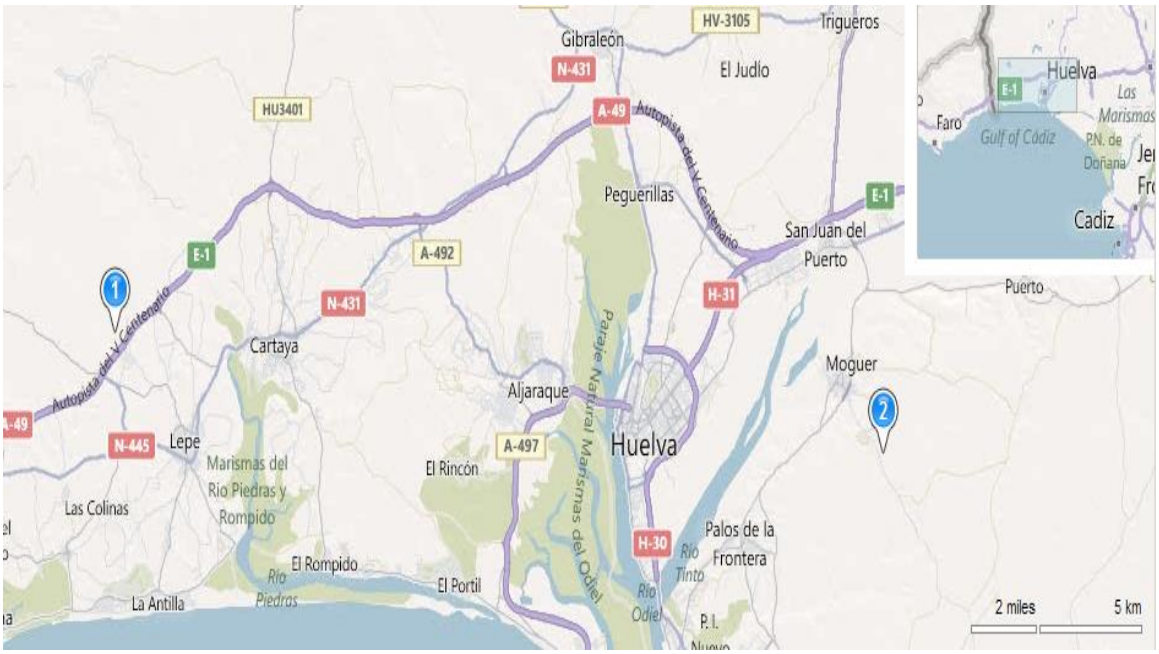

Figure A.1: SP1 (1) near Lepe and SP2 (2) near Moguer locations in Huelva region, Spain.

Figure A.2: S&A Produce (UK Site) location at Herefordshire UK.

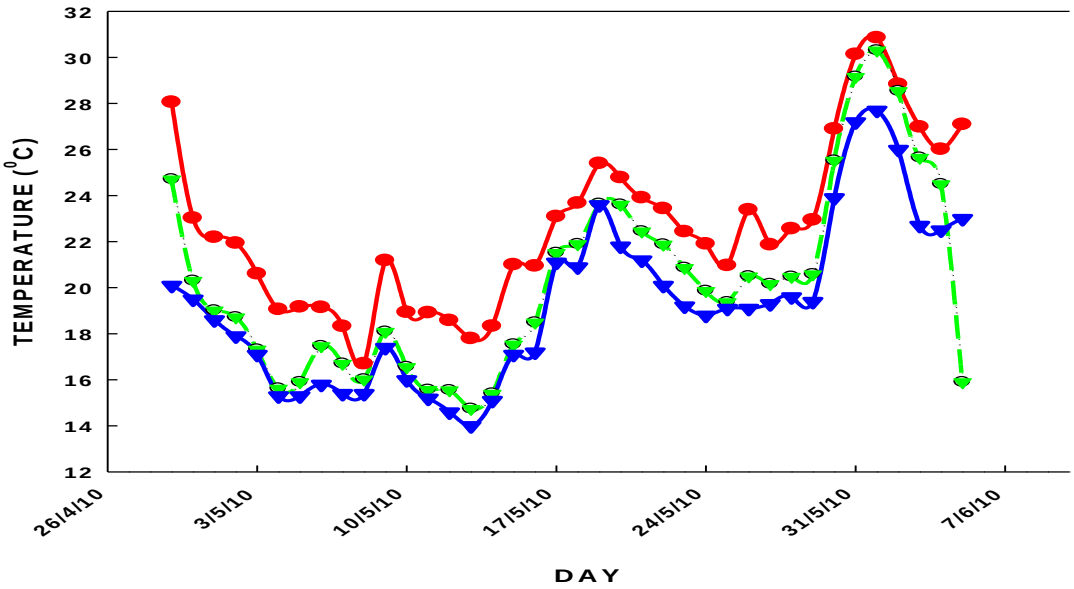

Figure A.3: Temperature comparisons (2010) at commercial strawberry growing Spanish site 1 (SP1) using loggers and weather station ≈3 Km away. Station (-▲-), field (-●-), tunnel (-●-).

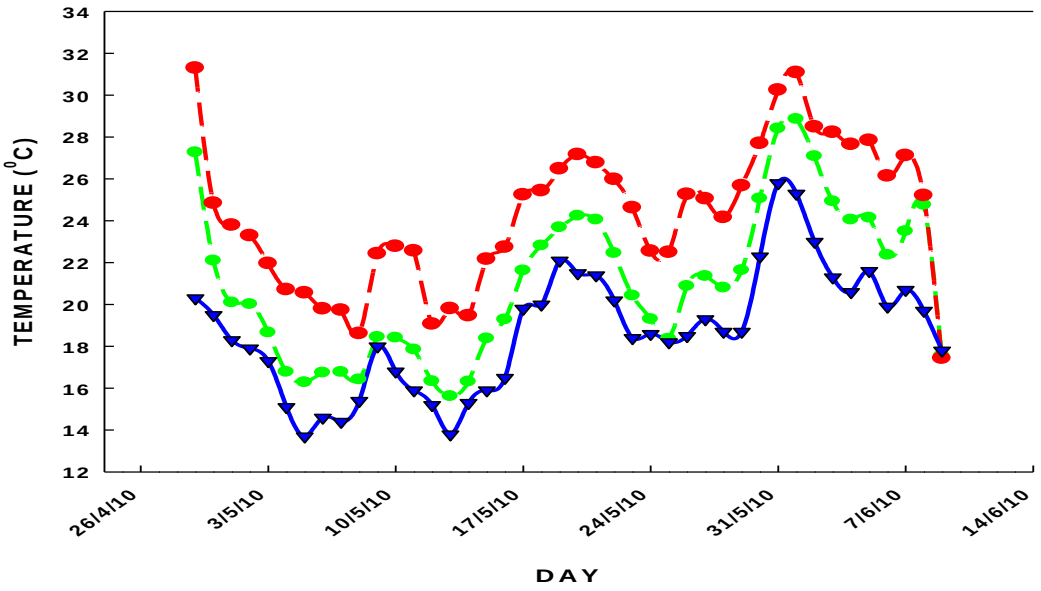

Figure A.4: Temperature comparisons (2010) at commercial strawberry growing Spanish site 2 (SP2) using loggers and weather station ≈5 Km away. Station (-▲-), field (-●-), tunnel (-●-).

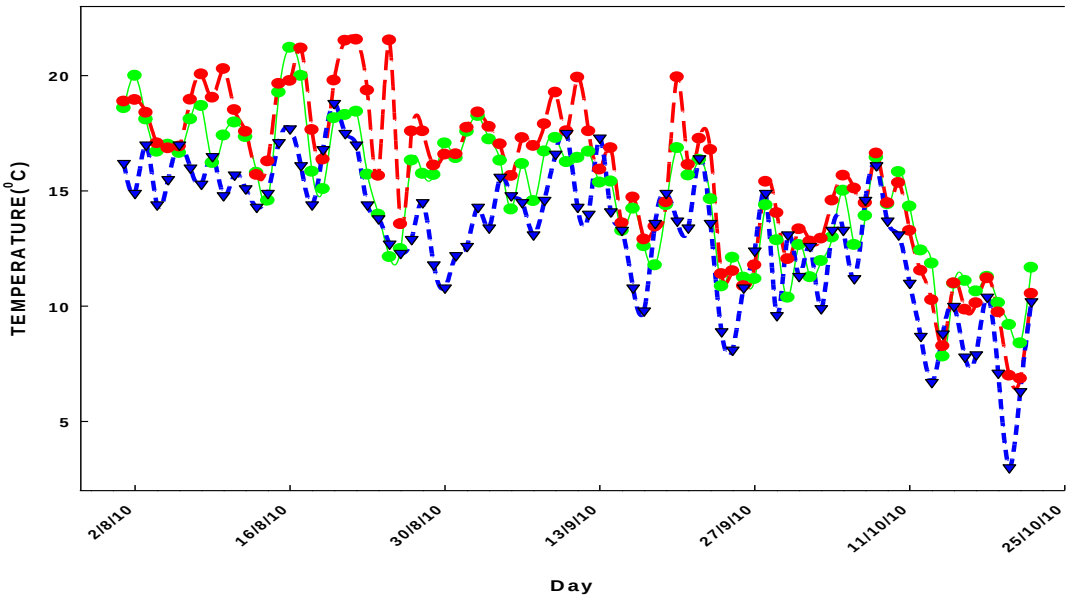

Figure A.5: Temperature comparisons (2010) at commercial strawberry growing UK site (UKH) using loggers and weather station ≈10 Km away. Station (-▲-), field (-●-), tunnel (-●-).

Table A.1: Significant differences for quality characteristics observed between SP1 and SP2 growing farms during pre-sampling.

Quality attribute	Firmness (Newtons)	TSS (°Brix)	*L**	*b**	Weight (g)
Site					
SP1	7.40	7.25	41.10	26.42	18.42
SP2	8.46	8.43	39.40	24.63	21.07

Table B.1: Linear firmness model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	Firmness=	Temperature (T)	VPD (V)	Irradiation (I)	Ozone (O)
SP1/ 1	0.4309	-0.4T-4.4V+0.2I -0.030 +14.9	<.001	0.003	0.042	0.021
SP1/ 2	0.1964	-0.4T-0.3I+6.8V -0.009O+19.8	0.051	0.003	0.056	0.87
SP1/ 1,2*	0.3161	-0.5T+0.1I -3.8V-0.0008O+15.5	<.001	<.001	0.040	0.94
SP2/ 1	0.2726	-0.5T+0.08I+0.2V-0.02O+15.0	<.001	0.85	0.15	0.25
SP2/ 2	0.3693	-0.3T+0.2I -10.7V -0.1O+ 22.7	0.002	0.005	0.13	<.001
SP2/ 3	0.4294	-0.8T+0.02I+7.2V -0.06O+21.5	<.001	0.89	0.003	0.012
SP2/ 1,2,3*	0.446	-0.7T -0.07I+4.2V-0.015O+ 20.0	<.001	<.001	0.16	0.103
UK/ 1	0.3878	0.04T+0.1I-22.3V+0.03O+7.5	0.83	0.26	0.025	0.62

* Mean across years

Table B.2: Linear firmness model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	Firmness=	Temperature	VPD (V)	Irradiation (I)	Ozone (O)
SP1/ 1	0.3486	-0.2T -0.09I+0.8V-0.07O+15.0	0.032	0.11	0.67	<.001
SP1/ 2	0.1981	-0.3T -0.3I+1.7V+0.01O+18.0	0.42	0.47	0.37	0.92
SP1/ 1,2*	0.2204	-0.3T-0.02I-3.5V_0.007O+15.0	<.001	0.003	0.66	0.52
SP2/ 1	0.2016	0.1T-0.2I+3.1V-0.05O +10.4	0.026	0.073	<.001	<.001
SP2/ 2	0.4347	0.7T-1.0I-3.7V+0.07O+10.9	0.059	0.025	0.012	0.56
SP2/ 3	0.4963	-0.4T+0.02I+9.8V -0.2O +25.2	<.001	0.14	0.92	<.001
SP2/ 1,2,3*	0.3774	-0.5T -0.09I+1.4V -0.04O+20.5	<.001	0.30	0.038	<.001
UK/ 1	0.3822	-1.8T-1.9I+144.8V-0.8O +40.9	<.001	<.001	<.001	<.001

* Mean across years

Table B.3: Linear TSS model and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	°Brix	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.0948	-0.03 T + 0.0004 I + 1.8 V + 0.02 O + 5	0.60	0.047	0.99	0.0031
SP1/ 2	0.1110	-0.7 T+ 0.2 I + 1.3 V + 0.02 O + 13	<0.001	0.27	0.0098	0.31
SP1/ 1&2	0.0315	-0.1 T +0.003 I +0.9 V + 0.02 +8	0.0012	0.18	0.93	<0.001
SP2/ 1	0.0990	0.2 T -0.03I + 0.6 V + 0.01 O + 6	<0.001	0.34	0.21	0.069
SP2/ 2	0.0784	0.3 T + 0.3 I -10.1 V -0.05 O + 8	0.084	0.025	0.071	0.042
SP2/ 3	0.0308	-0.2 T -0.03 I + 2.7 V -0.02067 O + 13	0.024	0.088	0.77	0.29
SP2/ 1,2&3	0.0257	-0.05T -0.07I + 1.6V -0.004O + 10.5	0.042	<0.001	<0.001	0.39
UK/ 1	0.2736	-0.07T + 0.2 I -16.1 V + 0.05 O + 9	0.51	0.055	0.0035	0.14

Table B.4: Linear TSS model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	°Brix	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1855	-0.2 T +0.004 I + 3.6 V + 0.03 O +7	<0.001	0.0016	0.95	0.016
SP1/ 2	0.0855	-0.4 T + -0.4 I + 0.6 V + 0.05 O + 7	<0.001	0.74	0.094	0.096
SP1/ 1&2	0.1221	-0.3 T+ 0.02 I+ 2.9 V + 0.04 O + 7	<0.001	<0.001	0.57	<0.001
SP2/ 1	0.1531	-0.02T + 0.2 I -2.0 V + 0.008 O + 5	0.79	0.047	<0.001	0.5000
SP2/ 2	0.1603	0.01 T+ 0.1 I -8.6 V -0.01O + 10	0.87	0.0030	0.37	0.56
SP2/ 3	0.0346	-0.2T + 0.1 I + 0.1 V -0.05O +13	0.13	0.98	0.51	0.099
SP2/ 1,2&3	0.0426	-0.22T+ 0.07I + 0.7V + 0.005O +9	<0.001	0.27	0.025	0.39
UK/ 1	0.2896	-0.6 T + 0.09 I + 10.8 V -0.05 O + 14	<0.001	0.19	0.42	0.33

Table B.5: Linear TSS model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	°Brix	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.3892	0.5 T + 0.08 IR -7.04 VPD + 0.02 OZ + 0.04	<0.001	<0.001	0.016	0.0092
SP1/ 2	0.1198	1.0 T -4.2 IR+ 0.9 VPD -1.3 OZ +4.08	<0.001	0.34	<0.001	0.19
SP1/ 1&2	0.0633	0.2 T -0.1 IR+ 0.7 VPD + 0.009 OZ + 4.5	<0.001	0.36	0.0013	0.20
SP2/ 1	0.2433	0.4 T +0.2 IR -5.9 VPD -0.03 OZ +2.9	<0.001	<0.001	<0.001	0.019
SP2/ 2	0.2137	1.2 T -1.09 IR -4.08 VPD+0.2 OZ -2.0	<0.001	0.0016	<0.001	0.076
SP2/ 3	0.0392	-0.07 T +1.4 IR -0.5 VPD -1.4 OZ + 8.4	0.67	0.59	0.15	0.16
SP2/ 1,2&3	0.0547	-0.005T + 0. 2IR -3.8 VPD -0.04OZ +10.5	0.83	<0.001	<0.001	<0.001
UK/ 1	0.2897	-1.8 T -0.9 IR +98.7 VPD -0.6 OZ + 35. 7	<0.001	<0.001	0.0084	0.0010

Table B.6: Linear Chroma model and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	Chroma	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.0871	+ 0. 3T +0.2 IR -7. 9 VPD -0.04 OZ+ 42.09	0.0048	<0.001	0.037	0.014439
SP1/ 2	0.3395	+1.13 T +0.3 IR -21.7VPD + 0.01 OZ+ 53.5	<0.001	<0.001	0.79	0.057643
SP1/ 1&2	0.1543	+0.5 T + 0.2 IR -12.1 VPD -0.01 OZ+40.1	<0.001	<0.001	<0.001	0.265930
SP2/ 1	0.1363	-0.05 T + 0.3 IR -3.2VPD + 0.04 OZ+ 38.3	0.57	0.0053	<0.001	0.009061
SP2/ 2	0.2443	+ 0.7 T + 0.1 IR -16.8 VPD + 0.05 OZ+ 38.0	0.0098	0.046	0.64	0.299232
SP 2/3	0.0847	-0. 3 T + 0.007 IR - 0.1VPD + 0.007 OZ+ 49.4	0.0016	0.94	0.94	0.695762
SP2/ 1,2&3	0.1328	-0.2 T -0.09 IR -1.3 VPD +0.08 OZ+45.7	<0.001	0.13	0.014	<0.001
UK/ 1	0.2896	+ 0.1T -0.04 IR -84.7 VPD + 0.15 OZ+ 53.5	<0.001	<0.001	0.79	<0.001

Table B.7: Linear Chroma model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	Chroma	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.0859	+0. 3 T + 0.4 IR -11.8 VPD - 0.02 OZ+39.7	0.019	<0.001	0.002	0.35
SP1/ 2	0.3395	+ 1.1T -0.07 IR -25.0VPD + 0.02 OZ+39.8	<0.001	<0.001	0.55	0.68
SP1/ 1&2	0.1211	+0.39 T + 0.1 IR -9.0 VPD -0.004 OZ+ 41.4	<0.001	<0.001	0.22	0.82
SP2/ 1	0.1517	-0.2 T + 0.3 IR -8.1 VPD + 0.08 OZ+39.7	0.16	<0.001	0.0023	0.00055
SP2/ 2	0.3046	+0.4 T-0.4 IR -9.02 VPD + 0.2OZ+ 38.7	0.0044	0.089	0.062	<0.001
SP2/ 3	0.1321	-0.5 T -0.2 IR + 7.5 VPD + 0.06 OZ+ 49.7	<0.001	0.0071	0.21	0.041
SP2/ 1,2&3	0.2314	- 0.3 T - 0.1 IR -1.7 VPD + 0.1 OZ + 45.5	<0.001	0.20	0.038	<0.001
UK/ 1	0.1241	+ 0.1 T+ -0.5 IR + 6.5 VPD -0.3 OZ+61.1	0.78	0.74	0.05	0.042

Table B.8: Linear Chroma model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	Chroma	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1948	+0.1T -0.09 IR + 9.05 VPD-0.1 OZ+46. 8	0.391316	0.000613	0.26	<0.001
SP1/ 2	0.343043	+1. 4T -0.5 IR -19.6 VPD +0.1 OZ+29.7	0.003528	<0.001	0.20	0.223725
SP1/ 1&2		+0.3T + 0.4IR -11.5 VPD -0.06 OZ+40.1	<0.001	<0.001	<0.001	<0.001
SP2/ 1	0.1289	+0.31T +0.2IR + -1.8 VPD - 0.07 OZ+40.4	0.009838	0.4875	0.0062	0.001544
SP2/ 2	0.2737	+0.6 T -0.1IR + -18.8 VPD + 0.1 OZ+39.3	0.283453	<0.001	0.86	0.558486
SP2/ 3	0.1861	+0.04T-0.6 IR + 38.8 VPD -0.25029 OZ+55.6	0.804805	<0.001	0.0045	0.00014
SP2/ 1,2&3	0.05721	-0.3 T + 0.2 IR - 6.5 VPD + 0.1 OZ + 46.2	<0.001	<0.001	0.0023	0.011334
UK/ 1	0.2968	+0.6 T -3.3 IR +154.5 VPD - 1.04OZ+74.5	0.366052	0.008952	<0.001	0.004062

Table B.9: Linear °Hue model and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	°Hue	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1131	-0.2T +0.2 IR + -7.5 VPD -0.06 OZ+ 38.6	0.139864	0.001307	0.095	0.005686
SP1/ 2	0.1985	+1.2 T + 0.3 IR -21.7 VPD + 0.1OZ+8.0	0.002202	<0.001	0.13	0.005754
SP1/ 1&2	0.05587	+0.2 T+0.2 IR -9.8 VPD -0.006 + OZ+31.3	0.131	<0.001	0.016	0.710
SP2/ 1	0.1226	-0.3 T+0.3 IR -2.1VPD - 0.01OZ+31.03	0.001335	0.081002	<0.001	0.306682
SP2/ 2	0.315584	-0.5 T -0.8 IR +16.8 VPD + 0.3OZ+29.2	0.066031	0.039609	0.0067	<0.001
SP2/ 3	0.0126	-0.04 T + 0.09 IR -2.2 VPD - 0.003OZ+ 35.7	0.704267	0.196739	0.42	0.883809
SP2/ 1,2&3	0.1047	-0.32 T -0.1 IR +0.5 VPD +0.08OZ+35.08	<0.001	0.65734	0.0085	<0.001
UK/ 1	0.3025	+ 1.5 T +0.3 IR -126.7VPD + 0.3OZ+ 36.3	<0.001	<0.001	0.045	0.001

Table B.10: Linear °Hue model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	°Hue	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1075	+0.02 T + 0.2 IR -8.1 VPD - 0.05OZ+35.4	0.885207	<0.001	0.17	0.008192
SP1/ 2	0.1615	+1.2 T -0.2 IR-19.4 VPD + 0.05OZ+21.3	<0.001	<0.001	0.34	0.583343
SP1/ 1&2	0.1072	+0.4T + 0.1 IR -10.7 VPD -0.04 OZ+32.5	0.0039	<0.001	0.21	0.0596
SP2/ 1	0.050323	-0.2 T + 0.08IR -3.9 VPD + 0.02 OZ+33.2	0.107236	0.064412	0.45	0.521887
SP2/ 2	0.1922	-0.2T + 0.06 IR -2.7 VPD + 0.2 OZ+23.9	0.298715	0.641855	0.77	<0.001
SP2/ 3	0.0528	-0.2 T -0. 6 IR + 11.0 VPD + 0.1 OZ+36.8	0.128317	0.001223	0.0035	0.001049
SP2/ 1,2&3	0.1846	-0.4 T -0.3 IR + 0.04 VPD +0.1 OZ+35.5	<0.001	0.980238	<0.001	<0.001
UK/ 1	0.2210	+ 1.0 T + 0.08 IR-48.7VPD - 0.1OZ+40.6	0.01153	0.012003	0.75	0.356666

Table B.11: Linear °Hue model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	°Hue	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.2050	-0.5 T -0.2 IR + 12.4 VPD -0.11736 OZ+ 44.0	0.000681	0.0001 5	0.081	<0.001
SP1/ 2	0.1763	+0.49 T -0.14 IR + -12.9 VPD + 0.39OZ+3.9	0.474555	0.0008 66	0.80	0.011051
SP1/ 1&2	0.0527	+0.06 T+ 0.4 IR -11.5 VPD - 0.06OZ+33.7	0.65472	<0.001	<0.001	0.00326
SP2/ 1	0.0580	-0.2 T -0.003 IR + 3.2 VPD -0.06 OZ+36.1	0.047018	0.2738	0.97	0.014236
SP2/ 2	0.2241 58	-1.2T + 1.0 IR -7. 9VPD + 0 3 OZ+21.3	0.04641	0.0032 8	0.13	0.146945
SP2/ 3	0.0557 02	+0.5 -0.8 IR + 33. 5 VPD - 0.2OZ+41.2	0.007075	0.0007 87	0.0019	0.023596
SP2/ 1,2&3	0.1033	-0.4 T -0.01 IR -2.8 VPD + 0.1 OZ+33. 7	<0.001	0.301	0.86	<0.001
UK/ 1	0.2524	+ 1. 8T-2.05 IR + 47.3 VPD -0.7 OZ+49.8	0.012701	0.4433	0.009	0.05682

Table B.12: Linear *L**model and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	*L**	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1104	+0.3 T +0.33 IR -8.1 VPD + 0.002 OZ+ 30.2	0.00277	<0.001	0.00027	0.884955
SP1/ 2	0.3653	+1.7 T +0.18 IR-23.0 VPD + 0.1OZ+10.2	<0.001	<0.001	0.18	0.000913
SP1/ 1&2	0.1985	+0.5T + 0.3 IR -11.7 VPD +0.05 OZ+27.6	<0.001	<0.001	<0.001	<0.001
SP2/ 1	0.1766	-0.06 T 0.2 IR -2. 9 VPD + 0.05 OZ+31.9	0.322497	0.001257	<0.001	<0.001
SP2/ 2	0.070335	-0.2T -0.5 IR + 10.5 VPD +0.07 OZ+40.6	0.354428	0.132396	0.029	0.047886
SP2/ 3	0.0169	-0.06T +0.02IR -1.2 VPD +0.002OZ+40.0	0.486706	0.374002	0.80	0.909224
SP2/ 1,2&3	0.0581	-0.2 T + 0.03 IR -0.48 VPD + 0.04 OZ+38.3	<0.001	0.517	0.26	<0.001
UK/ 1	0.2602	+ 0.8 T -0.02 IR -76.009VPD +0.15 OZ+45.5	<0.001	<0.001	0.88	0.007432

Table B.13: Linear *L**model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	*L**	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1269	+0.3 T +0.5 IR-11.9 VPD + 0.01OZ+29.3	0.126935	0.126935	0.13	0.126935
SP1/ 2	0.3378	+ 1.4T -0.2 IR -20.7 VPD + 0.07 OZ+22.4	<0.001	<0.001	0.14	0.224187
SP1/ 1&2	0.2132	+0.5 T + 0.2 IR -10.5 VPD + 0.04 OZ+29.1	<0.001	<0.001	0.015	0.00425
SP2/ 1	0.1610	-0.1 T +0.2IR -4.9 VPD + 0.06OZ+32.7	0.150243	0.000822	0.0011	0.000951
SP2/ 2	0.0548	+0.04 T +0.03 IR 6.8 VPD + 0.023OZ+38.0	0.724747	0.144524	0.84	0.672374
SP2/ 3	0.0393	-0.1 T -0.3 IR + 5.4 VPD + 0.08 OZ+ 39.9	0.239612	0.041811	0.028	0.004403
SP2/ 1,2&3	0.0895	-0.3 T - 0.02 IR +1.6 VPD +0.07OZ+38.3	<0.001	0.171	0.74	<0.001
UK/ 1	0.1659	+0.3 T -0.4 IR -6.28 VPD -0.2 OZ+50.7	0.316744	0.703187	0.046	0.102338

Table B.14: Linear *L**model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	*L**	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.2150	-0.1 T +0.2 IR+1.3 VPD -0.07 OZ+38.2	0.273411	0.571776	0.0013	<0.001
SP1/ 2	0.3747	+ 1.7T -0.8 IR-12.06 VPD + 0.3 OZ+ 8.9	<0.001	<0.001	0.036	0.003988
SP1/ 1&2	0.2272	+0.3 + 0.6 IR -14.3 VPD -0.02 OZ	0.00888	<0.001	<0.001	0.11478
SP2/ 1	0.1321	+0.23 T +0.2 IR -2.2 VPD -0.06 OZ+34.3	0.027243	0.285197	0.00096	0.001182
SP2/ 2	0.0934	+ 1.5T -1.4 IR -3.8 VPD +0.3 OZ+17.5	0.001334	0.073844	0.0057	0.041681
SP2/ 3	0.062447	+0.5 T -0.6 IR+22.0 VPD + -0.1OZ+44.1	0.000941	0.00435	0.0022	0.02213
SP2/ 1,2&3	0.0288	-0.2 T + 0.2 IR -1.6 VPD -0.0006 OZ+ 38.9	<0.001	0.303071	0.0026	0.96215
UK/ 1	0.2565	+ 0.7T -1.8 IR + 60.2 VPD -0.5 OZ+54.1	0.195809	0.233423	0.0043	0.124827

Table B.15: Linear weight model and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	Weight (g)	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.4631	-2.0 T -1.6 IR + 25.1 VPD + 0.05OZ+72.8	<0.001	<0.001	<0.001	0.125683
SP1/ 2	0.6309	-8.3 T +0.6 IR +42.3VPD +0.185199 OZ+112.2	<0.001	<0.001	0.020	0.009822
SP1/ 1&2	0.443	-2.4T +-1.4 IR +28.8 VPD +0.03 OZ+74.7	<0.001	<0.001	<0.001	0.195
SP2/ 1	0.2456	-0.8 T -0.3 IR +6.4 VPD - 0.09OZ+47.2	<0.001	0.002515	0.00011	0.000277
SP2/ 2	0.342	-1.9 T +1.5 IR -17.3 VPD - 0.4OZ+70.6	0.010364	0.42001	0.051	0.001061
SP2/ 3	0.423695	-1.4T -0.6IR + 15.01 VPD -0.12 OZ+54. 2	<0.001	<0.001	0.00071	0.00017
SP2/ 1,2&3	0.2277	-0.8 T -0.6 IR + 8. 6 VPD -0.05 OZ+48.7	<0.001	<0.001	<0.001	0.0113
UK/ 1	0.1739	+0.08 T +0.3 IR - 54.9VPD+0.3OZ+19.1	0.827564	0.05736	0.24	0.013917

Table B.16: Linear weight model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	Weight (g)	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.5043	-1.6 T -2.1 IR + 47.5 VPD - 0.01OZ+68.02	<0.001	<0.001	<0.001	0.74603
SP1/ 2	0.7143	-6.2 T +1.3 IR + 16.0 VPD +0.5OZ+52.3	<0.001	0.007348	<0.001	<0.001
SP1/ 1&2	0.4288	-2.6T - 0.8 IR + 28.006 VPD +0.03 OZ+ 64. 5	<0.001	<0.001	<0.001	0.348
SP2/ 1	0.2465	-0.9 T -0.6 IR +16.7 VPD -0.07512 OZ+49.0	<0.001	<0.001	0.00021	0.077402
SP2/ 2	0.371949	-1.7 T +1.6 IR -41.1 VPD - 0.7OZ+88.9	<0.001	0.003202	0.0023	<0.001
SP2/ 3	0.4030	-1.0 T+0.1 IR -0.7 VPD -0.3OZ+50.6	<0.001	0.894201	0.63	<0.001
SP2/ 1,2&3	0.2371	-0.5 T -0. 7 IR + 4.4 VPD -0.02 OZ+	<0.001	0.134	<0.001	0.511
UK/ 1	0.1959	-0.5 T +0.2IR -11.7 VPD + 0.2OZ+17.9	0.340421	0.681169	0.64	0.205347

Table B.17: Linear weight model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	Weight (g)	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.316037	+0.4 T -1.6 IR + 31.2VPD + 0.041OZ+29.6	0.11627	<0.001	<0.001	0.331564
SP1/ 2	0.7605	-5.3 T +0.3 IR + 4.2 VPD + 0.2OZ+85.0	<0.001	0.351679	0.62	0.396574
SP1/ 1&2	0.3614	-1.4T -1.5 IR +31.2 VPD +0.13371 OZ+48.9	<0.001	<0.001	<0.001	<0.001
SP2/ 1	0.2690	+0.3 T -0.9 IR + 7.8 VPD +0.1 OZ+0.3	0.149033	0.101893	<0.001	0.001858
SP2/ 2	0.3570	+0.8 T -2.7 IR -0.7VPD + 0.3OZ+37.8	0.580977	0.914812	0.084	0.475491
SP2/ 3	0.4621	-1.4 T+0.7 IR -44.0VPD - 0.04OZ+49.3	<0.001	0.003009	0.062	0.717428
SP2/ 1,2&3	0.3015	-0.4 T - 1.0 IR +3.1028 VPD + 0.08 OZ+43.9	<0.001	0.3733	<0.001	0.00652
UK/ 1	0.1925	-5. 3 T -2.2 IR + 254.1 VPD -0.9 OZ+78.3	<0.001	0.007108	0.06	0.129017

Table B.18: Linear total sugars model and significance level of environmental variables 2 days prior to harvest

Site/ Year	R^2	Total sugars mg/g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1125	+ 1.5T +0.2 IR -2.1 VPD - 0.08OZ+22.9	<0.001	0.711887	0.48	0.114254
SP1/ 2	0.2409	+0.9 T-1.3 IR + 25.7 VPD + 0.3 OZ+13.7	0.2954	0.3228	0.16	0.0159
SP1/ 1&2	0.1684	+ 1.6T + 0.2 IR -6.2 VPD - 0.04 OZ+20.1	<0.001	0.173	0.12	0.244
SP2/ 1	0.0811	+0.2 T -0.1 IR +6.8 VPD + 0.07OZ+40.3	0.4215	0.0256	0.30	0.0469
SP2/ 2	0.0903	+1.4 T - 0.1 IR -15.5 VPD + 0.5 OZ-15.5	0.182596	0.053538	0.84	0.000374
SP2/ 3	0.1586	+0.3 T+ 0.0003 IR + 16.8 VPD + 0.01 OZ +55.1	0.177659	<0.001	<0.001	0.831737
SP2/ 1,2&3	0.05916	+-0.09 T + 0.1 IR + 8.9 VPD + 0.002OZ+ 44.0	0.544308	<0.001	0.32	0.945893
UK/ 1	0.1247	+ 0.4T+0.30IR -78.6 VPD + 0.2OZ+53.5	0.4160	0.0358	0.36	0.1944

Table B.19: Linear total sugars model and significance level of environmental variables 7 days prior to harvest

Site/ Year	R^2	Total sugars mg/g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.0687	+0.9 T +0.6 IR -8.8 VPD -0.07 OZ +27. 6	0.0166	0.2399	0.13	0.3967
SP1/ 2	0.2326	+0.7 T + 0.2 IR -7.28 VPD +0.2 OZ+8.6	0.113	0.676	0.27	0.146
SP1/ 1&2	0.1630	+0.9 T+ 0.9 IR -14.7 VPD - 0.04OZ+22.5	<0.001	0.006201	<0.001	0.317406
SP2/ 1	0.1568	-0.4 T+ 0.4 IR + 6.1 VPD + 0.04OZ + 39.9	0.1609	0.1935	0.066	0.4689
SP2/ 2	0.0943	+0.5 T -0.1 IR -0.5 VPD +0.7OZ-7.1	0.374100	0.967019	0.73	<0.001
SP2/ 3	0.0846	+ 0.04T + 0.9 IR -2.41 VPD -0.2 OZ+49.1	0.88336	0.74699	0.040	<0.001
SP2/ 1,2&3	0.0942	-0.3 T +0. 8 IR + 0.8 VPD - 0.02OZ+39.6	0.0527	0.8133	<0.001	0.4952
UK/ 1	0.1172	-1.02 T-0.2 IR + 24.9 VPD + -0.1963 OZ+64.8	0.171	0.502	0.66	0.433

Table B.20: Linear total sugars model and significance level of environmental variables 21 days prior to harvest

Site/ Year	R^2	Total sugars mg/g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1733	+ 0.36T -0.09 IR + 16.3 VPD + 0.03 OZ +32.5	0.4274	0.0415	0.72	0.5340
SP1/ 2	0.2509	+ 1.2T -1.3 IR + 12.8 VPD + 1.1 OZ- 36.8	0.4922	0.1084	0.50	0.0544
SP1/ 1&2	0.2118	+ 0.8T + 0.1 IR +7.8 VPD + 0.03 OZ + 24.5	0.00293	0.0829 3	0.45	0.49415
SP2/ 1	0.1864	+0.7 T - 0.07 IR +6.5 VPD + 0.2OZ+27.0	0.01312	0.3617 4	0.75	0.36174
SP2/ 2	0.132	+ 0.7T -2.9 IR + 24.8 VPD + 1.7OZ- 45.4	0.67117	0.0099 1	0.037	<0.001
SP2/ 3	0.0299	+0.8 T+ 0.1 IR -18.3 VPD -0. 3 OZ	0.0751	0.4142	0.18	0.1288
SP2/ 1,2&3	0.0707	-0.02T + 0. 6 IR + 0.5 VPD -0.09 OZ+ 39.4	0.87967	0.9045	<0.001	0.82973
UK/ 1	0.1437	-3.7 T -3.9 IR +302.9 VPD -1.6 OZ+119.2	0.00783	0.0127 0	0.011	0.03148

Table B.21: Linear total acids model and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	Total acids mg/g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.2709	-0.01 T + 0.05 IR +-1.9 VPD + 0.2 O+5.8	0.946	0.613	0.81	<0.001
SP1/ 2	0.327	+ 0.8 T + 0.02 IR -14.1VPD + 0.02 OZ -2.8	0.0210	<0.001	0.021	0.6614
SP1/ 1&2	0.157	-0.04 T + 0.6 IR -8.7 VPD + 0.04 OZ+ 4.0	0.79823	0.00111	<0.001	0.06015
SP2/ 1	0.0732	+0.4 T + 0.02 IR -5.01 VPD - 0.00001 OZ+	0.00384	0.00699	0.76	0.99948
SP2/ 2	0.3679	-0.4 T + 0.2 IR + 0.09 VPD + 0.1 OZ	0.16461	0.99136	0.52	0.00496
SP2/ 3	0.205	-0.3 T + 0.03 IR + 3.2 VPD -0.06 OZ+ 17.2	0.002139	0.021035	0.75	<0.001
SP2/ 1,2&3	0.03367	+0.2 T + 0.04 IR -2.7 VPD -0.003 OZ+ 10.6	<0.001	0.019351	0.43	0.778912
UK/ 1	0.1165	-0.3 T -0. 20IR + 35.5 VPD -0.05194 OZ+ 14.2	0.1440	0.0361	0.22	0.3986

Table B.22: Linear total acids model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	Total acids mg/g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1931	-0.2T +0.1 IR -2. 5 VPD + 0.2OZ+6.8	0.36541	0.6270	0.59	0.00132
SP1/ 2	0.3316	+0.8 T +0.2 IR -14.8 VPD + 0.03 OZ+ 4.7	<0.001	<0.001	0.26	0.640802
SP1/ 1&2	0.1323	+ 0.03 T + 0.2 IR + 1.8 VPD + 0.02 OZ+ 7.0	0.86319	0.51102	0.14	0.48164
SP2/ 1	0.2016	+0.6 T -0.2 IR -5.6 VPD + 0.05OZ	0.000818	0.052980	0.12	0.164418
SP2/ 2	0.2285	-0.3 T + 0.8 IR -17.9 VPD -0.02 OZ+ 11.2	0.027148	0.003311	<0.001	0.702843
SP2/ 3	0.3265	+0.02 T + 0.2 IR -3. 1 VPD -0.1 OZ+ 18.2	0.870	0.224	0.23	<0.001
SP2/ 1,2&3	0.0762	+ 0.3 T + 0.02 IR -5.0 VPD -0.03 OZ+11.8	<0.001	0.00533	0.79	0.12012
UK/ 1	0.2957	+ 0.4 T -0.07 IR -6.1 VPD + 0.09 OZ + 9.7	0.2957	0.7194	0.75	0.4316

Table B.23: Linear total acids model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	Total acids mg/g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1149	+0.6 T + 0.6 IR -16.7 VPD - 0.04OZ+6.4	0.03356	0.00593	0.0032	0.29960
SP1/ 2	0.464	+2.1 T -1.4 IR -1.3 VPD + 0.3OZ - 14.7	<0.001	0.640382	<0.001	0.017036
SP1/ 1&2	0.1625	+ 0.6 T + 0.3 IR -4.4 VPD -0.1 OZ+ 9.0	0.00231	0.13421	0.022	<0.001
SP2/ 1	0.0954	-0.5 T - 0.02 IR + 6.9 VPD + 0.030 OZ+ 18.0	<0.001	0.085595	0.87	0.376716
SP2/ 2	0.09784	-1.2 T + 0.9 IR -2.8 VPD + 0.08695 OZ+ 11.4	0.0695	0.3427	0.20	0.6831
SP2/ 3	0.3522	-0.6T + 0.06 IR -33.6 VPD + 0.06 OZ+12.5	<0.001	<0.001	<0.001	0.311
SP2/ 1,2&3	0.0008622	+ 0.03 + 0.1 IR + VPD -0.03 OZ+ 13.4	0.6753	0.1705	0.079	0.1108
UK/ 1	0.1881	+1.07T +2.2 IR-155.4 +VPD + 1.0 OZ-16.4	0.08677	0.00386	0.0013	0.00370

Table B.24: Linear total anthocyanins model and significance level of environmental variables 2 days prior to harvest

Site/ Year	R^2	Total anthocyanins mg/100 g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1008	-1.3T -1.0 IR + 35.0 VPD + 0.2 OZ+47.9	<0.001	<0.001	<0.001	<0.001
SP1/ 2	0.0666	-0.4T -1.3IR +28.0 VPD -0.2 OZ+70.0	0.59432	<0.001	<0.001	0.05972
SP1/ 1&2	0.0646	-1.6T -0.7 IR +27.6 VPD +0.10240 OZ+55.9	<0.001	<0.001	<0.001	0.002309
SP2/ 1	0.0574	-0.02T +0.34 IR + 0.3 VPD - 0.2OZ+42.0	0.91001	0.91525	0.002	<0.001
SP2/ 2	0.0198	-0.3T -0.4 IR +10.8 VPD +0.01 OZ+ 41.6	0.00574	<0.001	<0.001	0.48432
SP2/ 3	0.0639	-0.5T -0.2 IR +9. 4 VPD -0.03 OZ+43.4	<0.001	<0.001	0.06	0.2312
SP2/ 1,2&3	0.0157	-0.07T +0.07 IR +1.4 VPD - 0.09OZ+40.7	0.492	0.401	0.30	<0.001
UK/ 1	0.2814	-1.7T -0.006 IR + VPD - 0.6OZ+221.7	<0.001	<0.001	0.98	<0.001

Table B.25: Linear total anthocyanins model and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	Total anthocyanins mg/100 g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.0463	-1.009T -1.3 IR +37.9 VPD + 0.1OZ+52.7	0.004562	<0.001	<0.001	0.149885
SP1/ 2	0.0465	-1.2T -0.8 IR +36.0 VPD +0.2OZ+37.9	0.016107	<0.001	0.010	0.145767
SP1/ 1&2	0.0543	-0.9T -0.9 IR +29.9 VPD - 0.9OZ+47.6	<0.001	<0.001	<0.001	0.007870
SP2/ 1	0.0931	-0.3T -1.4 IR +21.2 VPD +0.05OZ+56.7	0.318	<0.001	<0.001	0.343
SP2/ 2	0.0381	-0.4T -0.5 IR + 36.2 VPD - 0.006OZ+36.2	0.19260	0.0054	0.27	0.95149
SP2/ 3	0.0348	-0.08T +0.2IR -3.2 VPD - 0.1OZ+41.5	<0.001	0.4067	0.42	0.00104
SP2/ 1,2&3	0.0282	+0.3 T -0.6 IR +5.8 VPD -0.06 OZ+43.5	0.00407	0.0306 0	<0.001	0.01286
UK/ 1	0.3004	-0.3T +1. 9 IR -5.2 VPD +0.7 OZ- 6.9426	0.64734	0.8747 4	<0.001	0.00219

Table B.26: Linear total anthocyanins model and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	Total anthocyanins mg/100 g F.W.	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.1212	-0.7T +0.3 IR -27.2 VPD + 0. 4 OZ+33.6	0.066514	<0.001	0.27	<0.001
SP1/ 2	0.0817	+5.0T -3.0 IR + 6.1 VPD -1.4 OZ+ 114.0	<0.001	0.418636	0.0062	<0.001
SP1/ 1&2	0.0272	-0.7T -0.7 IR +13.3 VPD +0.2 OZ+43.6	0.015169	0.003108	<0.001	<0.001
SP2/ 1	0.0946	-1.8T -0.4 IR +13.2 VPD +0.1 OZ+59.4	<0.001	0.0383	0.081	0.0665
SP2/ 2	0.0380	-0.4T -0.5IR +36.2VPD -0.006 OZ+41.2	0.19260	0.0055	0.27	0.95149
SP2/ 3	0.0947	+0.4 T + 0.2 IR -45.2 VPD +0.06 OZ+40.8	0.0719	<0.001	0.45	0.5095
SP2/ 1,2&3	0.0139	-0.2T + 0.2 IR +1.7 VPD +0.009 OZ+ 41.4	0.0858	0.6152	0.038	0.7556
UK/ 1	0.3052	-0.7T +1.07 IR + VPD -0.1 OZ +12.0933	0.561	0.444	0.43	0.836

Table B.27: Linear % dry bruised fruits model, on arrival day and significance level of environmental variables 2 days prior to harvest.

Site/ Year	R^2	% Dry bruised fruits on arrival	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.6968	4.7T -2.2IR +82.7 VPD -0.5 OZ-62.9	<0.001	<0.001	0.0013	<0.001
SP1/ 2	0.5061	6.2 T + 2.7 IR -115.3 VPD + 1.0 OZ - 144.8	0.003651	<0.001	0.0062	<0.001
SP1/ 1&2	0.2654	3.7T + 0.8 IR +1.5 VPD -0.1 OZ-50. 2	<0.001	0.914195	0.16	0.220682
SP2/ 1	0.3247	5.0T -0.1 IR -30.4 VPD +0.2OZ-37.1	<0.001	<0.001	0.73	0.012589
SP2/ 2	0.3041	9.3 T +8.1 IR -258.34 VPD - 0.03 OZ-119.9	0.004588	0.006721	0.017	0.954281
SP2/ 3	0.3576	2.1T +1.3 IR -25.2 VPD -0.19893 OZ-13.9	<0.001	<0.001	0.0056	0.024430
SP2/ 1,2&3	0.3781	5.3 T -0.1 IR -28.9 VPD -0.3 OZ-43.2	<0.001	0.00107	0.71	0.00243
UK/ 1	0.1213	-0.6 T +0.7 IR +49.0 VPD +0.04 OZ-0.5	0.401	0.410	0.21	0.863

Table B.28: Linear % dry bruised fruits model, on arrival day and significance level of environmental variables 7 days prior to harvest.

Site/ Year	R^2	% Dry bruised fruits on arrival	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.6163	1.9T -1.1 IR + 70.4 VPD + 0.3OZ- 24.2	0.060629	<0.001	0.26	0.152678
SP1/ 2	0.5007	5.1 T + 2.7 IR -149.0 VPD + 0.5258 OZ- 84.2	<0.001	<0.001	0.0016	0.22269
SP1/ 1&2	0.4458	2.9 T + 0.3 IR + 22. 6 VPD - 0.3 OZ- 43.3	<0.001	0.059682	0.59	0.031890
SP2/ 1	0.3392	3.0T + 0.9 IR -13.3 VPD +0.3 OZ- 42.5	<0.001	0.24769	0.099	0.00381
SP2/ 2	0.3288	2.4T +0.4 IR +38.2 VPD -1.9 OZ- 72.5	0.106297	0.525297	0.88	<0.001
SP2/ 3	0.3313	3.1 T -0.8 IR -14.2 VPD +0.01 OZ- 5.5	<0.001	0.324	0.35	0.922
SP2/ 1,2&3	0.3832	3.3 T + 1.0IR -19.5 VPD+ 0.5 OZ- 50.2	<0.001	0.167404	0.15	<0.001
UK/ 1	0.09383	-0.2T +1.7 IR -52.3 VPD +0.6 OZ- 14.6	0.8922	0.3859	0.030	0.1141

Table B.29: Linear % dry bruised fruits model, on arrival day and significance level of environmental variables 21 days prior to harvest.

Site/ Year	R^2	% Dry bruised fruits on arrival	Temperature	VPD	Irradiation	Ozone
SP1/ 1	0.7239	3.1T + 0.3 IR -5.2 VPD +1.0OZ-67.0	<0.001	0.769760	0.54	<0.001
SP1/ 2	0.4918	2.5 T -2.9 IR -18.5 VPD +4.0 OZ-243.0	0.478	0.351	0.31	<0.001
SP1/ 1&2	0.463	3.1 T -0.1 IR +32.6 VPD -0.5 OZ-54.0	<0.001	0.012001	0.77	<0.001
SP2/ 1	0.2978	4.3 T +1.0 IR -27.8 VPD -0.008 OZ-31.0	<0.001	0.0664	0.082	0.9539
SP2/ 2	0.3755	-7.8 T -0.6IR +5.2 VPD +6.2 OZ-278.1	0.19964	0.84788	0.93	0.00195
SP2/ 3	0.3165	2.1 T + 0.5 IR + 65.2 VPD -0.6 OZ-11.6	0.0101	0.1256	0.62	0.0946
SP2/ 1,2&3	0.3401	4.8 T +0.8 IR -30.8 VPD -0. 2 OZ-41.5	<0.001	0.0921	0.22	0.2481
UK/ 1	0.1209	-4.0 T + 0.8 IR + 116.1 VPD -0.3401 OZ+ 38.2	0.0725	0.5487	0.75	0.7760

Table B.30: Ratio of Sucrose /Fuctose and Sucrose/Glucose as expressed on dry (DW) and fresh weight basis (FW), and Dry matter%, of strawberry fruits over sites and harvest seasons.

Site	Year	Dry Matter %	Sucrose/ fructose	Sucrose/ glucose	Dry Matter%
SP1	1	FW	0.80	0.95	11.8
		DW	0.81	0.96	
SP1	2	FW	0.43	0.59	11.3
		DW	0.44	0.60	
SP1	1,2*	FW	0.63	0.79	11.6
		DW	0.63	0.79	
SP2	1	FW	1.02	1.13	11.3
		DW	1.03	1.13	
SP2	2	FW	0.43	0.59	11.9
		DW	0.44	0.60	
SP2	3	FW	0.67	0.81	11.5
		DW	0.67	0.81	
SP2	1,2,3*	FW	0.71	0.86	11.6
		DW	0.71	0.87	
UK	1	FW	0.76	0.84	11.5
		DW	0.76	0.84	

* Mean across years

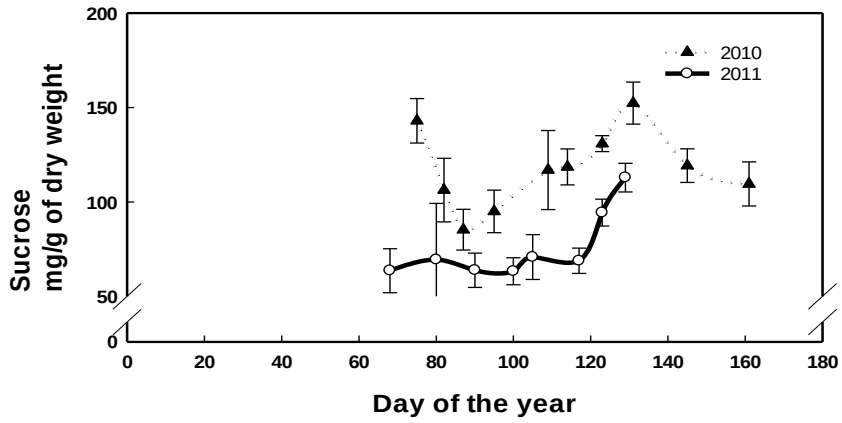

Figure B-1 Sucrose concentration expressed each sampling week over years 2010, 2011 of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP1.

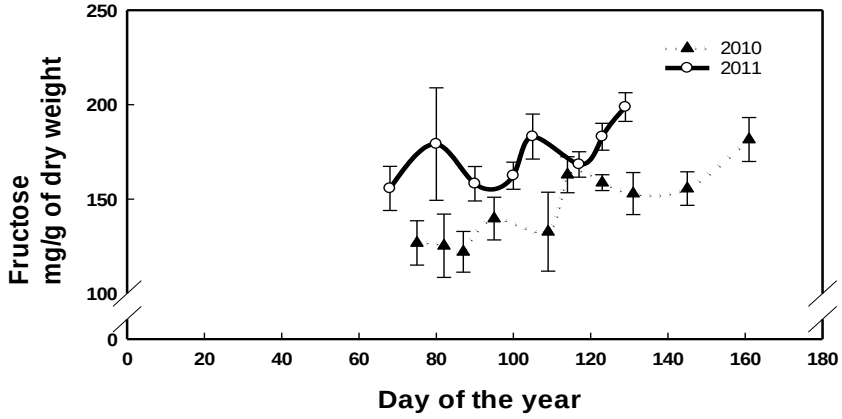

Figure B-2 Fructose concentration expressed each sampling week over years 2010, 2011 of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP1.

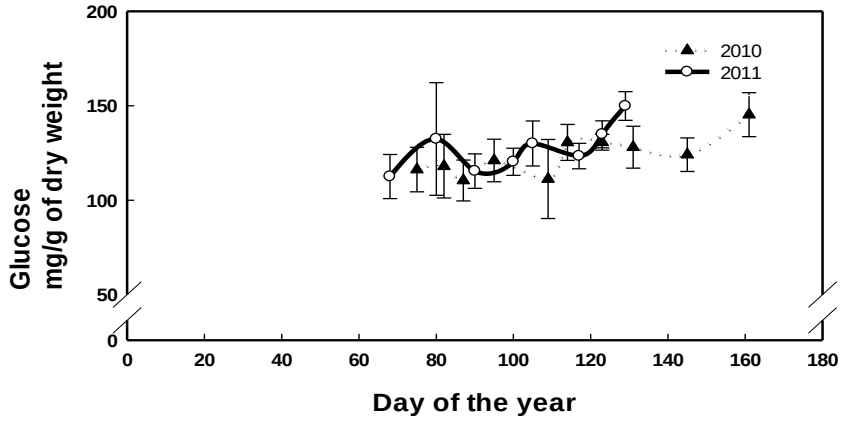

Figure B-3 Glucose concentration expressed each sampling week over years 2010, 2011 of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP1.

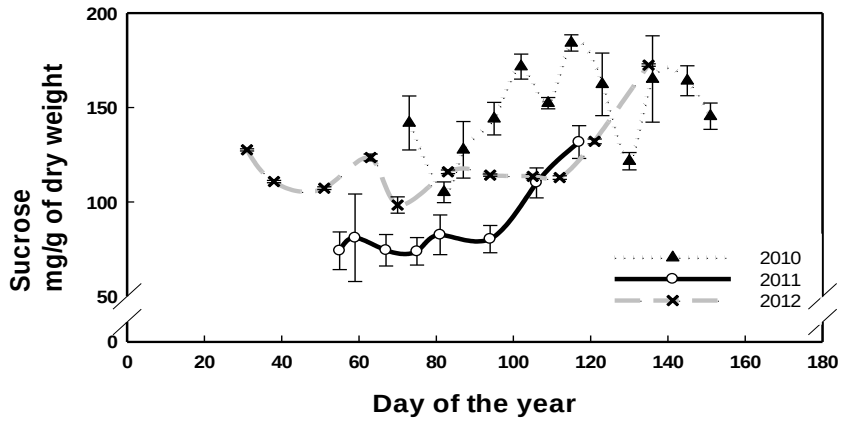

Figure B-4 Sucrose concentration expressed each sampling week over three years (2010,2011 and 2012) of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP2.

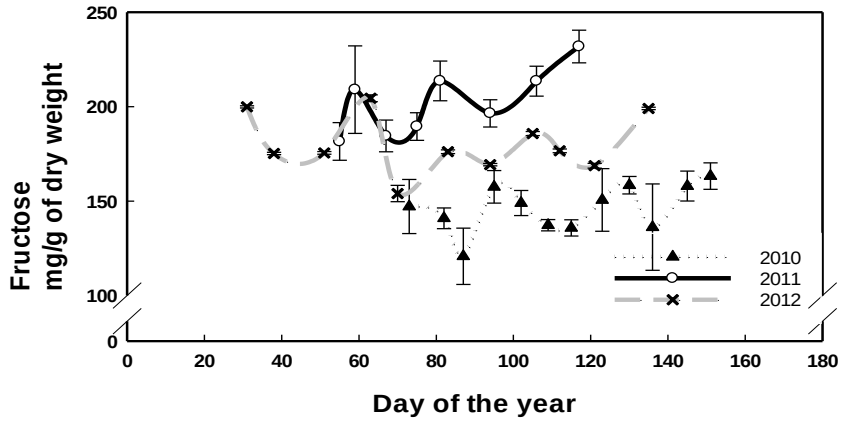

Figure B-5 Fructose concentration expressed each sampling week over three years (2010,2011 and 2012) of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP2.

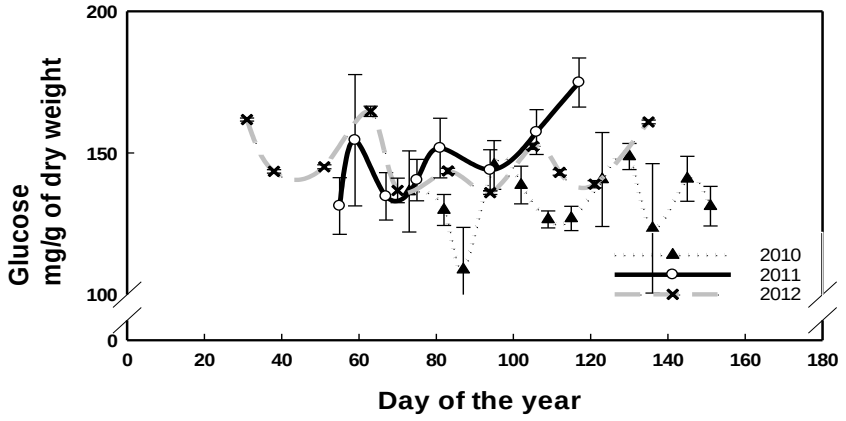

Figure B-6 Glucose concentration expressed each sampling week over three years (2010,2011 and 2012) of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP2.

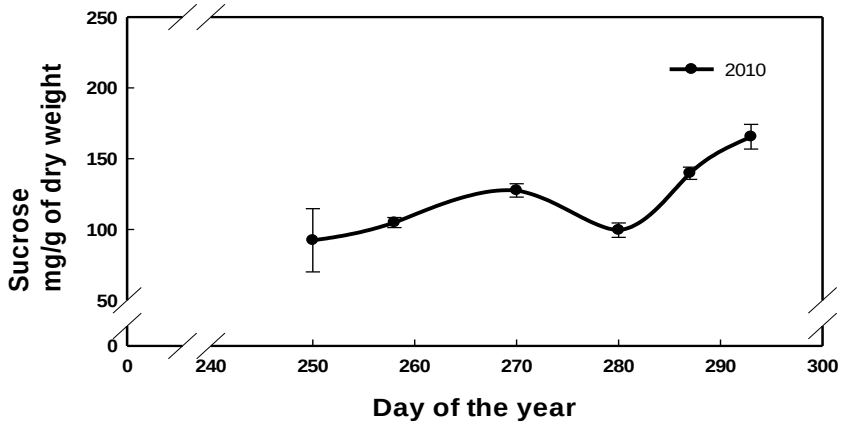

Figure B-7 Sucrose concentration expressed each sampling week over year 2010of cv Elsinore fruits (n=9 for each sampling week) for the UK site.

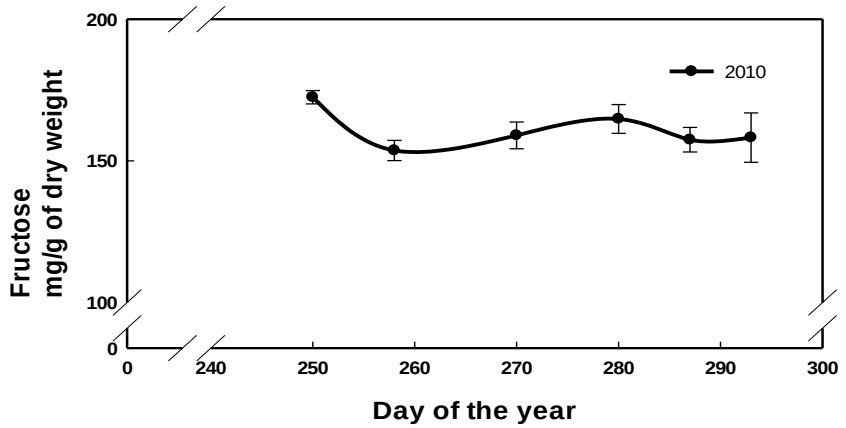

Figure B-8 Fructose concentration expressed each sampling week over year 2010of cv Elsinore fruits (n=9 for each sampling week) for the UK site.

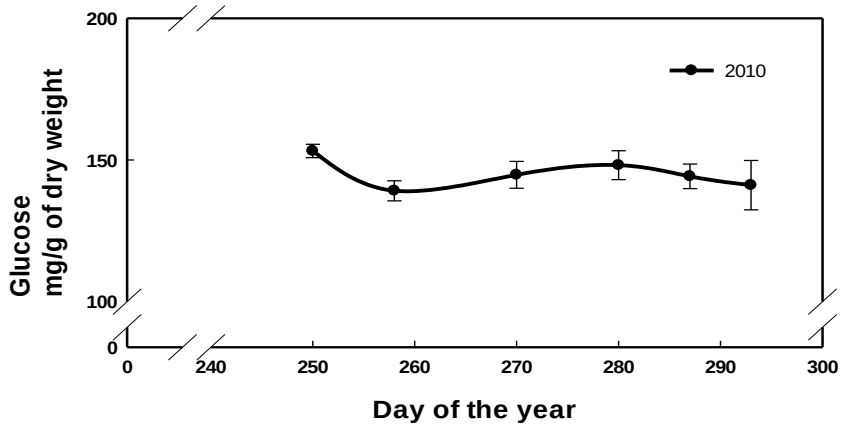

Figure B-9 Glucose concentration expressed each sampling week over year 2010of cv Elsinore fruits (n=9 for each sampling week) for the UK site.

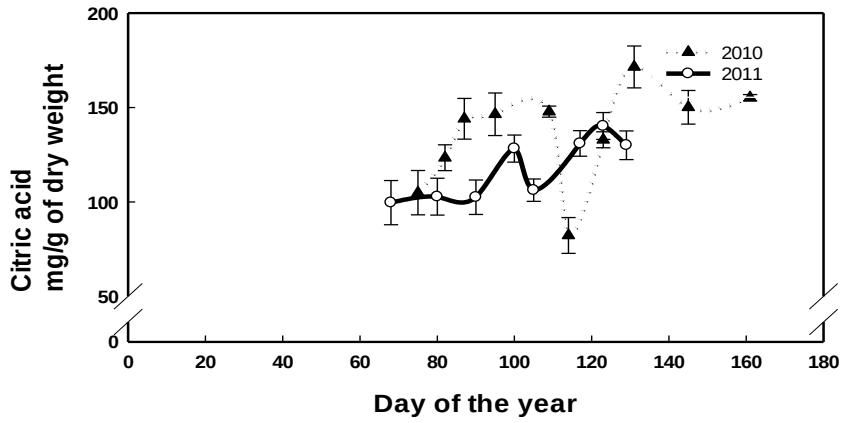

Figure B-10 Citric acid concentration expressed each sampling week over years 2010 and 2011 of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP1.

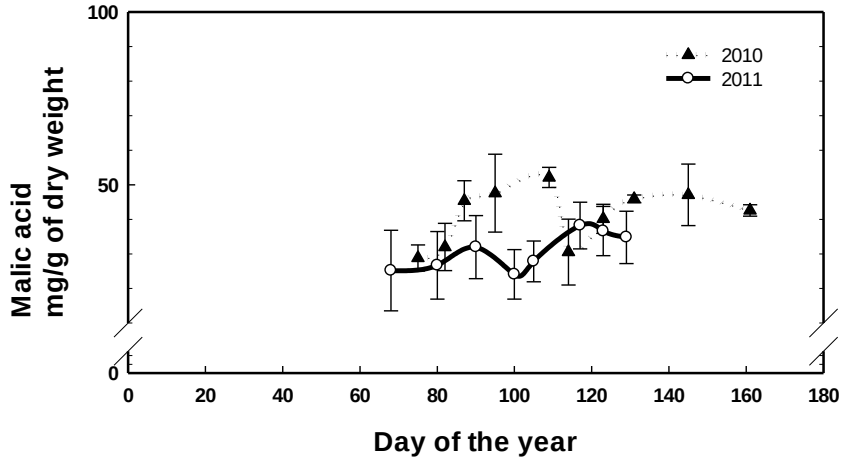

Figure B-11 Malic acid concentration expressed each sampling week over years 2010 and 2011 of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP1.

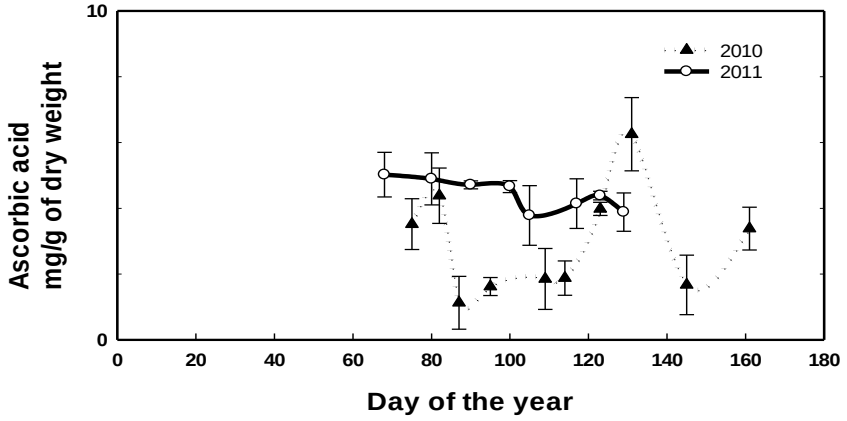

Figure B-12 Ascorbic acid concentration expressed each sampling week over years 2010 and 2011 of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP1.

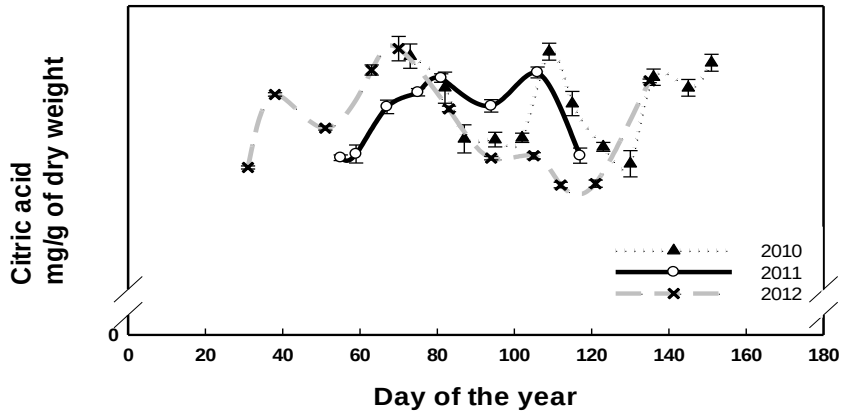

Figure B-13 Citric acid concentration expressed each sampling week over three years (2010,2011 and 2012) of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP2.

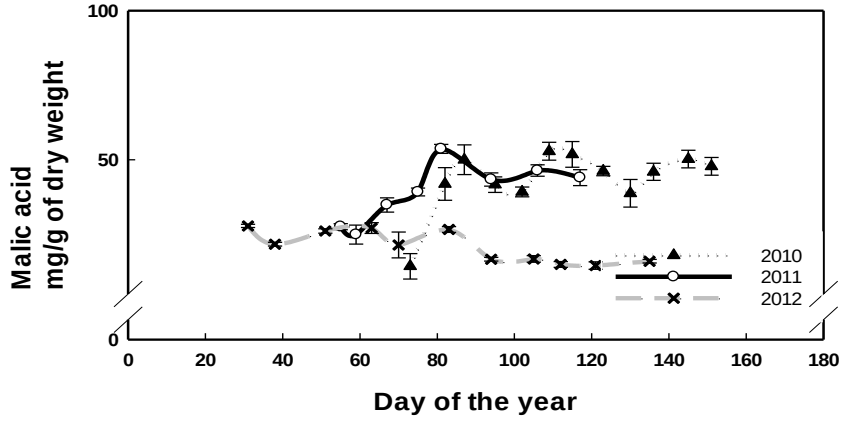

Figure B-14 Malic acid concentration expressed each sampling week over three years (2010,2011 and 2012) of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP2.

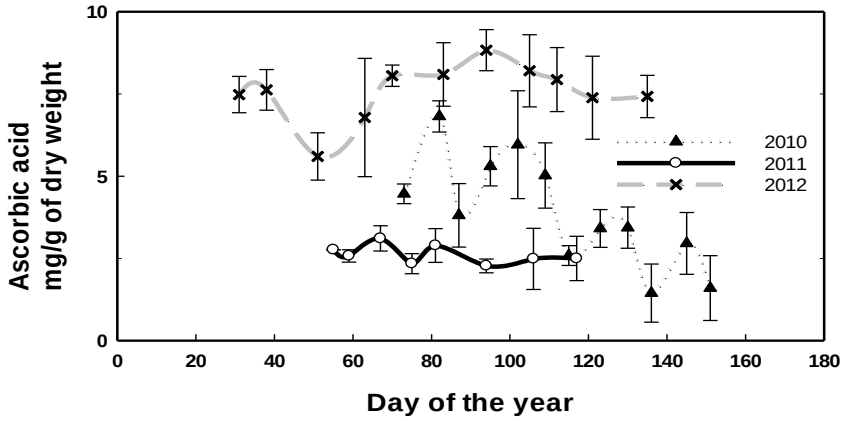

Figure B-15 Ascorbic acid concentration expressed each sampling week over three years (2010,2011 and 2012) of cv Candonga fruits (n=9 for each sampling week) for Spanish site SP2.

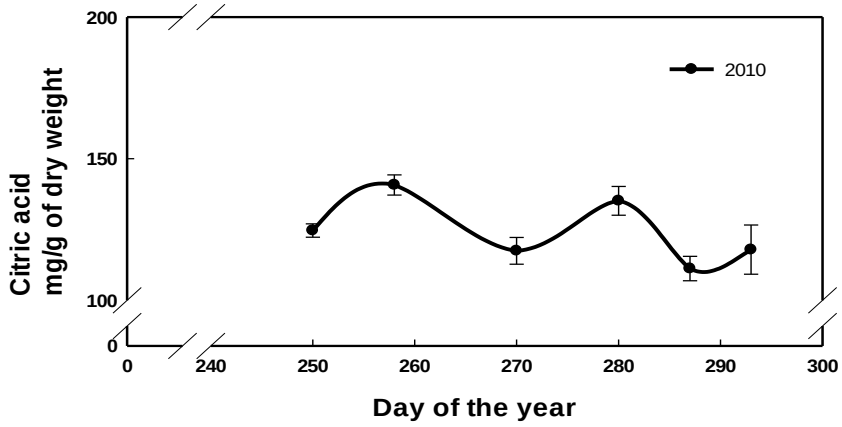

Figure B-16 Citric acid concentration expressed each sampling week for 2010 of cv Elsinore fruits (n=9 for each sampling week) for UK site.

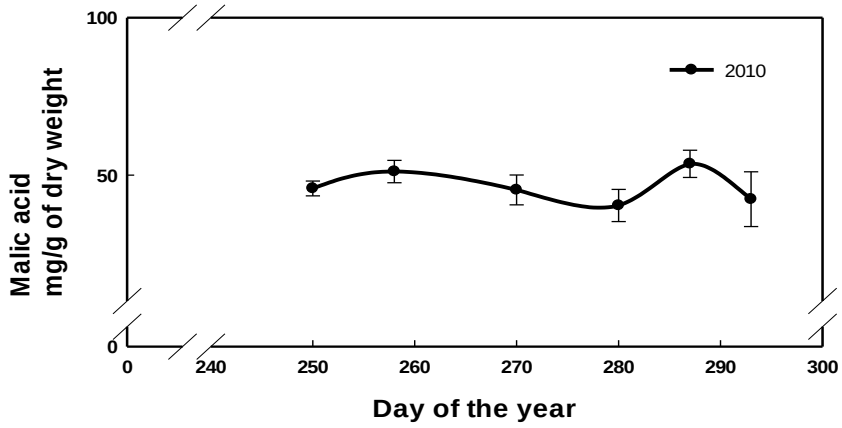

Figure B-17 Malic acid concentration expressed each sampling week for 2010 of cv Elsinore fruits (n=9 for each sampling week) for UK site.

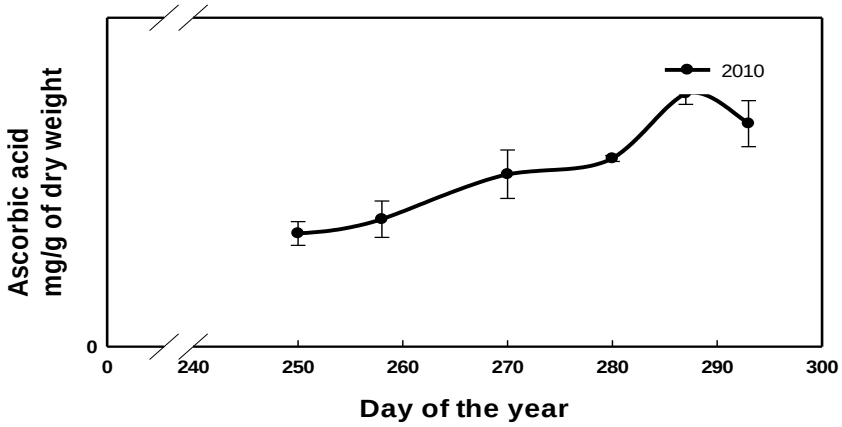

Figure B-18 Ascorbic acid concentration expressed each sampling week for 2010 of cv Elsinore fruits (n=9 for each sampling week) for UK site.

Appendix C

Pyrotis, S., Abayomi, L., Rees, D. Whitfield, C. and Orchard, J. Evaluating short term effects of temperature and light on strawberry 'ELSINORE®' firmness. (2012). Poster in 2nd Symposium on Horticulture in Europe

Evaluating Short Term Effects of Temperature and Light on Strawberry 'ELSINORE®' Firmness

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UNIVERSITY of GREENWICH

Natural Resources Institute

Objectives

Firmness is an important attribute of strawberries affecting consumer acceptance as well as postharvest quality. The effect of light and increased temperature on strawberry fruit firmness was evaluated.

Materials & methods

Strawberry plants of cv. Elsinore® were grown at a commercial farm. At the fruiting stage plants were transferred into growth cabinets where they were subjected to alternating 16 hour day / 8 hour night cycles, and to 12 hour low (20°C)/12 hour high (28 °C) temperature cycles, in succession. Three harvest days took place over a period of four weeks. For each harvest day, three fruit picks were undertaken, with a time interval of 4 hours between picks.
The conditions over the 4 hours immediately prior to harvest were: darkness and low temperature for the first pick; light and low temperature for the second pick; light and high temperature for the third pick (Fig. 1).

Results

Firmness of strawberry fruits was found to be significantly ($P<0.05$) different at the different pickings.
Fruits harvested under dark and low temperature conditions were found to be the most firm, followed by the fruits harvested after 4 hours of light and low temperature. Where picking followed an additional four hour period of light and increased temperature the fruits were least firm (Fig. 2).

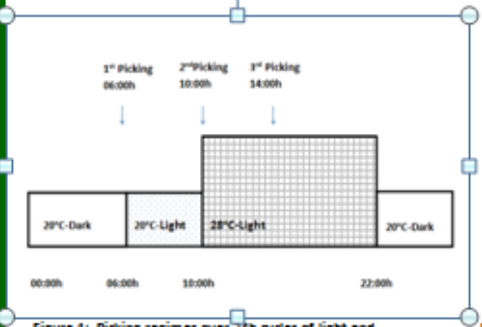

Figure 1: Picking regimes over 24h cycles of light and temperature in growth cabinets.

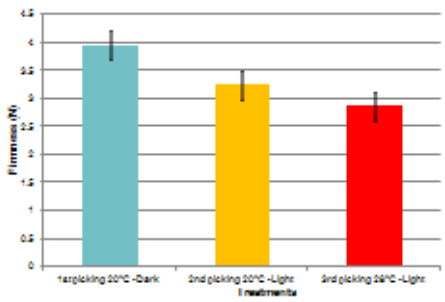

Figure 2: Postharvest fruit firmness of cv. 'Elsinore®' measured in response to three different treatments. $P<0.05$. n=14. s.e.d 0.51. LSD = 0.58.

Discussion

Loss of strawberry fruit firmness is associated with changes in cell turgor and pectolytic enzymatic activity. These factors are influenced by pre-harvest and post-harvest environmental conditions such as light and temperature. The short-term impact of temperature and light on fruit firmness were demonstrated.

Benefit to industry

These results demonstrate the importance of environmental management which could be incorporated into development of decision making tools to support marketing strategies and minimize post-harvest waste.

Project Partners: Natural Resources Institute, University of Greenwich, UK; Centro Innovazione Varietale, Italy

Pyrotis, S., Abayomi, L., Rees, D., Orchard, J. (2010) Effect of temperature and humidity on strawberry firmness at two different sites in the Huelva Region of Spain. In *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on* ***926***. 567–570.

Effect of Temperature and Humidity on Strawberry Firmness at two different Sites in the Huelva Region of Spain

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Abstract

The effects of ambient humidity and temperature for polytunnel grown strawberries (*Fragaria x ananassa* 'Candonga') and its impact on postharvest firmness were evaluated between March and June 2010 on two commercial sites in the Huelva region of Spain. A significant (r=0.87, P<0.05) negative impact of growing temperature on firmness was observed. Increased temperatures of 1ºC were found to decrease strawberry firmness by 0.5N and 0.7N at each site. However, humidity levels greater than 75%, caused by high levels of precipitation reversed that trend. Mean temperatures recorded during growth and development were between 11ºC-21ºC. Humidity varied between 56-81% and 60%-86%, firmness was in the range of 3.2N-9.9N and 5.4N-10.4N for Lepe and Moguer growing sites respectively.

Keywords: Candonga, postharvest quality, preharvest conditions, climate change

INTRODUCTION

Strawberries are one of the most popular berry fruits. Global production as well as in the UK has increased over recent years (FAO, 2010). This fact is not only owing their distinct flavor and aroma, but also because of their nutritional value (Torronen and Maata, 2002). The cultivar (cv.) Candonga is an important Spanish export strawberry to the UK. One of factors detrimental to strawberry quality is lack of firmness. Levels of UV radiation (Ordidge et al., 2008), humidity (Lieten, 2002), carbon dioxide (Harker, 2000), including cultural practices, such as fertilization (per. comm., Vogels, R., 2010) as well as postharvest conditions (Nunes et al. 2003; Shafieea et al. 2010) were found to influence strawberry firmness. Firmness affects both the textural properties as perceived when eating the fruit, including its susceptibility to bruising during harvesting and subsequent marketing. Efforts have been made both in understanding and manipulating this aspect of strawberry physiology (Burkhurt, 1943; Manning, 1998).

There are other factors associated with fruit firmness some of which include enzyme activity, namely pectate lyase (Jimenez-Bermudez, 2002; Figueroa et al., 2008), polygalacturonase and pectinmethilesterase, found to play an important role in deterioration of strawberry firmness (Fraeye, 2009). A three-year study is presently underway to gain better understanding of the effects of preharvest weather conditions and their extremes on strawberry (cv.) Candonga postharvest quality. Preliminary results are herein presented.

MATERIALS AND METHODS

Strawberry fruits (cv. Candonga) were grown using standard polytunnel practices on two commercial production sites, Lepe and Moguer in Huelva, Spain. Levels of precipitation (mm/day), mean temperature (˚C) and average humidity (%RH) were recorded from two weather stations ~3Km from the production sites, at the fields and within polytunnels (using Tinytag Plus and Tinytalk data loggers) at Lepe and Moguer. Ripe fruit were harvested, packed into 400g punnets and cooled to remove field heat. Fruit was then transported to the UK by road over 2 days at +3ºC and subsequently stored under standard packhouse conditions at +3ºC. Randomised samples were taken on average on a weekly basis from mid-march to mid-June 2010 from each production site. Fruits were sampled from 3 boxes per pallet (85 boxes per pallet), with 3 punnets taken from each box (12 punnets per box) for further sub-sampling. At each sampling interval, fruit firmness (N) was analysed using a hand-held penetrometer (Bishop Instruments Ltd) with an 8mm diameter probe. Strawberry fruit firmness was examined for whole fruits (n=27) on one side of each fruit. Fruit were sampled in a completely randomised design. Data were subjected to ANOVA using Genstat 13th edition (VSN International Ltd., UK). Least significant difference values (LSD; P = 0.05) were calculated for mean separation using critical values of t for two-tailed tests. Pearson's correlation was used to measure relationships between variables. All measurements were made in triplicate.

RESULTS

Mean temperature 7 days before harvest was found to have a significant ($r=0.87$, $P<0.05$) impact on firmness at both growing sites approximately 30Km apart (Fig. 1). Recorded data from weather stations differed by *ca*. 3.8°C compared with polytunnel temperature which was warmer. Similarly, mean relative humidity recorded at weather stations were on average 1% more humid for polytunnels at Moguer and 0.7% less humid for polytunnels at Lepe site. Generally, firmness levels of below 4N for cv. Candonga are undesirable. An average decrease in firmness of about 0.7N and 0.5N per increase in +1°C was observed for Lepe and Moguer sites respectively. The only exception to this trend was noted when elevated levels of precipitation (data not shown), accompanied by high mean relative humidity (RH) levels (Lepe site, 77% RH and Moguer site, 82% RH) were recorded. The average humidity that was recorded over sampling weeks was 67.6% for Lepe and 71.5% for Moguer sites. During the period of high humidity (>75% RH) at both sites, there was an increase in firmness of 0.48N and 1.1N for Moguer and Lepe grown fruit respectively despite the simultaneous temperature increase of 0.8 °C.

DISCUSSION

With few exceptions, fruits and vegetables generally display higher levels of firmness when grown and stored at lower temperatures (Bourne, 1982). Firmness values reported by others on other varieties include cv. Sweet Charlie' 3.5N-7N depending whether fruit was wrapped or not and using a

3mm probe (Nunes et al., 1995) and 2.1N-4.5 for cv. Chandler using a 5mm probe (Figueroa et al., 2009). Constant high humidity levels (>75%) were suggested to have a negative impact (reduction) on strawberry firmness grown in glasshouses at day temperatures of 22-28°C (Lieten, 2002). Fruit firmness is a complex phenomenon that is connected to several actions and its mechanism is not yet fully understood. Numerous factors may contribute to the loss of firmness at elevated growing temperatures. These have been shown to include enzyme activity and loss of cell turgor (Van Dijk, 2006). The combinations of different weather variables (temperature, humidity, CO_2 and solar radiation) will also have different effects (e.g. photosynthetic rate, evapotranspiration and metabolism of plant nutrients) on strawberry plants, resulting in different levels of firmness. Already, the impact of climate change on strawberry production is being studied in Huelva (Palencia et al. 2009) given the importance attached to this crop, with Spain being the second largest producer globally after the US. The threshold beyond which the quality of this economically important fruit is either positively or negatively affected at particular stages of plant growth in response to growing environment is still under investigation. The associated physiological responses (enzyme activity, biochemical profiles and expression of firmness related genes) during key growing conditions will subsequently be reported upon. Both consumers and producers would benefit from these findings as improved agronomic strategies and postharvest technology for extending shelf-life/maintaining quality could be adopted.

ACKNOWLEDGEMENTS

The authors would like to thank Rodanto Ltd. especially Mr Francisco Gonzalez, UK, including Frutas el Pinar S.L., Spain, Agromartin S.L., Spain for their contribution to the project through supply of strawberry fruit.

Literature cited

Bourne, M.C.1982. Effect of Temperature on Firmness of Raw Fruits and Vegetables. Journal of Food Science. 47(2): 440–444.

Burkhurt, L.N.D.1943. Firmness of strawberries as measured by a penetrometer. http://www.plantphysiol.org/cgi/reprint/18/4/693.pdf

Figueroa, C.R., Pimentel, P., Gaete-Eastman, C., Moya, M., Herrera, R., Caligari, P.D.S . and Moya-Leóna,M.A.2008. Softening rate of the Chilean strawberry (*Fragaria chiloensis*) fruit reflects the expression of polygalacturonase and pectate lyase genes. Postharvest Biology and Technology.49(2):210- 220.

Figueroa, C.R., Pimentel, P., Dottob, M.C., Civello, P.M., Gustavo A. Martínez, G.A., Herreraa, R. and Moya-Leóna,M.A. 2009. Expression of five expansin genes during softening of *Fragaria chiloensis* fruit: Effect of auxin treatment. Postharvest Biology and Technology. 53:51–57.

Food and Agricultural Organization. 2010. www.fao.org

Fraeye, I., Knockaert, G., Van Buggenhout, S., Duvetter, T., Hendrickx, M. and Van Loey, A. 2009. Enzyme infusion and thermal processing of strawberries: Pectin conversions related to firmness evolution. Food Chemistry. 114(4): 1371-1379.

Harker, R.F., Elgar, J.H., Watkins, C.B., Jackson, P.J. and Hallett, I.C.2000. Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. Postharvest Biology and Technology. 19(2):139-146.

Jimenez-Bermudez, S., Redondo-Nevado, J., Muñoz-Blanco, J., Caballero, J. L., López-Aranda, J. M., Valpuesta, V., Pliego-Alfaro, F., Quesada, M. A. and Mercado, J. A. 2002. Manipulation of strawberry fruit softening by antisense expression of a pectate lyase gene. Plant Physiology. 128: 751-759.

Lieten, P. 2002. The effect of humidity on the performance of greenhouse grown strawberry. Acta Hort. 567: 479-482.

Manning, K. 1998. Isolation of a set of ripening – related genes from strawberry: their identification and possible relationship to fruit quality traits. Planta. 205: 622-631.

Nunes, M.C.N., Emond, J.P. and Brecht, J.K. 2003. Quality of strawberries as affected by temperature abuse during ground, in-flight and retail handling operations. Acta Hort. 604: 239-246.

Ordidge, M. García-Macías, P., Vysini, E., Battey, N. H., Gordon, M.H., Hadley, P., John, P., Lovegrove, J.L. and Wagstaffe, A. 2008. Production and nutritional assessment of phytochemical-rich soft fruit and lettuce. Final Report. Workpackage No 3. August 2008. Development of surface colour and firmness of strawberry crops are UV light sensitive, but colour is not a good predictor of several quality parameters. University of Reading. 26-47.

Palencia, P., Martínez, F., Medina, J. J., Vázquez, E., Flores, F. and López-Medina, J. (2009). Effects of climate change on strawberry production. Acta Hort.838: 51-54.

Shafieea, M., Taghavi, T.S. and Babalar, M. 2010. Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry. Scientia horticulturae.124(1):40-45.

Törrönen, R. and Määttä, K. 2002. Bioactive substances and health benefits of strawberries. IV International Strawberry Symposium. Acta Hort .567:797-803.

Van Dijk, C., Boeriu, C., Peter, F., Stolle-Smits, T and Tijskens L.M.M.2006. The firmness of stored tomatoes (cv. Tradiro). 1. Kinetic and near infrared models to describe firmness and moisture loss. Journal of Food Engineering. 77(3), 575-584.

Vicentea, A.R., Costaa, M.L., Martínezb, G.A., Chavesa A.R. and Civello, P.M. 2005. Effect of heat treatments on cell wall degradation and softening in strawberry fruit. Postharvest biology and technology. 38(3): 213-222.

Figures

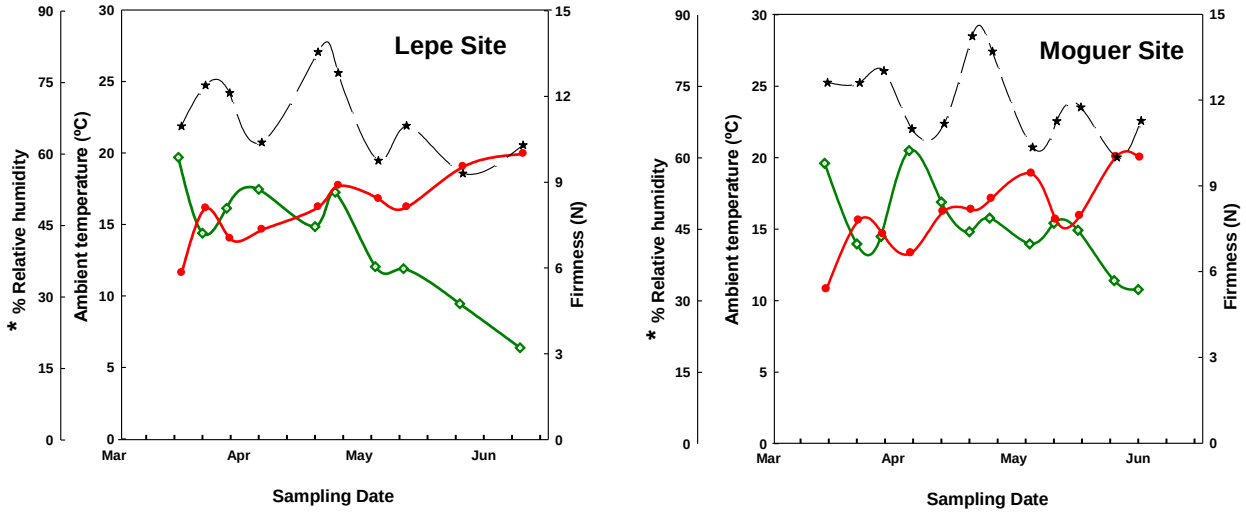

Figure 1: Postharvest fruit firmness measured 3 days after harvest (◊) in response to ambient growing temperatures (•) and humidity (*) for strawberry cv. 'Candonga 'on two commercial sites during March to June 2010. Data represents mean temperature and humidity conditions 7 days before fruit harvest.

LSD firmness-Lepe site = 1.0, P<0.001; Moguer site =1.0, P<0.001, n=27 replicates.

PLEASE DO NOT PARTICIPATE IF YOU HAVE ANY ALLERGY OR ANY OTHER PROBLEMS ASSOCIATED WITH STRAWBERRY CONSUMPTION OR IF YOU HAVE NEVER EATEN STRAWBERRIES BEFORE!!

Taste panel on strawberry fruits

You are invited to participate on a study about consumer's acceptability of strawberry fruits. The taste panel takes place as a part of a PhD/ MPhil project '**Evaluating the impact of climate variability on postharvest quality of strawberries'.** The project is taking place at Natural Resources Institute and it is founded by University of Greenwich. You will be asked to complete a consent form. After this you will be invited to taste strawberry fruits and evaluate-discuss their quality characteristics. You can ask the researcher questions at any time.

While we hope you will participate you are under no obligation to. If you choose to participate you are free to withdraw at any time and do not give any reason.

Your help is much appreciated.

Kind regards

In case that you problems associated with strawberry consumption please contact supervisor of the project.

Dr. Louise Abayomi l.abayomi@gre.ac.uk Work +44 (0)1634 88 3734

Description of the sensory terms

Sensory attribute	Definition
Big size	Size that is above normal
Red colour	Red colour intensity at the wider part of strawberry fruit (shoulder)
White colour	White colour intensity at the top of the fruit near leaves
Shiny surface	Ability of fruit to reflect light
Uniformity of colour	Even distribution of colour on the whole fruit
Bruised	Presence of colour changes on fruit surface
Irregular shape	Presence of miss formed areas on the fruit
Firm (hand)	Fruit requires high pressure to be squeezed
Firm texture (mouth)	Compact texture of fruit when eaten
Juicy texture	Fruit with high amount of juice when eaten
Strong strawberry aroma	Characteristic smell of strawberries
Sweet taste	Taste of sugar
Acidic taste	Taste of acid
Strong fruity taste	Characteristic taste of strawberries
Fermented taste	Characteristic taste of alcohol
Ripened	Overall perception of ripeness