VARIABILITY OF HEALTH AND TASTE PROMOTING COMPOUNDS IN STRAWBERRY (*FRAGARIA X ANANASSA*) FRUITS

A dissertation submitted to

ETH Zurich

for the degree of

Doctor of Sciences

presented by

PAMELA VIRGINIE ANN SARAH CRESPO

Dipl. Lebensmittel-Ing., ETH Zurich

born February 24\textsuperscript{th}, 1976

citizen of Corsier (GE)

Accepted on the recommendation of

Prof. Dr. Peter Stamp, examiner
Prof. Dr. Laura Nyström, co-examiner
Dr. Christoph Carlen, co-examiner

2010
Table of contents

ACKNOWLEDGMENTS................................................................. 5

ABBREVIATIONS ................................................................. 6

SUMMARY .................................................................................. 7

RÉSUMÉ .................................................................................... 9

CHAPTER 1 .................................................................................. 11

General introduction........................................................................ 11
1 Strawberry production and consumption ........................................ 12
2 Botany of the strawberry plant and fruit development...................... 12
3 Fruit quality................................................................................ 14
  3.1 Fruit composition affecting strawberry taste .................................. 14
  3.2 Strawberries as a source of health promoting compounds............... 15
4 Factors influencing fruit quality......................................................... 16
5 Objectives and structure of the thesis.................................................. 17

CHAPTER 2 .................................................................................. 18

Genotype stability of health and taste promoting compounds............... 18
1 Introduction................................................................................ 19
2 Material and methods..................................................................... 20
  2.1 Plant material and trial environments........................................... 20
  2.2 Harvest and sampling................................................................. 23
  2.3 Soluble solids content and titratable acidity.................................. 23
  2.4 Sample preparation for phytochemical analysis and determination of the dry matter...... 24
  2.5 Extraction and quantification of the antioxidant capacity, total phenolic compounds and total anthocyanin contents ................................................................. 24
  2.6 Analysis of individual anthocyanins.............................................. 25
  2.7 Extraction and HPLC determination of total vitamin C...................... 26
  2.8 Validity of the analytical methods................................................ 26
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9</td>
<td>Statistical methods</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>Results</td>
<td>27</td>
</tr>
<tr>
<td>3.1</td>
<td>Genotype and environment effects and their interactions on agronomic and outer quality traits</td>
<td>27</td>
</tr>
<tr>
<td>3.2</td>
<td>Variation in fruit inner quality parameter</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Discussion</td>
<td>33</td>
</tr>
<tr>
<td>4.1</td>
<td>Agronomic aspects</td>
<td>33</td>
</tr>
<tr>
<td>4.2</td>
<td>Inner quality traits: taste related attributes</td>
<td>34</td>
</tr>
<tr>
<td>4.3</td>
<td>Inner quality traits: health related compounds</td>
<td>35</td>
</tr>
<tr>
<td>4.4</td>
<td>Cultivar rating</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>Conclusions</td>
<td>38</td>
</tr>
</tbody>
</table>

CHAPTER 3 ........................................................................................................................................................ 39

**Characterisation of major taste and health related compounds of four strawberry genotypes grown at two different Swiss production sites** ........................................................................................................ 39

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>Material and methods</td>
<td>41</td>
</tr>
<tr>
<td>2.1</td>
<td>Cultivation sites</td>
<td>41</td>
</tr>
<tr>
<td>2.2</td>
<td>Plant materials and cultivation practices</td>
<td>42</td>
</tr>
<tr>
<td>2.3</td>
<td>Fruit sampling</td>
<td>43</td>
</tr>
<tr>
<td>2.4</td>
<td>Analysis of sugars</td>
<td>43</td>
</tr>
<tr>
<td>2.5</td>
<td>Analysis of malic, citric and ascorbic acid</td>
<td>44</td>
</tr>
<tr>
<td>2.6</td>
<td>Analysis of total vitamin C</td>
<td>44</td>
</tr>
<tr>
<td>2.7</td>
<td>Analysis of individual anthocyanins</td>
<td>44</td>
</tr>
<tr>
<td>2.8</td>
<td>Extraction and quantification of total antioxidant capacity</td>
<td>45</td>
</tr>
<tr>
<td>2.9</td>
<td>Data analysis</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>Results and discussion</td>
<td>46</td>
</tr>
<tr>
<td>3.1</td>
<td>Variation among cultivars in taste and health related compounds</td>
<td>46</td>
</tr>
<tr>
<td>3.2</td>
<td>Effect of production sites on agronomical traits and fruit taste and health related compounds</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>Conclusions</td>
<td>55</td>
</tr>
</tbody>
</table>
Evolution of fruit quality during the harvest: influence of the fruit order and time from flowering to harvest

1 Introduction
2 Material and methods
   2.1 Plant material and sampling
   2.2 Analysis of taste and health related quality parameters
   2.3 Statistical methods
3 Results
   3.1 Plant to plant variability
   3.2 Weekly evolution of the fruit quality during the harvest
   3.3 Influence of the fruit order on taste and health related parameter
   3.4 Effects of the fruit development and ripening time on health related parameter
4 Discussion
   4.1 Variation in SSC and acidity during harvest
   4.2 Variation in anthocyanin contents
   4.3 Variation in antioxidant capacity
5 Conclusions

CHAPTER 5

General conclusions
1 Taste related compounds
2 Health related compounds

LITERATURE CITED
ACKNOWLEDGMENTS

This study was supported by the State Secretariat for Education and Research SER of Switzerland and the European Science Foundation (COST 863 Euroberry). All field experiments were conducted at the research station Agroscope Changins-Wädenswil ACW in Conthey, Switzerland.

I would like to thank Prof. Dr. Peter Stamp for his guiding advice and permanent support throughout my PhD. I am further grateful to my advisor Dr. Christoph Carlen for providing me the opportunity to do this PhD thesis, for sharing his knowledge, for the fruitful discussions and for his statistical advices. I would also like to thank Prof. Dr. Laura Nyström for offering her expertise as external co-examiner.

Thank you to Dr. Leon A. Terry for hosting me in his laboratory in Cranfield University and to Jordi Giné Bordonaba for his great support during and after my short term scientific mission in Cranfield.

Gratitude goes to my colleagues Dr. Cédric Camps and Dr. Danilo Christen for their valuable advices and their friendship throughout my PhD.

I am also grateful to my colleagues from Wädenswil, Dr. Anna Bozzi and her group for the very interesting and motivating discussions.

I would like to thank the team “Berries” of the research centre of Conthey in particular André Ançay for managing the field trials. Special thanks go to the harvest team: Dominique, Eliane, Loulou, Rita and Véronique, as well as to the interns and students who helped me in the lab: Alicia Blanc, Sarah Perren and Tiffany Géroudet.

Finally, my warmest gratitude goes to my parents for believing in me and for their support during my studies. Last but not least my dearest thanks go to Alexandre Délez for his interest in my work, his encouragements and his love, which immensely contributed to the success of this thesis.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Antioxidant Capacity</td>
</tr>
<tr>
<td>AsA</td>
<td>Ascorbic Acid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>Cya-3-gluc</td>
<td>Cyanidin-3-glucoside</td>
</tr>
<tr>
<td>DHAA</td>
<td>Dehydro Ascorbic Acid</td>
</tr>
<tr>
<td>DM</td>
<td>Dry Matter / dry weight</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-Diphenyl-1-picrylhydrazyl</td>
</tr>
<tr>
<td>FW</td>
<td>Fresh Weight</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic Acid Equivalent</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometer</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetic active radiation</td>
</tr>
<tr>
<td>Pg-3-gluc</td>
<td>Pelargonidin-3-glucoside</td>
</tr>
<tr>
<td>Pg-3-mal</td>
<td>Pelargonidin-3-malonylglucoside</td>
</tr>
<tr>
<td>Pg-3-rut</td>
<td>Pelargonidin-3-rutinoside</td>
</tr>
<tr>
<td>PPFD</td>
<td>Photosynthetic Photon Flux Density</td>
</tr>
<tr>
<td>RID</td>
<td>Refractive Index Detector</td>
</tr>
<tr>
<td>SSC</td>
<td>Soluble Solid Contents</td>
</tr>
<tr>
<td>TA</td>
<td>Titratable Acidity</td>
</tr>
<tr>
<td>TAC</td>
<td>Total Anthocyanin Content</td>
</tr>
<tr>
<td>TE</td>
<td>Trolox Equivalent</td>
</tr>
<tr>
<td>TPC</td>
<td>Total Phenolic Content</td>
</tr>
</tbody>
</table>
Strawberries are very popular in Switzerland where they are the fourth most consumed fruits after apples, oranges and bananas. Swiss production exclusively targets the local fresh market and relies on the high eating quality of the berries. Since recent studies have shown that a diet rich in antioxidant reduces risk of cancer and of cardiovascular diseases, strawberries can be considered as an important dietary source because of their high level of vitamin C, anthocyanins, ellagic acid and other antioxidant compounds. However, significant variations of quality parameters like sugars, acids, phenolic compounds and vitamin C have been observed and criticised during the last decade. The aim of this study was to evaluate the variability in health and taste quality traits in strawberry fruits by assessing several parameters during two years: influence of the genotype, role of the environment, harvest period, fruit position on the inflorescence and number of days from flowering to full ripeness on sugars, acids, phenolic compounds and vitamin C.

To assess the genotypic variation, ten cultivars (viz. Antea, Asia, Clery, Darselect, Elsanta Manille, Matis, Sonata, Sveva and Yamaska) were evaluated in open field, plastic tunnel and high altitude cultivation. Significant variations were observed in their vitamin C contents (41.8 to 85.0 mg/100 g), their antioxidant capacity (772 to 1327 μmol Trolox equivalent/100 g) and their anthocyanin contents (14.1 to 25.9 mg/100 g), which strongly suggests a genetic heterogeneity. The Soluble Solid Contents (SSC) varied in a much lesser extent with values comprised between 8.3% and 9.5% Brix. Most cultivars had titratable acidity values ranging from 0.61 to 0.76 g citric acid equivalent/100 g, except the late cultivars Sveva and Yamaska which showed higher acidity (0.86 and 0.82 g citric acid equivalent/100 g respectively). Although none of the cultivars combined good taste, high nutritional value and high productivity; Manille, Clery and Sonata received the best overall score. Furthermore the large genotypic variation in health promoting compounds provides numerous possibilities for breeding cultivars with specific nutritional traits. The difference in climate between the two years of investigation did not allow the assessment of environmental effects. However, some cultivars like Clery showed low variation in all traits and should be favoured for a stable production.

To assess the chemical variation with altitude of cultivation, four cultivars (viz. Antea, Asia, Clery and Matis) were grown in two production sites during one year at low and high altitude. Differences between the cultivars were observed in the composition of individual sugars (viz. glucose, fructose and sucrose) and acids (viz. malic, citric and ascorbic). Pelargonidin-3-glucoside remained the major anthocyanin present in all cultivars, representing between 75% and 94% of the total anthocyanin content. The presence and levels of other pelargonidin and cyanidin derivatives appeared to be genotype dependent. The plants cultivated in the mountain had a larger leaf area and produced higher yields over a shorter period. According to our results, the variation of the fruits composition in
different production sites was genotype specific. Antea was the most affected by the production site, showing a lower content of all analysed compounds at high altitude.

To evaluate the plant to plant variability in the cultivar Clery, fruits were sampled from thirty plants cultivated next to each other. Despite identical growing condition, an important variation in titratable acidity (0.60 to 0.87 g citric acid equivalent/100 g), SSC (7.6% to 11.2% Brix) and total anthocyanin contents (16.8 to 23.7 mg/100 g) was observed. The total antioxidant capacity varied in a lesser extent with values comprised between 1018 and 1360 μmol Trolox equivalent/100 g. These results suggest that additional factors other than genotype and production site could be responsible for inconsistencies in strawberry quality.

SSC, titratable acidity and total anthocyanin content varied significantly during the five harvest weeks, while the total antioxidant capacity remained stable. The changing proportion of the fruits from different inflorescence position (primary, secondary and tertiary fruits) during the harvest period could not explain the observed variations. In fact, primary, secondary and tertiary fruits had similar SSC, acidity and anthocyanin contents. Furthermore tertiary fruits showed a higher antioxidant capacity (+16% in 2008 and +9% in 2009) which was probably linked with the higher achene proportion in the fruit. Changes in taste related compounds during the harvest period appeared to be related to the changing of fruit load on the plant, which indicates a strong competition for assimilates between fruits at higher fruit load. Anthocyanin variation was shown to be positively correlated with the number of days from flowering to full fruit ripeness. According to our observations, fruits with slower development and ripening appeared to accumulate more anthocyanins, while fruit size did not affect taste or health promoting compounds in strawberry fruits. This denies the common belief that small fruit supposedly have a higher concentration in nutrients.

In conclusion, the choice of cultivar turns out to be the most important factor to increase health and taste promoting compounds in strawberry fruits. In the context of unpredictable environmental changes, the choice of stable cultivars appears of primary importance. In the future, increasing taste and health related components in strawberry fruits could be achieved by breeding new cultivars or by acting on plant biosynthetic pathway.
La fraise est un fruit très apprécié en Suisse où elle apparaît en quatrième position après la pomme l’orange et la banane. La production Suisse vise exclusivement le marché frais local et repose sur une qualité gustative élevée des baies. Depuis que de nombreuses études ont montré qu’une alimentation riche en antioxydants réduit le risque de cancer et de maladies cardio-vasculaires, la fraise peut être considérée comme une importante source de composés bénéfiques pour la santé en raison de sa teneur élevée en vitamine C, en anthocyanes, en acide ellagique et en autres composés phénoliques. Toutefois, au cours de la dernière décennie, l’importante fluctuation de la qualité de la fraise a souvent été observée et critiquée. La teneur en composés liés au goût et la valeur nutritionnelle, tels que sucres, acides, vitamine C et composés phénoliques est un paramètre essentiel pour la qualité des fraises. Toutefois, la fluctuation de ces composés est difficile à contrôler car de nombreux facteurs sont impliqués dans leur biosynthèse. L’objectif de cette étude a été d’évaluer différentes sources de variabilité des composés liés à la valeur gustative et nutritionnelle des fraises.

Afin d’évaluer la variabilité génétique de la valeur gustative et nutritionnelle des fraises, dix variétés (Antea, Asia, Cléry, Darselect, Elsanta Manille, Matis, Sonata, Sveva et Yamaska) ont été évaluées durant deux ans en plein champs, sous tunnel plastique et en altitude. Une variation significative a été observée parmi les génotypes quant à leur teneur en vitamine C (de 41.8 à 85.0 mg/100 g), leur capacité antioxydante (de 772 à 1327 μmol équivalent Trolox/100 g) et leur teneur en anthocyanes (de 14.1 à 25.9 mg/100 g). La teneur en matières solubles a varié dans une moindre mesure, avec des valeurs comprises entre 8.3% et 9.5% Brix. La plupart des variétés ont montré des valeurs d’acidité allant de 0.61 à 0.76 g équivalent acide citrique/100 g, sauf les variétés tardives Sveva et Yamaska qui ont montré une acidité plus élevée (0.86 et 0.82 g équivalent acide citrique/100 g, respectivement). Malgré qu’aucune des variétés testées n’a combiné une valeur gustative et nutritionnelle élevée avec une productivité importante, Manille, Cléry et Sonata ont été les variétés les mieux évaluées. De plus, la grande variabilité génétique concernant les composés bénéfiques pour la santé offre de nombreuses possibilités pour la sélection de variétés à valeur nutritionnelle particulière. L’importante variation climatique annuelle n’a pas permis de tirer des effets environnementaux évidents, mais elle a souligné l’avantage de variétés stables telles que la variété Cléry qui a montré une qualité constante.

Pour analyser la variation de la composition chimique due à la culture en altitude quatre variétés (Asia, Antea, Cléry et Matis) ont été cultivées à basse et haute altitude pendant un an. Des différences ont été observées entre les génotypes dans la répartition des sucres (glucose, fructose et saccharose) et des acides (malique, citrique et ascorbique). La pélargonidine-3-glucoside s’est avérée être l’anthocyanine prédominante dans toutes les variétés représentant entre 75% et 94% de la teneur en anthocyanes totale; la présence d’autres dérivés de la pélargonidine et de la cyanidine ainsi que leur concentration dans le fruit ont paru dépendre du génotype. Les plantes cultivées en montagne ont eu une plus grande
surface foliaire et ont produit des rendements plus élevés dans un laps de temps plus court. D’après nos résultats, la variation de la composition des fruits en fonction du site de production a été spécifique pour chaque génotype. Ainsi, Antea a été la variété la plus affectée par le site de production avec une teneur généralement plus faible de tous les composés analysés lors de sa production en altitude.

Afin d’évaluer la variation entre les plants d’une même variété (Clery), les fruits de trente plants ont été échantillonnés individuellement. En dépit de conditions de croissance identiques, une grande variation de l'acidité titrable (0.60 à 0.87 g équivalent acide citrique/100 g), de la teneur en matières solubles (7.6% à 11.2% Brix) et de la teneur en anthocyanes (de 16.8 à 23.7 mg/100 g) a été observée entre les fruits de différentes plantes. La capacité antioxydante totale a varié dans une moindre mesure, avec des valeurs comprises entre 1018 et 1360 μmol équivalent Trolox/100 g. Ces résultats indiquent que des facteurs supplémentaires, autres que le génotype et le site de production, pourraient être responsables des incohérences observées dans la qualité des fraises.

La teneur en matières solubles, l’acidité et la teneur en anthocyanes dans les fruits ont fortement varié au cours des cinq semaines de récolte, tandis que la capacité antioxydante est restée stable. Les changements de proportion des différents types de fruits (primaires, secondaires et tertiaires) au cours de la récolte n’ont pas pu expliquer la variation observée dans la qualité des fruits. En effet, le taux de matières solubles, l'acidité titrable et la teneur en anthocyanes sont resté semblables entre les différents types de fruits. D'autre part, les fruits tertiaires ont eu une plus forte capacité antioxydante (+16% en 2008 et +9% en 2009) sans doute liée à la proportion plus élevée d’akène. Les changements influençant la qualité gustative au cours de la période de récolte ont semblé être liés à la charge en fruits sur la plante, indiquant une forte concurrence pour les métabolites primaires entre les fruits à forte charge. La teneur en anthocyanes a corrélé avec le nombre de jours entre la floraison et à la maturité des fruits. D’après nos observations, les fruits qui ont eu un développement et une maturation plus lente ont semblé accumuler d’avantage d’anthocyanes. En outre, la taille des fruits n’a pas semblé affecter la valeur gustative et nutritionnelle des fruits, contredisant ainsi une opinion populaire selon laquelle de petits fruits sont un concentré de nutriments.

Pour conclure, le choix de la variété apparaît comme étant l'approche la plus efficace pour augmenter à la fois la valeur gustative et nutritionnelle de la fraise. Dans un contexte de changements climatiques difficilement prévisibles le choix de variétés stables apparaît d'une importance primordiale. Dans le futur, la qualité gustative et nutritionnelle des fraises pourrait être améliorée par la sélection de nouvelles variétés et en agissant sur la biosynthèse des métabolites recherchés.
CHAPTER 1

General introduction
1 Strawberry production and consumption

The garden strawberry (*Fragaria x ananassa* Duch.) is the most common strawberry species cultivated worldwide. The first producer of strawberries in the world is USA, while Spain and Poland are the largest producers in Europe. With ca. 5400 t per year cultivated on approximately 425 ha, the Swiss strawberry production supplies essentially the local fresh market. However, 2/3 of the strawberries consumed in Switzerland are imported (source: Fruit Union Suisse, 2010). Imported strawberries for fresh consumption are available before the start of the local production mainly in March and April. Nearly all the strawberries used for industrial processing are also imported. During a short period corresponding to the peak of production (between middle of May and end of June depending on the year), Swiss strawberries are exclusively promoted on the market. Earlier and later in the season, Swiss strawberries are in competition with imported strawberries. Producing fruits of very high quality is therefore essential for the Swiss growers in order to stay competitive on the market due to the higher production costs.

The principal production regions in Switzerland are Thurgau with 70 ha, Berne with 58 ha, Zurich with 44 ha and Valais with 43 ha. Elsanta and Darselect are the two most produced cultivars. They represent 33% of the total produced strawberries in Switzerland. In the last years the newly released cultivar Clery has gained in importance and is situated now on the third position representing 13% of the produced strawberries with a production surface of 55 ha (source: Fruit Union Suisse, Beerenanbauflächen 2010). In Valais, Marmolada was so far the most widely planted cultivar. However, despite its satisfactory visual aspect, the poor taste quality of this cultivar is often criticised by the consumer. Cultivar Clery is today considered as the candidate about to replace Marmolada in Valais.

In Switzerland strawberries are the most important consumed berries with 4 kg per person per year (source: Fruit Union Suisse, Guide des petits fruits 2007). They come in the fourth position in the most consumed fruits after bananas, oranges and apples; but in contrast to the latter, strawberries are typical seasonal fruits and their consumption occurs therefore during a short period.

2 Botany of the strawberry plant and fruit development

The garden strawberry is an octoploïd species belonging to the family of *Rosaceae*. It resulted from an accidental cross between a wild species *Fragaria virginiana* Duchesne originated from North America and *Fragaria chiloensis* (L.) Mill. from Chile. Today over 1000 strawberry varieties are cultivated worldwide.

The strawberry plant is a perennial plant characterised by an evolutionary morphology (vegetative growth, formation of runners, fructification). Strawberry cultivars can be divided into two groups
according to the type of photoperiod inducing flower initiation: short day and day-neutral cultivars. Short day cultivars are the most widely produced in temperate climates such as in middle Europe. For these plants floral induction occurs during the autumn, when the length of the day is shorter than the critical photoperiod of 14 h and the temperatures are cooler. Temperature is important for floral induction: under short days the optimum temperatures is between 15 and 18 °C, while below 10 °C and above 25 °C floral induction is rather ineffective (Sønsteby & Heide 2006). As soon as the temperature drops below 7 °C at the end of autumn, the plant starts its dormancy period. A defined amount of hours with temperatures below 7 °C (chilling requirement) are necessary for the short day plant to start the next vegetation period. The chilling requirement varies between cultivars and reflects their regional adaptation. Following the dormancy period, the plant flowers and produces fruits in spring.

Day-neutral varieties have been developed as strawberry production expanded in further regions in the world. They produce crowns and flower buds approximately three months after planting regardless of the day length. The optimal conditions for flower induction are between 15 and 21 °C, however temperatures higher than 27 °C have an inhibitory effect on flowering (Sønsteby & Heide 2009).

In every leaf axillary of the strawberry plant a bud is located, which can create, depending on the development phase of the plant, either runners, or a new shoot axis or an inflorescence (Naumann & Seipp 1989). The strawberry inflorescence is as such a modified stem and is terminated with the primary blossom. Following the primary blossom there are typically two secondary and four tertiary blossoms decreasing in size. Their flowering and fruiting time is slightly delayed.

The flowers of most strawberry cultivars are hermaphroditic and self pollinating. The resulting seeds are the achenes and form the true fruits, while the fruit receptacle constitutes the strawberry flesh. The receptacle is composed of an epidermal layer, a cortex and a pith. The latter two layers are separated by vascular bundles that supply nutrients to the developing embryos (Hancock 1999).

Strawberry fruits have an initial phase of cell division and cell enlargement followed by a ripening phase during which important biochemical changes occur in the fruit (Montero et al. 1996). Strawberry fruits represent the most competitive sink in the plant and accumulate 20%-40% of the total plant dry weight (Hancock 1999). During the rapid period of fruit growth fruit dry weight accumulation may exceed the assimilatory capacity of the plant and continued fruit growth is maintained by translocation from other plant parts, as for example the roots (Hancock 1999).

Soluble Solid Contents (SSC) continually increase in the fruit during its development but the main changes occur between 21 and 28 days after fruit set when fruit ripening take place (Montero et al. 1996). Glucose, fructose and sucrose are the major soluble sugars found in the fruit during all ripening stages. Sugars are primary accumulated in the developing fruit by translocation from leaves to fruit (Villareal et al. 2010). A decrease of glucose, fructose and sucrose is observed in the crown during
fruit development (Macias-Rodriguez et al. 2002) indicating their translocation to the fruit. Glucose and fructose are found in almost equal concentration and their contents rise continuously during fruit development. They represent 83% of the total sugars in ripe fruits (Wrolstad & Shallenberger 1981). Sucrose levels in the developing fruit diminish at 21 days after anthesis, probably under the action of invertase (Montero et al. 1996). Final levels of sucrose in ripe fruits seem to be highly variable.

Titratable acidity gradually declines as fruit matures. The proportion of the individual organic acids seems to vary during fruit development but citric acid remains by far the major organic acid in all stages of fruit development (Montero et al. 1996). As a consequence to the evolution of sugars and acids during fruit ripening, their ratio has been investigated as index of fruit development and ripening (Perez et al. 1997).

Phenylalanine ammonialyase (PAL) is a key enzyme in the phenolic biosynthesis. The development stage of the plant seems to regulate PAL activity. A high concentration of phenolic compounds is found in unripe fruits during their phase of early growth and extensive cell division (Williner et al. 2003). A sharp decrease is observed during the successive phases of fruit development until the ripening phase. In the ripening phase the total phenolic content remains stable (Ferreyra et al. 2007) or slightly increases due to the accumulation of anthocyanins in the fruit (Montero et al 1996). Anthocyanins start to appear in the white stage and from this stage their concentration increases exponentially (Ferreyra et al. 2007) giving the characteristic red colour of the strawberry fruit.

Ripe strawberries contain in average 89.5% water and 0.4% ash. They contain 3.7%-8.5% sugar and high levels of pectin (0.5%-1.36%) but no starch (Herrmann 2001). Compared to other fruits, strawberries are extremely rich in Vitamin C (60-100 mg/100 g FW) and in anthocyanins, especially pelargonidin-3-glucoside (pg-3-gluc) and cyanindin-3-glucoside (cya-3-gluc). However the concentration of the fruit components reported in different literature sources is highly variable (Herrmann 2001).

3 Fruit quality

3.1 Fruit composition affecting strawberry taste

Colour, texture, odour and the balance between sweetness and sourness have been identified as essential parameters for the overall quality rating of strawberry fruits (Shamaila et al. 1992). While colour and texture are important characteristics for the retail market and influence strongly consumer choices (Naumann & Seipp 1989), taste is mainly affected by aroma, sugars and acids. Wozniak et al. (1997) showed a correlation between the overall sensory quality of some Californian cultivars and the sugar:acid ratio in maturing fruits. Carlen & Ançay (2003), as well as Pelayo-Zaldívar et al. (2005) reported that consumer preferences were related to higher sugar and volatile contents. Jouquand et al.
(2008) showed the dominant role of SSC and/or sugar:acid ratio as indicators of flavour liking. However, the same authors underlined the complex role of volatiles, such as esters, which, in certain cases, can influence the overall acceptability of strawberry fruits.

Sugars are the main contributors to SSC in strawberries, followed by organic acids and soluble pectins (Pelayo-Zaldivar et al. 2005). As mentioned earlier, SSC is widely accepted as a good indicator for fruit taste. However, the relative proportions of the individual sugar components such as glucose, fructose and sucrose may influence the perception of sweetness. In fact, different sweetness coefficients have been attributed to sugars: glucose = 1, fructose = 2.3 and sucrose = 1.35 (Keutgen & Pawelzik 2007). Further the sugar:acid ratio can act as important component of fruit flavour and, as such, an index of consumer acceptability (Keutgen & Pawelzik 2007).

3.2 Strawberries as a source of health promoting compounds

Cancer and cardiovascular diseases represented with 62% the major cause of death in Switzerland in 2005 (source: Bundesamt für Statistik). Numerous epidemiological studies showed that consumption of fruits and vegetables have a protective effect against these degenerative diseases (Hannum 2004). The possible explanation for this effect is that many degenerative diseases associated with aging are caused by toxic oxygen radicals. Oxygen radicals can react with lipids, protein and DNA. The antioxidants present in fruits and vegetables are able to maintain low cellular levels of oxygen radicals by preventing their formation, scavenging them or promoting their decomposition (Hancock et al. 2007). Compared to other fruits, berries possess high antioxidant activity and are naturally rich in a variety of phytochemicals, in particular phenolic compounds (Häkkinen et al. 1999, Koponen et al. 2007, Jakobek et al. 2007). Being the most consumed berry, strawberry can therefore be considered as an important dietary source of health promoting compounds.

Around forty different phenolic compounds have been identified in strawberry fruits by Aaby et al. (2007) which could be grouped in different chemical family: flavonols, anthocyanins, flavanols, hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, ellagic acid, ellagic acid glycosides and ellagitannins. Within this great number of bioactive compounds, the authors underlined the predominant role of vitamin C, ellagitannins and anthocyanins as the most important contributors to the antioxidant capacity of strawberries. Further, Zhang et al. (2008) investigated antiproliferative activities of individual phenolic compounds in vitro. Anthocyanins appeared in addition to being the most powerful antioxidants, to possess the ability to inhibit the growth of human colon, prostate and oral tumour cells in a dose dependent manner.

Twenty-five anthocyanins have been identified in five strawberry cultivars by Lopez da Silva et al. (2007). Most of them contained pelargonidin and some of them cyanidin in the aglycon part. Glucose was the most usual substituting sugar. Rutinose and arabinose were also found and acylation with
different aliphatic acids occurred in certain cases. Pg-3-gluc constituted 77%-90% of the anthocyanins present in strawberry extracts, followed by pelargonidin-3-rutinoside with 6%-11% and cya-3-gluc with 3%-10%. Presence of pelargonidin-3-malonylglucosid ranging from 5% to 24% was mentioned by Yoshida et al. (2002) in Japanese cultivars.

4 Factors influencing fruit quality

Bioactive compounds are synthesised in the plant often as a result of environmental stress. Due to their sedentary nature, plants have to cope with unavoidable environmental changes such as drought, high radiation, extreme temperature, soil salinity or attack by insects or pathogens (Atkinson et al. 2005). Those stress situations results in an increased production of free radicals by the cell metabolism. The synthesis of antioxidants by the plant is one of their strategies to protect their DNA against those reactive oxygen species or to directly act as antimicrobial defence against pathogens. These compounds have been subjected to natural selection during the evolution, when the presence of a particular compound has conferred a selection advantage for the plant. For this reason today, genetic variability exist in the content of bioactives in plants.

In cultivated species such as strawberries, agronomical practices can influence the environment in which plants are growing through modifying temperature, light intensity, fertilisation level or pathogens pressure and therefore affect the level of antioxidants in the fruit. Various authors have studied the effect that the use of plastic tunnel (Kadir et al. 2006, Karhu et al. 2007, Voca et al. 2009, Ordidge et al. 2010), specific plastic mulch (Moor et al. 2005, Atkinson et al. 2006), organic farming (Häkkinen & Törrönen 2000, Anttonen & Karjalainen 2006, Olsson et al. 2006, D’Evoli et al. 2010) and fertilisation (Wang & Lin 2003, Anttonen et al. 2006) may have on fruit composition with specific emphasis on health promoting compounds. Those studies underline how minor cultivation changes can increase or decrease the content of phenolics. Therefore, they indicate that opportunities exist for agronomical practices to optimise the content of health promoting compounds. On the other hand the specificity of a production region and yearly variable meteorological conditions can often not be chosen or controlled and those factors have been shown to influence the fruit quality in strawberries and other berries (Häkkinen & Törrönen 2000, Connor et al. 2002, Davik et al. 2006, Rieger et al. 2008, Zheng et al. 2009).

2007, Kafkas et al. 2007, Hernanz et al. 2007, Tulipani et al. 2008). Additionally, recent studies have analysed genotype x environment interactions affecting fruit quality and suggests environmental response to be genotype specific (Connor et al. 2002, Davik et al. 2006, Carbone et al. 2009). Furthermore specific field conditions combine multiple factors which can influence in one or the other way the quality of the fruits. This fact complicates the interpretation of the results of such studies. Therefore predicting the effect the growing environment may have on the fruit quality remains challenging. In this context, the genotype stability of interesting fruit quality traits can become an essential parameter to consider when selecting a cultivar. However, to date and to the best of our knowledge, no information exists on the genotypic stability of taste and health promoting compounds in strawberries.

Finally, very little is known on plant physiological aspects affecting fruit quality and bioactive compounds in strawberries. Inconsistencies exist within cultivars in their quality traits (Ford et al. 1997, Lopez da Silva et al. 2007) which could be attributed to plant individual differences or to changes in the fruit quality during the harvest period. Anttonen et al. (2006) investigated the influence of the fruit position on the inflorescence on its quality. The results of his study allow considering plant physiological aspects as a possible reason for the quality fluctuation of fruits of a same genotype.

5 Objectives and structure of the thesis

The aim of this study was to explore the variability in taste and health promoting compounds of strawberry fruits. For this purpose the effect of the following factors on specific health and taste target parameter of the fruits were evaluated such as

1. the genotype and the genotypic stability of specific quality traits (Chapter 2)
2. the environment and the interaction between environment and genotype (Chapter 2 and 3)
3. plant physiological aspects like the harvest period, the fruit position on the inflorescence and the number of days from flowering to full ripeness (Chapter 4).
CHAPTER 2

Genotype stability of health and taste promoting compounds
1 Introduction

Health and enjoyment are the two main reasons for eating berries. In Switzerland the strawberry consumption reaches 4 kg per person per year (source: Fruit Union Suisse, Guide des petits fruits 2007), being one of the most consumed fruits after less seasonal ones like apples, oranges and bananas.

Standard quality characteristics for strawberry can be classified in visual or outer quality traits and inner quality traits. As outer quality traits, fruit size and homogeneity, colour and brightness seem to strongly influence consumer choices (Naumann & Seipp 1989) and thus are important characteristics for retail. On the other hand, the inner quality traits include the chemical composition influencing fruit taste. Strawberry taste has been shown to be mainly related to sugars, acids and aroma contents of the fruits (Kallio et al. 2000, Azodanlou et al. 2003, Jouquand et al. 2008). For these reasons the common standards to assess inner strawberry quality are the soluble solids contents (SSC) and titratable acidity (TA) of the fruit juice.

During the last years, the external quality traits, transportation tolerance and yield performance of commercial strawberries have been considerably improved contrary to their inner quality traits. As a consequence, strawberry organoleptic properties, especially their inconsistency at the points of purchase are often criticized by the consumer.

Furthermore, recent scientific evidences point out the importance of health promoting compounds in strawberries in relation to their high level of antioxidants including vitamin C and phenolic compounds combined with their wide consumption. In fact these compounds have protective effects against cancer, cardiovascular and other chronic diseases (Hannum 2004, Szajdek & Borowska 2008, Basu et al. 2010). Therefore improved knowledge about existence and quantity of inner quality traits is becoming desirable for the consumer.

A great variety of phenolic compounds such as anthocyanins, flavonols, flavanols and derivatives of hydroxycinnamic and ellagic acid have been identified in strawberries (Macheix et al. 1990). Anthocyanins are quantitatively the most important type of phenolic compounds present in strawberries (Seeram et al. 2006, Aaby et al. 2007, Lopez da Silva et al. 2007) and they are also responsible for the bright red colour of strawberry flesh. Beside the quantification of specific health promoting compounds, global tests have been developed to assess the overall antioxidant capacity of the fruits (Singleton & Rossi 1965, Cao et al. 1993, Brandwilliams et al. 1995).

To date, there is substantial evidence that reveals the genotype as the main source of variation in the composition of berry fruits (Yoshida et al. 2002, Kosar et al. 2004, Scalzo et al. 2004, Tulipani et al. 2008, Capocasa et al. 2008, Crespo et al. 2010). In addition to genotypic differences, numerous studies have shown that environmental parameters like light conditions, temperature, irrigation, fertilization or...
Genotypic stability of health and taste promoting compounds

Cultivation systems can affect the concentration of anthocyanins and antioxidant activity in strawberries and other berry crops (Wang & Zheng 2001, Davik et al. 2006, Terry et al. 2007). A recent study conducted on blackcurrant berries (Zheng et al. 2009) demonstrated that the genotype crucially influenced the response to weather conditions at different latitudes. Investigation on the stability of genotype specific quality traits may be crucial to optimise the cultivation of high quality fruits satisfying the requirements of the market.

The aim of this study was to characterise the genetic effect on fruit quality (market quality and health related parameter) and yield of newly released and standard June-bearing strawberry cultivars as well as the genetic stability of these traits in different environments. The variations in environmental conditions were obtained by cultivating these genotypes in upland and lowland conditions, in the latter case with and without plastic tunnel covering.

2 Material and methods

2.1 Plant material and trial environments

Ten strawberry cultivars were selected for their diversity in parentage and origin and for their commercial importance (Table 1). For nine cultivars A+ frigo plants were planted at the beginning of July 2007 and 2008. For cultivar Asia plug plants were used at the beginning of August 2007 and 2008 for the 2008 and 2009 harvest, respectively at all sites because no frigo plants were available. In Bruson (upland condition) the strawberry cultivars were planted one month earlier as commonly practiced in the mountains due to the earlier and longer winter. All cultivars were short day plants that are affected by a photoperiod of less than 14 hours, corresponding here to the period after the 20th of August.
Table 1: Origin and parentage of the ten strawberry cultivars used in this study.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Origin</th>
<th>Pedigree (♀ x ♂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antea</td>
<td>Italy</td>
<td>FB6L-3 x Onebor</td>
</tr>
<tr>
<td>Asia</td>
<td>Italy</td>
<td>Maya x selection</td>
</tr>
<tr>
<td>Clery</td>
<td>Italy</td>
<td>(Elsanta x FB6L-3) x (Agathe x Sweet Charlie)</td>
</tr>
<tr>
<td>Darselect</td>
<td>France</td>
<td>Elsanta x Parker</td>
</tr>
<tr>
<td>Elsanta</td>
<td>Netherlands</td>
<td>Gorella x Holiday</td>
</tr>
<tr>
<td>Manille</td>
<td>France</td>
<td>Gariguette x selection (incl. Mara des bois)</td>
</tr>
<tr>
<td>Matis</td>
<td>France</td>
<td>Mara Des Bois x (Douglas x Belrubi)</td>
</tr>
<tr>
<td>Sonata</td>
<td>Netherlands</td>
<td>Elsanta x Polka</td>
</tr>
<tr>
<td>Sveva</td>
<td>Italy</td>
<td>EM 483 x 87.734.3</td>
</tr>
<tr>
<td>Yamaska</td>
<td>Canada</td>
<td>Pandora x Bogota</td>
</tr>
</tbody>
</table>

The trials were conducted from 2007 to 2009 (two harvests) in three different environments:

- under polyethylene tunnel in Conthey
- in an open field (without tunnel) in Conthey
- in an open field (without tunnel) in Bruson.

The two sites where situated in Switzerland (Conthey, 46°12’ N/7°18’ E and Bruson, 46°04’ N/7°18’ E), and were characterized by different soil and climatic conditions but principally differed in their elevation over sea level (Table 2).

Table 2: Characteristic of the trial sites.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conthey</th>
<th>Bruson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>480 m</td>
<td>1060 m</td>
</tr>
<tr>
<td>Latitude / longitude</td>
<td>46°12’ N/7°18’ E</td>
<td>46°04’ N/7°18’ E</td>
</tr>
<tr>
<td>Granulometric soil composition</td>
<td>23% clay, 44% silt, 33% sand</td>
<td>16% clay, 35% silt, 49% sand</td>
</tr>
<tr>
<td>Soil fertility</td>
<td>Phosphorus: optimum, Potassium: above optimum, Magnesium: above optimum</td>
<td>Phosphorus: optimum, Potassium: optimum, Magnesium: optimum</td>
</tr>
<tr>
<td>Soil pH</td>
<td>7.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Soil organic matter (%)</td>
<td>3.6%</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

Plants were planted on raised beds covered with black plastic mulch in one row plots, containing 28 plants in 2008, increased to 37 plants in 2009, taking into account a considerable heterogeneity in
2008 at a density of 4 plants per m². Rows were separated by 1.25 m and the raised beds had an inner width and height of 60 cm and 15 cm respectively. Treatments were arranged in a randomized block design in each environment with 4 repetitions.

The tunnels were 5 m wide, 3 m high and 52 m long, covered with new polyethylene plastic sheets (200 mu, 7.25 EVA Patilux, Italy) each year at beginning of March. The tunnel ends and the sides remained open during the day when temperature inside reached 20 °C. Temperature inside and outside the tunnel was continuously recorded from March to June. Daily radiation data, expressed on the basis of energy (W/m²) of the photosynthetic active radiation (PAR) (source: Agrometeo.ch) have been converted into photosynthetic photon flux density (PPFD) (µmol/m²·s) according to a linear regression model performed with the data of one month in 2008 using following equation:

- For Bruson: \[ PPFD\text{ Bruson} = 0.1256 \times \text{rad Bruson} - 36.06 \]
- For Conthey open field: \[ PPFD\text{ Conthey} = 0.0967 \times \text{rad Conthey} - 7.4684 \]
- For Conthey tunnel: \[ PPFD\text{ Tunnel} = 0.064 \times \text{rad Conthey} - 52.31 \]

Water and nutrients were given by fertigation (drip irrigation, T-Tape with emitters spaced 0.2 m apart) based on the recommendations for strawberries with a fruit yield of 2 kg/m² (100 N, 15 P, 50 K and 20 Mg kg/ha). Phytosanitary treatments were made according to the recommendation for the Integrated Production System (Steffek et al. 2003).

Year 2008 was characterised by slightly higher temperatures in October than 2007 (10.6 °C against 9.6 °C in 2007). The first frost took place 8 days later in Conthey and 12 days later in Bruson in 2008 compared to 2007 (Conthey: November 24th against November 16th, Bruson: November 22nd against Nov 10th). Theses facts resulted in a higher temperature sum during the short day period that induces initiation and differentiation of flowers.

In both years the plants started to grow again in the first week of March in Conthey, and one month later in Bruson. This period corresponded with the set up of the tunnel for the covered treatment in Conthey. Compared to the spring 2008, 2009 was characterized by higher temperatures in April in both sites while the temperature in May and June remained similar both years (Table 3). In Bruson, 2008 was wet with a total of 104 mm rainfall from restart of growth until final harvest, while 2009 was rather dry with only 65 mm. The tunnel protection reduced the PPFD by 42%. In Bruson fruits ripened around one month later, leading PAR values comparable to Conthey in the open field throughout the fruiting season.
Genotypic stability of health and taste promoting compounds

Table 3: Temperatures (monthly average in °C), precipitations (mm) and photosynthetic photon flux density (PPFD) (mol/m²s) during flowering and fruiting in each environment.

<table>
<thead>
<tr>
<th></th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conthey tunnel</td>
<td>Conthey open field</td>
</tr>
<tr>
<td>Harvest start (earliest)</td>
<td>07.05</td>
<td>12.05</td>
</tr>
<tr>
<td>Temperature in May</td>
<td>19.8</td>
<td>16.8</td>
</tr>
<tr>
<td>Temperature in June</td>
<td>22.7</td>
<td>18.9</td>
</tr>
<tr>
<td>Temperature in July</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of daily temperatures until harvest start&lt;sup&gt;3&lt;/sup&gt;</td>
<td>639</td>
<td>786</td>
</tr>
<tr>
<td>Total precipitations&lt;sup&gt;2&lt;/sup&gt;</td>
<td>NA 88</td>
<td>104 NA 83</td>
</tr>
<tr>
<td>PPFD May</td>
<td>308</td>
<td>537</td>
</tr>
<tr>
<td>PPFD June</td>
<td>326</td>
<td>565</td>
</tr>
</tbody>
</table>

<sup>3</sup> only daily mean temperatures > 7°C are counted during the period from beginning of the year to the harvest.

<sup>2</sup> From end of winter to harvest start. End of winter was defined as March 1<sup>st</sup> in Conthey and April 1<sup>st</sup> in Bruson (both years) when the plant started to develop again.

2.2 Harvest and sampling

Strawberries were harvested three times a week. At each harvest the yield of marketable and excluded fruits was recorded for the whole plot and the average fruit weight was measured by weighing 25 marketable fruits. Excluded fruits were smaller than 25 mm diameter or malformed. Samples for chemical analysis were taken 14 days after the first harvest and mainly consisted of secondary and tertiary ripe fruits, i.e. when fully red. The samples were prepared for further analysis as described by Tulipani et al. (2008). Briefly, within three hours following harvesting, the samples (whole fruits) were stored at -20 °C for one month except for the fruits used to measure the colour, which were analysed immediately. Leaf area of five consecutive plants was measured after the last harvest with a leaf area meter (Area meter 300, LiCor).

2.3 Soluble solids content and titratable acidity

SSC and TA were measured on the thawed fruit samples after extracting the juice with a commercial Juice Master (Hapag, Switzerland). SSC expressed as % Brix was analysed with a refractometer (Atago, PR-1, Kunzmann, Switzerland) and TA by titration of 10 g of clear fruit juice with NaOH 0.1 N until a pH of 8.2 with an automated titrator (Titrino DMP 785, Metrohm AG, Schweiz). The result was expressed in g citric acid equivalent per 100 g juice.
2.4 Sample preparation for phytochemical analysis and determination of the dry matter

For all the phytochemical analysis the frozen berries were dipped into liquid nitrogen and milled with a laboratory blender (IKA® A 11 basic, Staufen, D). The obtained powder was filled in plastic bottles and stored at -80 °C until analysis.

The dry matter (DM) of the powder was measured in triplicate by drying 5 g of the fresh frozen powder at 105 °C until constant weight (4-6 hours) and expressed as g DM/100 g Fresh Weight (FW).

2.5 Extraction and quantification of the antioxidant capacity, total phenolic compounds and total anthocyanin contents

Antioxidants were extracted according to the method of Tulipani et al. (2008) with slight modifications. Briefly, 5 g of frozen strawberry powder were weighed in 25 g of the extraction solution containing methanol, water and formic acid (80:19:1, v:v:v). The obtained slurry was sonicated in a with ice-cooled (0-4 °C) water bath for 15 minutes then centrifuged 5 minutes at 9000 rpm. The supernatant was filtered through a LS 14 ½ filter (Schleicher and Schuell, Germany). The extracts were stored up two 3 days at -80 °C and were used for the determination of antioxidant capacity (AC), total phenolic contents (TPC) and total anthocyanin contents (TAC).

The determination of antioxidant capacity with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is based on the properties of DPPH, which in its radical form has an absorption band at 517 nm and disappears upon reduction by an radical scavenging compound. The determination of AC was performed according to the method described by Brandwilliams et al. (1995). All chemicals used in this section were purchased by Sigma-Aldrich (Buchs, Switzerland). Briefly, 100 μl extracts were added to 10 ml of a 0.1 mM DPPH solution stirred well and left to react at room temperature. The absorption at 517 nm was measured after 30 minutes against the methanol/water extraction mixture (blank). Quantification was performed with a Trolox standard calibration curve (0-2.4 μmol/ml) and the results were calculated in μmol Trolox Equivalent (TE) per g DM or FW.

The determination of TPC with the Folin-Ciocalteu assay is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The method was performed as originally described by Singleton & Rossi (1965) with some modifications. Briefly, 500 μl extracts or standards were mixed with 7.5 ml H2O and 0.5 ml of Folin-Ciocalteu reagent (F-9252, Sigma-Aldrich, Switzerland). After 10 minutes incubation at room temperature, 0.5 ml of a 20% Na2CO3 in water solution was added and the samples were incubated at 40 °C for further 20 minutes. The reaction was stopped by transferring the samples into an ice container and the absorbance was read at 755 nm.
against water. The quantification was performed with a gallic acid standard calibration curve (0-2.9 μmol/ml) and results were expressed as μmol Gallic Acid Equivalent (GAE) per g DW or FW.

For the quantification of TAC the method described by Lee et al. (2005) was used. This method is based on the structural change of anthocyanin chromophore between pH 1 and 4.5. PH 1 buffer was a 0.025 M potassium chloride solution. PH 4.5 buffer was a 0.4 M sodium acetate solution. 800 μl of the strawberry extract was diluted with 3200 μl of each buffer solution. Absorbance was measured at 520 and 700 nm between 20 to 50 minutes following the preparation against distilled water as blank. Anthocyanin pigments concentration was calculated as pelargonidin-3-glucoside (pg-3-gluc) using the molar absorptivity of 22400 L/mol cm. The results were expressed in mg per 100 g FW or per g DW.

2.6 Analysis of individual anthocyanins

Determination of individual anthocyanins was performed on a HPLC Agilent 1200 series. 12 g of frozen strawberry powder was diluted with 25 ml extraction solution containing methanol, water and formic acid (80:19:1, v:v:v) and 2 g ascorbic acid. The obtained slurry was sonicated for 15 minutes and centrifuged at 4000 rpm for 10 minutes. The supernatant was diluted to 50 ml with an ascorbic acid water solution (2 g/L). In each sample 100 μl of naringin (10 mg/ml) was added as an internal standard. The extracts were filtered through a 0.45 μm filter and injected (50 μl) into a C18 column of 250 mm x 4.6 mm diameter, 5 μm particle size (type Luna part number, Phenomenex, CA, USA). The mobile phase consisted of HPLC-grade water (A), ACN (B) and 10% (v/v) HCOOH (C). Flow rate, column temperature was set up at 0.7 ml/min and 30 ºC respectively. Finally, eluted anthocyanins were detected at 500 nm. The presence and relative abundance of each individual anthocyanin was identified by comparing peak area with standards of cyanidin-3-glucoside (cya-3-gluc) and pg-3-gluc (Extrasynthese, Lyon, France) using Agilent ChemStation Rev. B.02.01, except for pelargonidin-3-mangolylglucoside (pg-3-mal) which was identified by MS analysis.

Separation of pg-3-mal was performed under identical separation condition as described above with a HP series 1100 equipped with a MSD API and an ESI interface for the identification. The gas flow was 12 L/min, the nebulizer pressure 45 psi, the drying gas temperature 350 ºC and the capillary voltage 3500 V. The anthocyanin compounds were analysed in positive mode. Pg-3-mal eluted after pg-3-gluc at 15 min and its absorbance maxima were recorded at 280, 430 and 502 nm. The most abundant ions detected at a retention time of 15 min were 519 and 271 (m/z). The identification of pg-3-mal was confirmed by comparing absorbance spectrum, MS data and elution order with literature (Aaby et al. 2007) and pg-3-mal was quantified with the standard calibration curve of pg-3-gluc.
2.7 Extraction and HPLC determination of total vitamin C

The determination of vitamin C included the ascorbic acid (AsA) and the dehydroascorbic acid (DHAA) form. Vitamin C was extracted with a phosphate buffer solution (36 mM, pH 5.0) containing 1 g/L DL-Dithiothreitol (Fluka 43819, Sigma-Aldrich, Switzerland) for one hour at room temperature allowing the reduction of DHAA into AsA (Brause et al. 2003). Extracts were filtered through a 0.45 μm filter and injected on a Varian Prostar 230 HPLC pump system equipped with a diode array detector Varian Prostar 335 and a reverse phase column (Nucleodur C18, 4.5 x 250 mm, 5μm particle size, Macherey-Nagel, Switzerland) with a flow rate of 0.6 ml/min. The mobile phase consisted of the same buffer adjusted to a pH value of 2.5 to maintain AsA in the reduced form. The absorbance was measured with an UV-detector at 254 nm and the AsA peak area was quantified with the Software Galaxie Chromatography Data System Vers.1.9-Rev.2 on the basis of an external standard calibration curve (0-60 mg/L).

2.8 Validity of the analytical methods

The repeatability of the different HPLC methods including the extraction procedure was tested by calculating the coefficient of variation of five parallel samples. For pg-3-gluc the coefficient of variation was 0.6%, for pg-3-mal 2.7%, for cya-3-gluc 7.8% and for vitamin C, 0.9%.

In addition to the four field replicates which were used to calculate the mean value of each parameter, every 10th sample was extracted and analysed twice in order to have a continuous control the repeatability of the analytic over the time of the experiment.

2.9 Statistical methods

All statistical analyses were carried out using XLSTAT Version 2007.5 (Addinsoft, Paris, France). All data were subjected to analysis of variance and the differences between the means were assessed with Fishers Least Significant Difference (LSD) at P<0.05. The Coefficient of Variation (CV) was calculated for each genotype out of 6 mean values (3 environments x 2 years) and expressed as percentage of the mean. CV was adopted instead of the S²d (deviation from the regression mean square) to assess the genotype stability over the different environments because CV is the standardized S²d and is easier to comment on. Relationships between factors were analysed by the Simple Linear Regression and by the Coefficient of Determination (r²), calculated from the Pearson Product Moment Correlation Coefficient (r).
3 Results

3.1 Genotype and environment effects and their interactions on agronomic and outer quality traits

There were significant main effects (genotype, site and year) for agronomic and outer quality parameter (Table 4). The genotype was the factor with the strongest influence on all measured parameter, followed by the production site. The year affected essentially yield and fruit size. All interactions were significant for yield and fruit size. The genotypic effects on fruit colour parameter and leaf area were stable but no strong interactions were found with the environment.

Table 4: Significance of the factors and their interactions from analysis of variance (ANOVA) for agronomic and external quality traits.

<table>
<thead>
<tr>
<th></th>
<th>leaf area</th>
<th>yield</th>
<th>fruit size</th>
<th>fruit colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Site</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Year</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Genotype x Site</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Genotype x Year</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Site x Year</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Genotype x Site x Year</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>ns</td>
</tr>
</tbody>
</table>

a 4 genotypes only (Antea, Clery, Manille, Matis). Significant effect and interactions: *** at p<0.001, ** at p<0.01, * at p<0.05 and ns for not significant differences.

Among the genotypes studied Sonata was the most productive with an average yield of 624 g fruits per plant followed by Matis, Clery, Elsanta and Asia, whereas Manille had the lowest fruit yield of 386 g per plant (Table 5). Clery had the most stable yield with the lowest variation between site and years while Asia varied most with a CV reaching 46%. There were significant interactions between genotype and environment, genotype and year and genotype environment and year (Table 4) resulting in different cultivar ranking according to the environment. While Sonata reached both years its highest yield when cultivated in Conthey in an open field, Manille reached both years its highest yield when grown in Bruson. Both, production site and trial year had significant effect on the yield. However the variation caused by the year calculated as CV between years (26%) was more important than the variation caused by the genotype (13%) or the site (6%).

The total fruit yield was 45% higher in 2009 than in 2008; a trend towards higher yields was observed for plants under polyethylene tunnel but the difference with the field cultivation was not significant (results not shown).
Genotypic stability of health and taste promoting compounds

Strawberry fruit weight tends to decrease from the beginning to the end of the harvest for this reason the results presented herein are based on an average fruit weight calculated over the entire harvest.

Big fruits characterised Asia, while Manille had the smallest ones. The largest variation in fruit weight according to the environment was found in Sveva with fruit weights varying between 11.3 and 25.6 g per fruit and resulting in a CV of 27%. Elsanta had the most stable fruit weight with a variation of only 11%. The variation over years was especially marked in Bruson with an increase of 25% of the fruit weight in 2009. On the other hand a significant trend towards smaller fruits in the open field in Conthey was observed (results not shown).

Table 5: Genotypic variation (mean of three sites and two years) in fruit yield and single fruit weight and coefficient of variation (CV) between environments (n=6).

<table>
<thead>
<tr>
<th></th>
<th>yield mean g/plant</th>
<th>CV(%)</th>
<th>single fruit weight mean g/fruit</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antea</td>
<td>463</td>
<td>25</td>
<td>14.8</td>
<td>17</td>
</tr>
<tr>
<td>Asia</td>
<td>524</td>
<td>46</td>
<td>26.4</td>
<td>24</td>
</tr>
<tr>
<td>Clery</td>
<td>540</td>
<td>13</td>
<td>17.4</td>
<td>16</td>
</tr>
<tr>
<td>Darselect</td>
<td>513</td>
<td>22</td>
<td>20.6</td>
<td>18</td>
</tr>
<tr>
<td>Elsanta</td>
<td>531</td>
<td>28</td>
<td>17.5</td>
<td>11</td>
</tr>
<tr>
<td>Manille</td>
<td>386</td>
<td>43</td>
<td>11.8</td>
<td>19</td>
</tr>
<tr>
<td>Matis</td>
<td>554</td>
<td>17</td>
<td>17.8</td>
<td>19</td>
</tr>
<tr>
<td>Sonata</td>
<td>624</td>
<td>27</td>
<td>17.5</td>
<td>17</td>
</tr>
<tr>
<td>Sveva</td>
<td>462</td>
<td>36</td>
<td>19.6</td>
<td>27</td>
</tr>
<tr>
<td>Yamaska</td>
<td>486</td>
<td>38</td>
<td>20.0</td>
<td>15</td>
</tr>
<tr>
<td>LSD_{0.05}</td>
<td>153</td>
<td>3.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2 Variation in fruit inner quality parameter

There were significant main effects (genotype, site and year) for most inner quality parameter (Table 6). The genotype and year were the two factors with the strongest influence on all measured parameter, followed closely by the production site. All interactions were significant concerning the fruit dry weight, the acidity and the anthocyanin content.
Table 6: Significance of the factors and their interactions from analysis of variance (ANOVA) for agronomic and external quality traits.

<table>
<thead>
<tr>
<th></th>
<th>DW</th>
<th>SSC</th>
<th>TA</th>
<th>TPC</th>
<th>vit. C</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Site</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Year</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Genotype x Site</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Genotype x Year</td>
<td>**</td>
<td>ns</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Site x Year</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Genotype x Site x Year</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
</tbody>
</table>

DW: Dry weight, SSC: Solubles solids contents, TA: titratable acidity, TPC: total phenolic compounds, TAC: Total anthocyanin contents. Significant effect and interactions: *** at p<0.001, ** at p<0.01, * at p<0.05 and ns for not significant differences.

3.2.1 Taste related compounds

The DM content of the fruits varied between the genotypes from 8.1 g/100 g FW in Matis to 9.8 g/100 g FW in Manille (Table 7). Despite the fact that one of the less productive cultivar (Manille) was also the cultivar with highest DM content, no negative correlation could be found between yield and dry matter content. Additionally an important variation was found in DM contents between years and environment. An average of 9.9 g DM/100 g FW was found in 2009 compared with 8.1 g DW/100 g FW found in 2008; in Bruson DM content was significantly lower with 8.5 g DM/100 g FW compared to both treatments in Conthey with values of about 9.4 g DM/100 g FW. On the other hand no significant differences were found between tunnel and open field in Conthey.

All cultivars showed similar SSC ranging from 8.9% to 9.5% Brix, except Matis and Yamaska, (Table 8). However, genotype x environment x year interactions were observed and the cultivar ranking differed largely between the environments. Sonata had one of the lowest SSC in the open field in Conthey in 2008 (7.8%) and the highest SSC in Bruson in 2009 (9.7%). Manille had as well very variable SSC showing one of the lowest values observed in Bruson in 2008 (7.5%), as well as the highest value in the tunnel the same year (11.1%). The coefficient of variation for those cultivars was accordingly high. On the other hand, Matis had low but stable SSC ranging between 7.7% and 8.7%.

Both, production site and trial year had significant effects on the SSC. However the SSC variations caused by year and site were low (3%) and were in the same range as the genotypic variation (4%). Generally the fruits had a higher SSC in 2009 than in 2008 and the fruits from Bruson had lower SSC than the fruits from Conthey following the same trend than the DM content.
Genotypic stability of health and taste promoting compounds

The TA varied between genotypes significantly and ranged from 0.61 to 0.86 g/100 g (Table 8), representing a variation of ca. 11%. With acidity values of respectively 0.86 and 0.82 g/100 g, Sveva and Yamaska were the most acidic cultivars and their SSC/TA ratio was accordingly the lowest (10.8 and 10.2). The lowest acidity values were found in Matis (0.61 g/100 g).

Finally, TA values were slightly higher in 2009 except in Conthey open field, were the years did not differ significantly. A tendency towards lower acidity values in Bruson was observed especially in 2008. Year and site both induced same range of variation in TA (7% and 6% respectively) remaining low compared to the genotypic effect.

Table 7: Genotypic variation of the dry matter content (DM), soluble solid content (SSC), titratable acidity (TA) and sugar:acid ratio (SSC/TA) and coefficient of variation (CV) between environments (n=6).

<table>
<thead>
<tr>
<th></th>
<th>DM content</th>
<th>SSC</th>
<th>TA</th>
<th>SSC/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g FW</td>
<td>CV %</td>
<td>% Brix</td>
<td>CV %</td>
</tr>
<tr>
<td>Antea</td>
<td>9.1</td>
<td>18</td>
<td>9.1</td>
<td>9</td>
</tr>
<tr>
<td>Asia</td>
<td>9.4</td>
<td>11</td>
<td>9.1</td>
<td>5</td>
</tr>
<tr>
<td>Clery</td>
<td>8.5</td>
<td>10</td>
<td>8.9</td>
<td>7</td>
</tr>
<tr>
<td>Darestlect</td>
<td>9.5</td>
<td>15</td>
<td>9.2</td>
<td>5</td>
</tr>
<tr>
<td>Elsanta</td>
<td>9.3</td>
<td>15</td>
<td>9.0</td>
<td>7</td>
</tr>
<tr>
<td>Manille</td>
<td>9.8</td>
<td>14</td>
<td>9.5</td>
<td>12</td>
</tr>
<tr>
<td>Matis</td>
<td>8.1</td>
<td>16</td>
<td>8.3</td>
<td>5</td>
</tr>
<tr>
<td>Sonata</td>
<td>9.2</td>
<td>17</td>
<td>8.9</td>
<td>7</td>
</tr>
<tr>
<td>Sveva</td>
<td>9.5</td>
<td>10</td>
<td>9.3</td>
<td>9</td>
</tr>
<tr>
<td>Yamaska</td>
<td>8.7</td>
<td>10</td>
<td>8.4</td>
<td>7</td>
</tr>
<tr>
<td>LSD.05</td>
<td>0.9</td>
<td>0.9</td>
<td>0.08</td>
<td>1.39</td>
</tr>
</tbody>
</table>

Compared to the agronomic parameters, taste related parameters, especially SSC, showed a greater stability through the environments; their variation was in the range of 5% to 18% while yield and that of fruit size between 11% and 46% variation (Table 5 and 7).

3.2.2 Variation in health related components

Because of the large variation in DM content between the regions and years the fruit chemical composition was related to the DM in order to assess real enhanced/decreased synthesis of specific compounds and not dilution effects due to changes in water content.

AC measured with the DPPH assay and TPC measured with Folin-Ciocalteu assay showed similar values and were closely correlated ($r^2 = 0.766$, p<0.0001).
A great genotypic variability was found in the AC and TPC values (Table 8). Antea showed the highest AC and TPC values (152.4 μmol TE/g DM and 138.2 μmol GAE/g DM, respectively) and Sonata the lowest (89.7 μmol TE/g DM and 94.8 μmol GAE/g DM).

Beside the genotype, the year had a significant effect on the AC and TPC with 12% and 14% variation, respectively. AC and TPC were higher in 2008 compared to 2009. Finally, production site had smaller impact on AC and TPC with only 5% and 4% variation. No significant interaction was found between site and genotype but there were strong site x year interactions. In 2008, the fruits grown under tunnel cultivation showed lower values, however in 2009 the three production sites did not differ significantly in their AC and TPC.

Genotype affected strongly the vitamin C content of the fruits (Table 8). For instance, Antea showed with 9.7 mg/g DM (85 mg/100 g FW) the highest vitamin C content, 1.9 times more than Matis (5.1 mg/g DM or 41.8 mg/100 g FW). The vitamin C content was well correlated with the antioxidant capacity as well as with the total phenolic content ($r^2 = 0.618^{***}$ and $0.497^{***}$ for DPPH and Folin respectively for both $p<0.0001$). The ranking of the cultivars was similar for both parameters: exceptions were Yamaska with the second highest vitamin C content (8.4 mg/g DM, 72.9 mg/100 g FW) but only an average antioxidant capacity of 114.8 μmol TE/g DM, and Sveva with the second highest antioxidant capacity but a relatively low vitamin C content (7.0 mg/g DM, 65.5 mg/100 g FW).

As reported for the AC and TPC values, there were generally greater variations in vitamin C content between years than between production sites with higher contents in 2008 than in 2009. Slight differences between tunnel and open field were found in 2008; however, these differences could not be confirmed in 2009. There were no significant interactions between genotype and environment, genotype and year or between genotype environment and year.

Anthocyanin content was correlated neither with the antioxidant capacity nor with the total phenolic content. The genotypes differed not only in their total anthocyanin content (Table 8) but also in their anthocyanin profile (Table 9). Total anthocyanin content varied up to 2.7 times ranging from 1.5 mg/g DM (14.1 mg/100 g FW) in Sveva to 3.1 mg/g DM (25.8 mg/100 g FW) in Clery.

The proportion of pg-3-gluc to the total anthocyanin content varied between genotypes ranging from 67.9% in Sonata to 97.8% in Asia, but remained quantitatively the most important anthocyanin present in the fruit. The production of pg-3-mal was almost absent in Antea and Asia whereas Manille and Sveva contained the greatest amount of cya-3-gluc with relative contents around 4 times higher than in Clery and Yamaska.

Regarding the stability of the health related compounds over environmental variation, genotypic differences were observed. Clery was the most stable cultivar with CVs of different health related
compounds ranging from 7% to 18%. On the other hand Sonata showed the highest variability with CV ranging from 20% to 31%.

The highest anthocyanin contents were found in Bruson in 2008. Despite significant differences between the sites in 2008, the differences were not significant within individual genotypes except for Manille between the sites of Bruson and Conthey tunnel. In 2009, lower anthocyanin contents were measured and the differences between the three environments were less marked. Slight differences in the ranking of the cultivars between the sites and year resulted in significant genotype environment interaction.

**Table 8:** Genotypic variation in antioxidant capacity (AC), total phenolic compounds (TPC), Vitamin C and total anthocyanins contents (TAC) and coefficient of variation (CV) between the environments. Results are given per g dry matter.

<table>
<thead>
<tr>
<th></th>
<th>AC (μmol TE/g)</th>
<th>CV %</th>
<th>TPC (μmol GAE/g)</th>
<th>CV %</th>
<th>Vitamin C (mg/g)</th>
<th>CV %</th>
<th>TAC (mg/g)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antea</td>
<td>152.4</td>
<td>16</td>
<td>138.2</td>
<td>15</td>
<td>9.7</td>
<td>12</td>
<td>1.9</td>
<td>19</td>
</tr>
<tr>
<td>Asia</td>
<td>102.2</td>
<td>10</td>
<td>95.0</td>
<td>5</td>
<td>6.1</td>
<td>13</td>
<td>2.4</td>
<td>6</td>
</tr>
<tr>
<td>Clery</td>
<td>116.8</td>
<td>10</td>
<td>111.4</td>
<td>7</td>
<td>7.1</td>
<td>10</td>
<td>3.1</td>
<td>18</td>
</tr>
<tr>
<td>Darselect</td>
<td>129.5</td>
<td>16</td>
<td>129.2</td>
<td>14</td>
<td>7.3</td>
<td>15</td>
<td>2.3</td>
<td>18</td>
</tr>
<tr>
<td>Elsanta</td>
<td>122.8</td>
<td>12</td>
<td>123.7</td>
<td>13</td>
<td>8.2</td>
<td>12</td>
<td>2.3</td>
<td>19</td>
</tr>
<tr>
<td>Manille</td>
<td>105.0</td>
<td>9</td>
<td>97.5</td>
<td>8</td>
<td>6.7</td>
<td>7</td>
<td>2.7</td>
<td>15</td>
</tr>
<tr>
<td>Matis</td>
<td>95.7</td>
<td>17</td>
<td>100.4</td>
<td>18</td>
<td>5.3</td>
<td>17</td>
<td>2.7</td>
<td>18</td>
</tr>
<tr>
<td>Sonata</td>
<td>89.7</td>
<td>20</td>
<td>94.8</td>
<td>21</td>
<td>5.4</td>
<td>20</td>
<td>2.2</td>
<td>31</td>
</tr>
<tr>
<td>Sveva</td>
<td>129.4</td>
<td>11</td>
<td>129.2</td>
<td>8</td>
<td>7.0</td>
<td>12</td>
<td>1.5</td>
<td>14</td>
</tr>
<tr>
<td>Yamaska</td>
<td>114.8</td>
<td>13</td>
<td>113.8</td>
<td>10</td>
<td>8.4</td>
<td>11</td>
<td>2.4</td>
<td>11</td>
</tr>
<tr>
<td>LSD,05</td>
<td>20.7</td>
<td></td>
<td>20.6</td>
<td></td>
<td>1.2</td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>
4 Discussion

4.1 Agronomic aspects

Yield and fruit size are financially important parameter for strawberry growers and the single fruit weight directly influences the harvest speed and therefore may affect the production cost. Yield is affected by various factors such as number and size of fruit. In this study, the factor responsible for the greatest variation in yield was the year, followed by the genotype whereas production site had the weakest effect on the yield. This indicates a principal influence, probably of temperature dynamics, during early and late development while the modulation of temperature by the altitude was of inferior importance. Doving (2004) showed temperature variation during flowering and ripening to be of lesser importance, while temperatures in autumn allowed early and reliable yield prediction for the following season. Short day plants used in this study initiate flower buds when day length is shorter than 14 hours while their vegetative growth (leaf area and runners) is favoured by long days (Hancock 1999). In the latitude of this trial, short day periods favourable to floral initiation (less than 14 hours) started around August 20th. From the planting time to this date the photoperiod was favourable for the leaf and runners development. For this reason the strawberry plants planted in Bruson beginning of June had an extended long day period compared to the plant planted in Conthey one month later. This site related fact may have favoured leaf area development for the plants grown in the mountain. On the other hand the higher temperatures in each environment during the short day period especially in October 2008 compared to 2007 might explain the higher yields in 2009 compared to 2008. Such correlations have
Genotypic stability of health and taste promoting compounds

already been observed by Doving (2004). A further indication of the importance of autumn conditions was missing principal differences between tunnel and open field since tunnels were set up in spring. The individual yield response of each genotype to the environment suggests that the transduction and translation of such climatic signals are strongly modulated by the genotype and therefore subject to selection. While most cultivars showed higher yield when cultivated under the protective tunnel, Sonata had higher yield when cultivated in open field condition indicating differences in hardiness. Interestingly, the reduced radiation in the tunnel compared to the open field did not negatively impact yield or leaf area.

Temperature in March affected the harvest start as the sum of the days with temperatures above 7 °C during the month of March was 14 days in 2008 and 12 days in 2009. The earliness of flower and development is strongly related with temperature in spring, for this reason the harvest starts when a certain temperature sum is reached and therefore similar sum of temperature were measured in each environment between the beginning of plant development in spring and the harvest begin.

4.2 Inner quality traits: taste related attributes

The relation between sugars content and sensory quality of strawberry fruit has been studied by various authors (Azodanlou et al. 2003, Jouquand et al. 2008, Kader 2008) and sugar content has been shown to be strongly related to taste. Furthermore a linear correlation between the total sugar content and the soluble solids (% Brix) has been shown by Kallio et al. (2000).

Despite genotypic and environmental variations, SSC values were always above the critical limit of 7.5% considered as good according to the model for assessment of strawberry quality described by Azodanlou et al. (2003) and above the minimum SSC requirement of 7% for acceptable flavour rating recommended by Mitcham et al. (2000). The genotypic differences in SSC were small and only two genotypes differed significantly from the others. Additionally, the low variation observed between environments suggests SSC to be a stable property over years and environment.

However, SSC values of some standard cultivars (Elsanta, Clery and Asia) were higher than found by Wozniak et al. (1997) and Voca et al. (2009). The former group found 6.7% for Elsanta fruits cultivated under plastic tunnel and lower SSC (5.9%-6.3%) when cultivated in the greenhouse (soil-less). Concerning Clery and Asia, SSC values found by the latter group were 8.8% and 8.0% in an open field and 10% (both cvs.) than under plastic tunnel, respectively. Large variation in SSC for a same genotype according to the environment and season has been observed by Ford et al. (1997) in different Australian farms. Further, Jouquand et al. (2008) observed harvest date and seasonal variation for SSC in various studied genotypes.

Previous observations by Shaw (1990) explained the reduced genotypic consistency for SSC by large genetic x harvest date interactions. In our study, however, the standardisation of the sampling
procedure allowed eliminating genetic x harvest date effects. Considering the climatic variation, 2008 was exceptionally wet in Bruson and the higher rainfall and humidity resulted in significant loss of SSC. The differences were less drastic in 2009, as the climate in Bruson was dryer and thus more comparable to the climate in Conthey. Similar results have been reported by Kallio et al. (2000) while comparing strawberries (Senga Sengana) grown in Finland and Poland during two years.

Acid contents may influence the perception of sweetness and thus have an impact on the taste (Terry et al. 2005). TA values over 0.8% are considered as too acidic for an acceptable flavour by Mitcham et al. (2000). This was the case for Sveva and Yamaska, the two most acidic cultivars. Under specific conditions, Elsanta and Antea reached also TA values over 0.8%. The variation in TA expressed as CV values were greater than for SSC but remained below 20% and thus were low compared to other parameters.

Because of its effect on perceived sweetness the ratio between sugar and acids in strawberries and other berries can act as an important indicator of fruit taste (Terry et al. 2005, Giné Bordonaba & Terry 2008), fruit ripeness (Perez et al. 1997) or even as an index of consumer acceptability (Keutgen & Pawelzik 2007). However this parameter cannot be considered independently of the other taste related parameters. For example Matis showed a generally high SSC/TA ratio, but compared to the other cultivars with similar SSC/TA ratio, Matis has a low SSC and low acidity values, which despite the good sugar acid balance could result in a negative impact on the taste. Davik et al. (2006) and Jouquand et al. (2008) found both a negative correlation between sugar:acid ratio and maximal temperature. However, such correlation could not be confirmed with our data thus indicating that the observed variability relies on other factors or factor combinations.

4.3 Inner quality traits: health related compounds

Genotypic variation in health related compounds has been previously reported (Yoshida et al. 2002, Scalzo et al. 2005, Tulipani et al. 2008) however only few cultivars have been characterised so far and new cultivars are being released every year. Several references exist on the nutritional quality of Elsanta. Therefore this cultivar was considered as a standard and the results were used to compare our values with literature data.

The total phenolic contents found in Elsanta fruits (2.0 mg GAE/g FW) were in the lower range of the values found by Terry et al. (2007) with values between 2.1 and 3.5 mg GAE/g FW according to fruit order and irrigation regime, or Skupien & Ozimanski (2004) and Keutgen and Pawelzik (2008). Sveva has been already characterised in a study of Tulipani et al. (2008) and the total phenolic contents reported (2.5 mg GAE/g FW) are as high as or slightly higher than ours for the same cultivar.

Strawberries are known to be very rich in vitamin C compared to other fruits and vegetables (Hancock 1999). However a great variation in their vitamin C content was observed among genotypes. High
vitamin C contents in Elsanta fruits have been previously reported and varied from 53.2 to 87 mg/100 g FW depending on the sample preparation and analytical procedure used. The values found here (72.7 mg/100 g FW) were close to the values found by Terry et al. (2007) and Atkinson et al. (2006), slightly higher than the values found by Keutgen & Pawelzik (2008) (53.17 mg/100 g FW) and lower than those found by Skupien & Oziomansky (87.0 mg/100 g FW) who analysed the fresh fruits directly after the harvest. In our study, only one cultivar (Antea) had higher vitamin C content than Elsanta. This result confirms Elsanta as a vitamin C rich cultivar to be used in breeding program having high vitamin C cultivars as objective. Vitamin C seems to be the main contributor to antioxidant capacity in strawberry fruit (Aaby et al. 2007, Tulipani et al. 2008). This fact was confirmed by the strong correlation found between the vitamin C content and the antioxidant capacity of the fruits extracts here ($r^2 = 0.618, p<0.001$).

Besides genotypic differences, AC, TPC and vitamin C contents were more affected by the year than by the production site, suggesting meteorological condition as being more important for the formation of antioxidant compounds than fixed site related factors. This fact underlines the importance of the choice of stable cultivars like Clery, Manille and Asia that showed the greatest stability over year and environment in their antioxidant capacity.

Anthocyanins, as well, have been shown to possess high antioxidant capacity (Wang et al. 1997) and are after vitamin C important contributors to the antioxidant capacity of strawberries (Aaby et al. 2007, Tulipani et al. 2008). However the anthocyanin content did not correlate with antioxidant capacity measured with DPPH assay neither with the total phenolic contents measured with Folin-Ciocalteu assay. This may be related to the fact that, when assessing the total phenolic content of the extracts, vitamin C and sugars are interfering factors, as discussed by Wrolstad et al. (2005).

The anthocyanins content of Sveva (14.1 mg/100 g FW) was slightly higher than reported by Tulipani et al. (2008) who found 13.7 mg/100 g FW. Concerning the anthocyanin profile, these authors detected small quantities of pg-3-rutinoside representing 0.6% of the total anthocyanin content, which could not be detected with our method. On the other hand we found greater proportion of cya-3-gluc (4.16% against 1.5%).

Total anthocyanin contents found in Elsanta fruits by Skupien & Oziomanski (2004) was 25 mg/100 g FW and Terry et al. (2007) reported values between 14.8 and 20.1 mg/100 g. It is to be noted that both studies were based on one year results and that our values varied widely between the two trial years. Strong year to year variation in anthocyanin contents have already been reported by Moor et al. (2005), and Koponen et al. (2007) for other cultivars. Among the studied genotypes, only one cultivar (Clery) had fruits with higher anthocyanin contents than Elsanta and most cultivars showed similar contents (Asia, Darselect, Manille, Matis, Sonata and Yamaska).
Genotypic stability of health and taste promoting compounds

Anthocyanins like other antioxidants are synthesised by the plant to protect cells from stress thus may be influenced by several factors. It has been shown for example that anthocyanin biosynthesis in plant tissue requires light or appears to be enhanced by it (Mancinelli 1985, Atkinson et al. 2005). In 2008 the reduced PAR under the plastic tunnel seemed to have a negative impact on the anthocyanin content of strawberry fruits. However this fact could not be confirmed in 2009 as a majority of the cultivars had their highest anthocyanin content when cultivated under tunnel.

The fruits collected in 2009 were generally characterised by lower nutritional values having less anthocyanins, less vitamin C and less antioxidant capacity but higher SSC and acidity. The differences between the production sites were more marked in 2008 because of specific meteorological condition.

4.4 Cultivar rating

None of the cultivars combined all positive expression in traits like good taste, high nutritional value and high productivity (Table 10). Concerning taste related attributes, Manille seemed to be the cultivar with highest potential of acceptance by the consumer through its high SSC values combined with high sugar:acid ratio. Additionally its high anthocyanin contents despite the low antioxidant capacity can be an interesting property concerning health promoting compounds. However its low yield performance combined with small fruit size makes it financially less interesting for growers unless this can be compensated with a higher price of the berries. Its high yield variability reflects the need of this cultivar to be grown in a favourable environment, which in this study was the case in Bruson. The high yield performance of Sonata combined with acceptable taste attributes makes this cultivar a valuable option for both grower and consumer. Finally Clery was the cultivar with most desired properties associating high fruit quality with a medium but stable yield and thus may be recommended for grower having as objective to produce high quality fruits at an affordable cost.

Table 10: Relative cultivar rating for the main taste related parameters, health related parameters and yield performance. High levels correspond to values above 75% of the maximal value, medium for values between 25 and 75% and low for values below 25%. Desired properties are highlighted.
5 Conclusions

The results of this study support the fact that the genotype strongly influences the overall quality and the nutritional value of strawberry fruits. However the observed genotype x site x year interactions, lead to inconsistency of the inner quality of strawberries across environments and the strong variation between the years does not allow after two seasons to draw clear trends between the different environmental effects.

Environmental factors affecting the yield such as meteorological conditions in autumn are rather unpredictable. Furthermore the discussed variations in agronomic related traits underline the importance of choosing a genotype which shows high stability in its productivity since this cannot be easily manipulated by the choice of the production site.

Influencing strawberry fruit taste by the mean of preharvest factors or even by breeding tasty cultivars seems challenging. On the other hand the great stability of taste related parameter of fruits at mid harvest can be regarded as a significant advantage for growers. Further information regarding the other factors influencing the sugar contents such as physiological status of the plant like harvest period, fruit order is needed.

The data reported in this work reveals a large genotypic variation in the content of chemicals with health promoting properties. With genotypic variation of up to 1.9 times, the individual intake of vitamin C through strawberry consumption may not be easily quantified. On the other hand the large genotypic variation in nutritional value has important implications for strawberry breeding programs considering nutritional value as a selection criterion.

Finally this study has shown that very few cultivars associate high nutritional values, a good taste and interesting agronomical traits today. The observed interactions challenge strawberry breeders and complicate cultivar recommendation. However, it offers opportunities to raise interesting properties through genotypes specially adapted to a given area or minimising the effect of an unfavourable year through the cultivation of stable genotypes.
CHAPTER 3

Characterisation of major taste and health related compounds of four strawberry genotypes grown at two different Swiss production sites

P. Crespo, J. Giné Bordonaba, L.A. Terry, C. Carlen

1 Introduction

Epidemiological studies suggest that consumption of fruit and vegetables contributes towards reducing risk of certain types of human cancer and cardiovascular diseases (Bazzano et al. 2002). Among fruits, berries are popular because of their good taste and their known nutritional value. Indeed, strawberry fruits have been shown to contain high amounts of vitamin C and phenolic compounds, if compared to other fruits and vegetables, which are known to provide protection against free radicals when tested in vitro (Meyers et al. 2003). Several studies have identified a wide range of phenolic compounds in strawberry fruits (Seeram et al. 2006, Aaby et al. 2007), but anthocyanins remain quantitatively the most important type in ripe fruits. Anthocyanins belong to the flavonoid group and are responsible for the bright red colour of strawberry fruits. Despite a great number of anthocyanins being identified in strawberry fruits, pelargonidin-3-glucoside (pg-3-gluc), pelargonidin-3-rutinoside (pg-3-rut) and cyanidin-3-glucoside (cya-3-gluc) represent over 95% of the total anthocyanin bulk present in most strawberry fruits (Lopez da Silva et al. 2007).

Nowadays, a large number of research studies have been conducted with an aim to elucidate the mechanisms for increased synthesis of bioactive compounds in the fruits and hence potentially healthier berries. This said, there seems to be a lack of information which addresses how genotype and cultivation systems affect the concentration of sugars and acids in the fruit since they can act as an index of consumer acceptability (Azodanlou et al. 2003, Pelayo-Zaldivar et al. 2005, Jouquand et al. 2008, Keutgen & Pawelzik 2008). Sugars in strawberry fruits are mainly mono- and disaccharides (viz. glucose, fructose and sucrose) (Perez et al. 1997, Kallio et al. 2000, Terry et al. 2007, Giné Bordonaba & Terry 2009) and the relative proportion of these individual sugars is important for governing the perception of sweetness (Keutgen & Pawelzik 2008).

To date, there is substantial evidence that reveals genotype as the main source of variation in composition, specifically on both anthocyanin and sugar contents, of berry fruits (Yoshida et al. 2002, Kosar et al. 2004, Capocasa et al. 2008, Giné Bordonaba & Terry 2008, Tulipani et al. 2008, Giné Bordonaba & Terry 2009). However, commercially available cultivars are changing rapidly and hence constant updating of information is required to quantify important taste- and health-related compounds in fruits from newly released cultivars. In addition to differences among genotypes, numerous works have shown that exogenous factors such as environmental parameters (viz. light conditions, temperature, irrigation, fertilization or cultivation systems) can affect the concentration of anthocyanins and antioxidant activity in strawberries and other berry crops (Wang & Zheng 2001, Davik et al. 2006, Terry et al. 2007, Crespo et al. 2009). A recent study conducted on blackcurrant berries (Zheng et al. 2009) demonstrated that genotype crucially influenced the composition of fruits as a response to weather conditions at different latitudes. The effect of altitude at the same latitude was
Analysed for wild populations of common elderberries and bilberries (Rieger et al. 2008). Nevertheless, thus far no other studies have elucidated the effect that production site, principally differing in altitude, may have on strawberry fruit composition. Given that strawberry production in middle or Eastern Europe, including countries such as Italy, Switzerland and Turkey is carried out in mountain regions, understanding the relationship between genotype and environmental conditions may be crucial to optimising the cultivation of high quality fruits which can satisfy the requirements of the market. Accordingly, this study aimed to characterise the fruit quality of four newly released June-bearing cultivars (viz. Antea, Asia, Clery and Matis) when plants were grown on two different production sites at different altitudes.

2 Material and methods

2.1 Cultivation sites

The trials were conducted at two different sites in Switzerland (Conthey, 46°12’ N / 7°18’ E and Bruson, 46°04’ N / 7°18’E) during 2008, characterized by different soil and climatic conditions but principally differing in elevation over sea level (Table 1) and different harvest periods.
2.2 Plant materials and cultivation practices

The cultivars used in this study were selected for their genetic diversity (different parentages) and growing commercial importance. A+ frigo plants from cultivars Antea (FB6L-3 x Onebor, Salvi Vivai, Italy), Clery ((Elsanta x FB6L-3) x (Agathe x Sweet Charlie), Salvi Vivai, Italy) and Matis (Mara Des Bois x (Douglas x Belrubi), Marionnet, France) were planted at the beginning of June 2007 in Bruson and one month later in Conthey. The earlier plantation in Bruson was done due to the earlier start of winter in the mountain region. For Asia (Maya x selection, New Fruits, Italy), plug plants were planted beginning of August for both sites. Plants were planted on raised beds covered with black plastic mulch at a density of 4 plants per m². Distance between the raised beds was 1.25 m (centre to centre) and strawberries were planted in one row with a distance of 0.2 m (Crespo et al. 2009). For each cultivar four replications of twenty eight plants were planted in both production sites. During plant growth and fruit production the same fertigation and phytosanitary treatments were applied for both sites. Water and nutrients were given by fertigation with drip irrigation at a flow of 1 L/h with emitters spaced 0.2 m apart (T-Tape, T-systems, USA). Nutrients were applied once a week during the growing period based on the recommendations for strawberries with a yield of 2 kg/m² (total nutrients applied: 100 N, 15 P, 50 K and 20 Mg kg/ha). Vacuum gauge tensiometers (Irrometer Co., Riverside, USA)
were used to schedule irrigation at 200 hPa measured at 0.2 m soil depth in the middle of the raised beds below the drip tube. Applications of phytosanitary treatments were made according the Swiss Integrated Production System (Steffek et al. 2003) to control spider mites, powdery mildew and grey mould.

2.3 Fruit sampling

Fruits were harvested three days a week and the yield at each harvest recorded. The total leaf area of five consecutive plants per replication was measured at the end of the harvest with an I-3100 area meter (LI-COR Biosciences, inc. Lincoln, Nebraska, USA). Samples for analysis were taken 14 days after the first harvest and mainly consisted of secondary and tertiary fully ripe fruits (when fully red). The samples were prepared for further analysis as described by Tulipani et al. (2008). Briefly, within three hours following harvesting, the samples (whole fruit) were stored at -20 °C for one month. The frozen berries were then dipped into liquid nitrogen and milled with a pre cooled laboratory blender (IKA A 11 basic, Staufen, Germany). The obtained powder was subsequently stored at -80 °C until analysis. The determination of total antioxidant capacity and vitamin C was conducted on wet samples, whereas determination of individual anthocyanins, organic acids and sugars was performed on freeze-dried samples as described by Terry et al. (2007).

2.4 Analysis of sugars

Sugars were extracted using 62.5% (v/v) aqueous methanol as described by Terry et al. (2007). Sugar content in strawberry extracts was determined using an Agilent 1200 series HPLC binary pump system (Agilent, Berks., UK), equipped with an Agilent refractive index detector (RID) G1362A. Strawberry extracts (20 μl) were diluted (1:10), and injected into a Rezex RCM monosaccharide Ca+ (8%) column of 300 mm x 7.8 mm diameter (Phenomenex, California, USA; Part no. 00H-0130-K0) with a Carbo-Ca2+ guard column of 4 mm x 3 mm diameter (Phenomenex; Part no. AJ0-4493). Temperature of the column was set at 80 ºC using a G1316A thermostated column compartment. The mobile phase used was HPLC grade water at a flow rate of 0.6 ml/min (Terry et al., 2007, Giné Bordonaba & Terry, 2008). Temperature of the optical unit in the detector was set up at 30 ºC and temperature of the autosampler at 4 ºC using an Agilent cooled autosampler G1330B. The presence and abundance of fructose, glucose and sucrose were automatically calculated by comparing sample peak area to standards (0.025-2.5 mg/ml) using ChemStation Rev. B.02.01.

The sweetness index was calculated by multiplying the sweetness coefficient of each individual sugar (glucose = 1, fructose = 2.3 and sucrose = 1.35) as described by Keutgen & Pawelzik (2007).
2.5 Analysis of malic, citric and ascorbic acid

Extracts for organic acids determination were prepared as described elsewhere (Terry et al. 2007, Giné Bordonaba & Terry 2008). Briefly, freeze-dried strawberry extracts (50 mg) were dissolved into 3 ml of HPLC grade water. Samples were kept at room temperature (25 ºC) for 10 min and then filtered through a 0.2 µm syringe driven filter (Millipore Corporation, Massachusetts, USA). Citric, malic acid and ascorbic acid contents in extracts were detected at 210 nm using the same HPLC system as described earlier, in this case equipped with an Agilent DAD G1315B/G1365B photodiode array with multiple wavelengths detector. Extracts (20 µl) were injected into an Alltech Prevail Organic Acid column 250 mm x 4.6 mm diameter, 5 µm particle size (Alltech, California, USA; Part no. 88645) with an Alltech Prevail Organic Acid guard column of 7.5 mm x 4.6 mm diameter (Alltech; Part no. 96429). The mobile phase was analytical grade degassed 0.2% (w/v) metaphosphoric acid in H2O (Giné Bordonaba & Terry, 2008). The flow rate of the mobile phase was 1 ml/min under isocratic conditions and the column temperature was set up at 35 ºC. The presence and quantity of ascorbic, citric and malic acid was calculated against a calibration curve obtained by using external standards for each acid (0.02-2.0 mg/ml) using ChemStation Rev. B.02.01.

2.6 Analysis of total vitamin C

The vitamin C was determined in strawberry samples before freeze drying (fresh frozen). The determination of vitamin C included the ascorbic acid (AsA) and the dehydroascorbic acid (DHAA) form. Vitamin C was extracted with a phosphate buffer solution (36 mM, pH 5.0) containing 1 g/L DL-Dithiothreitol (Fluka 43819, Sigma-Aldrich, Switzerland) during one hour at room temperature allowing the reduction of DHAA into ASA (Brause et al. 2003). Extracts were filtered through a 0.45 µm filter and injected on a Varian Prostar 230 HPLC pump system equipped with a diode array detector Varian Prostar 335 and a reverse phase 18 column (Nucleodur C18, 4.5 x 250 mm, Macherey-Nagel, Switzerland) with a flow rate of 0.6 ml/min. The mobile phase consisted of the same buffer adjusted to a pH value of 2.5 to maintain AsA in the reduced form. The absorbance was measured with an UV-detector at 254 nm and the AsA peak area was quantified with the Software Galaxie Chromatography Data System Vers.1.9-Rev.2 on the basis of an external standard calibration curve (0-60 mg/L).

2.7 Analysis of individual anthocyanins

Individual anthocyanins were extracted as described elsewhere (Terry et al. 2007, Giné Bordonaba & Terry 2008) by mixing 150 mg of freeze-dried sample with 3 ml of 70% (v/v) methanol and 0.5% (v/v) HCl in HPLC-grade water. The slurry obtained was held at 35 ºC in a water bath with constant
shaking for 1.5 h; mixing the samples every 15 min. Finally, the flocculate obtained was filtered through a 0.2 μm Millex-GV syringe driven filter unit and the clear extract analyzed by HPLC. The anthocyanin profile of strawberry fruits was determined according to the method described by Gine Bordonaba et al. (publication submitted). Briefly, the separation was performed on an Agilent 1200 series system as described for organic acids determination. Strawberry diluted (1:5 v:v) extracts were injected (10 μl) into a Zorbax Eclipse XDB-C18 column of 250 mm x 4.6 mm diameter, 5 μm particle size with an XDB-C18 guard column of 12.5 mm x 4.6 mm diameter. The mobile phase consisted of 2% (v/v) acetic acid in HPLC-grade water (A) and 2% (v/v) trifluoroacetic in methanol (B). Flow rate, column temperature and temperature of the autosampler were set up at 1 ml/min, 35 ºC, and 4 ºC, respectively. Finally, eluted anthocyanins were detected at 520 nm. The presence and abundance of cya-3-gluc and pg-3-gluc was calculated by comparing peak area against a calibration curve obtained by using external standards of cya-3-gluc and pg-3-gluc respectively (Extrasynthese, Lyon, France) and Agilent ChemStation Rev. B.02.01. Unknown peaks were quantified using the external calibration curve of pg-3-gluc.

2.8 Extraction and quantification of total antioxidant capacity

Antioxidants were extracted according to the method of Tulipani et al. (2008) with slight modifications. Briefly, 5 g of snap-frozen strawberry powder (wet) were weighted in 25 g of the extraction solution containing 80% methanol and 1% formic acid in water (v:v). The obtained slurry was sonicated in a cooled water bath during 15 minutes then centrifuged 5 minutes at 10000 x g. The supernatant was filtered through a LS 14 ½ filter (Schleicher and Schuell, Germany).

The determination of antioxidant capacity with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is based on the properties of DPPH which in its radical form has an absorption band at 517 nm and disappears upon reduction by an antiradical compound. The determination of antioxidant capacity was performed according to the method described by Brandwilliams et al. (1995). All chemicals used in this section were purchased by Sigma-Aldrich (Buchs, Switzerland). Briefly, 100 μl extracts were added to 10 ml of a 0.1 mM DPPH solution stirred well and leaved to react at room temperature. The absorption at 517 nm was measured after 30 minute against blank. Quantification was performed with a Trolox standard calibration curve (0-2.4 μmol/ml) and the results were calculated in μmol Trolox Equivalent (TE)/g FW or DW.

2.9 Data analysis

All statistical analyses were carried out using XLSTAT Version 2007.5 (Addinsoft, Paris, France). All data were subjected to a two-way analysis of variance and the means were compared using Tukey test at significant level of 95% (P = 0.05). Relationships between factors were analysed by the Simple
3 Results and discussion

3.1 Variation among cultivars in taste and health related compounds

In the present study, strawberry compounds directly related to taste- or health-related properties of the fruit strongly differed between genotypes (Table 2). On a FW basis, Asia showed the highest total sugar content (51.8 mg/g FW mean of both sites) followed by Clery (48.3 mg/g FW), Antea (41.4 mg/g FW) and Matis (40.9 mg/g FW). Three main sugars (viz. fructose, glucose and sucrose) were quantified in each cultivar with fructose being quantitatively the most important. The proportion of each individual sugar to the total sugar concentration was similar in Antea and Asia. Lower concentrations of sucrose as compared to the rest of cultivars were encountered in Matis that resulted in fruits from this genotype having the greatest monosaccharide/disaccharide ratio (5.2 relative units). Despite differences in the sugar distribution between the cultivars, the cultivar ranking for the sweetness index was similar to the cultivar ranking for the total sugar content ranging from 81.7 to 65.5 relative units.
Characterisation of major taste and health related compounds of four strawberry genotypes
grown at two different Swiss production sites

Table 2: Sugars content of strawberry fruits on a fresh weight (FW) and dry matter (DM) basis from different cultivars and production sites. The sweetness index was calculated on the fresh weight basis.

<table>
<thead>
<tr>
<th>cultivar</th>
<th>site</th>
<th>sucrose (mg/g FW)</th>
<th>glucose (mg/g FW)</th>
<th>fructose (mg/g FW)</th>
<th>total sugars (mg/g FW)</th>
<th>(fru+glu)/sucrose</th>
<th>sweetness index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antea</td>
<td>Conthey</td>
<td>14.7 a</td>
<td>16.0 a</td>
<td>18.3 a</td>
<td>49.0 a</td>
<td>2.6 b,c</td>
<td>77.9 a</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>12.0 a b c</td>
<td>10.1 b</td>
<td>11.6 b</td>
<td>33.7 b</td>
<td>1.8 c</td>
<td>53.1 b</td>
</tr>
<tr>
<td>Asia</td>
<td>Conthey</td>
<td>14.0 a b</td>
<td>18.1 a</td>
<td>19.6 a</td>
<td>51.5 a</td>
<td>2.9 b,c</td>
<td>81.9 a</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>17.9 a</td>
<td>16.2 a</td>
<td>17.8 a</td>
<td>52.0 a</td>
<td>1.9 c</td>
<td>81.5 a</td>
</tr>
<tr>
<td>Clery</td>
<td>Conthey</td>
<td>11.9 a b c</td>
<td>18.2 a</td>
<td>20.6 a</td>
<td>50.9 a</td>
<td>3.3 b</td>
<td>83.4 a</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>13.1 a b</td>
<td>15.3 a</td>
<td>17.2 a</td>
<td>45.6 a b</td>
<td>2.5 b,c</td>
<td>72.6 a</td>
</tr>
<tr>
<td>Matis</td>
<td>Conthey</td>
<td>7.2 b c</td>
<td>16.0 a</td>
<td>18.2 a</td>
<td>41.4 a b</td>
<td>4.9 a</td>
<td>67.6 a b</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>6.3 c</td>
<td>16.0 a</td>
<td>18.0 a</td>
<td>40.3 a b</td>
<td>5.4 a</td>
<td>65.9 a b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cultivar (C)</th>
<th>site (S)</th>
<th>C x S</th>
</tr>
</thead>
<tbody>
<tr>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>***</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sugar content is an important taste attribute for strawberries and is highly correlated with consumer acceptance (Azodanlou et al. 2003, Jouquand et al. 2008). To date, no information is available on the sugar and acid content of fruits from these newly released cultivars (viz. Antea, Asia, Clery, Matis) however fructose and glucose contents were comparable to that reported for other cultivars (Perez et al. 1997, Kallio et al. 2000, Wang et al. 2002, Skupien & Oszmiański 2004, Davik et al. 2006, Keutgen & Pawelzik 2008, Giné Bordonaba & Terry 2009, Giné Bordonaba & Terry 2010). This said, the concentrations of sucrose described herein were higher than that found in some of the above-mentioned studies. Besides differences among cultivars, greater sucrose content may be partially attributed to differences in the extraction procedures used between this and other studies; the greater solubility of sucrose in methanol as compared to fructose and glucose has already been pointed out by others (Terry et al. 2007, Giné Bordonaba and Terry 2009).
Taste in strawberry fruits is, however, not only influenced by sugars. Acids within the fruit are part of the soluble solids pool and are also important contributors to strawberry taste and flavour (Cordenunsi et al. 2002). In the present study, three major organic acids were found within the cultivars studied corresponding to citric, malic and ascorbic acid (Table 3). Citric acid was the major acid, with concentrations on a FW basis ranging from 3.8 mg/g FW (Matis) to 5.7 mg/g FW (Asia) and accounting for 62.7% (Clery) to 71.7% (Matis) of total acid content, and was in agreement with that found in the literature (Perez et al. 1997, Terry et al. 2007, Keutgen & Pawelzik 2008, Giné Bordonaba & Terry 2009). Similarly, malic acid concentrations were ca. 24% of the total acid concentration and significantly differed between cultivars. Antea had 1.5-fold greater malic acid content than Matis. As compared to other acids, ascorbic acid (AsA) was present in all cultivars in lower actual amounts (ca. 7% of total acids). The AsA contents were also highly variable between the genotypes studied: the greatest content of AsA was encountered in fruits from cultivar Antea (0.6 mg/g FW), showing 1.7 more AsA than Matis. Similarly, up to 2-fold difference was found by Tulipani et al. (2008) when comparing nine strawberry cultivars and selections, with contents varying from 0.3 to 0.5 mg/g FW. Variations in organic acid metabolism have been reported for many fruits (Zheng et al. 2009) and several genetic studies have shown that the accumulation of organic acids (i.e. malic acid) is controlled by genes which differ not only between species but also between cultivars (Saradhulhat & Paull 2007).
Characterisation of major taste and health related compounds of four strawberry genotypes
grown at two different Swiss production sites

Table 3: Organic acids and vitamin C contents of strawberry fruits on a fresh weight (FW) and dry matter (DM) basis from different cultivars and production sites.

<table>
<thead>
<tr>
<th>cultivar</th>
<th>site</th>
<th>malic acid (mg/g FW)</th>
<th>citric acid (mg/g FW)</th>
<th>ascorbic acid (mg/g FW)</th>
<th>total acids (mg/g FW)</th>
<th>vitamin C (mg/g FW)</th>
<th>sugar:acid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antea</td>
<td>Conthey</td>
<td>1.9 a</td>
<td>4.8 abc</td>
<td>0.7 a</td>
<td>7.4 abc</td>
<td>0.9 a</td>
<td>6.7 abc</td>
</tr>
<tr>
<td></td>
<td>Brunson</td>
<td>1.8 a</td>
<td>3.6 cd</td>
<td>0.5 ab</td>
<td>6.0 bcd</td>
<td>0.7 b</td>
<td>5.7 c</td>
</tr>
<tr>
<td>Asia</td>
<td>Conthey</td>
<td>1.8 a</td>
<td>6.1 a</td>
<td>0.5 b</td>
<td>8.4 a</td>
<td>0.7 b</td>
<td>6.2 bc</td>
</tr>
<tr>
<td></td>
<td>Brunson</td>
<td>1.8 a</td>
<td>5.3 ab</td>
<td>0.5 b</td>
<td>7.6 ab</td>
<td>0.6 bc</td>
<td>6.9 ab</td>
</tr>
<tr>
<td>Clery</td>
<td>Conthey</td>
<td>1.2 ab</td>
<td>4.6 bcd</td>
<td>0.5 b</td>
<td>6.3 bcd</td>
<td>0.7 b</td>
<td>8.2 ab</td>
</tr>
<tr>
<td></td>
<td>Brunson</td>
<td>1.4 ab</td>
<td>3.9 cd</td>
<td>0.4 bc</td>
<td>5.7 cd</td>
<td>0.5 bcd</td>
<td>8.0 ab</td>
</tr>
<tr>
<td>Matis</td>
<td>Conthey</td>
<td>1.5 ab</td>
<td>4.1 bcd</td>
<td>0.3 cd</td>
<td>5.8 cd</td>
<td>0.4 cd</td>
<td>7.1 abc</td>
</tr>
<tr>
<td></td>
<td>Brunson</td>
<td>1.1 b</td>
<td>3.5 d</td>
<td>0.2 d</td>
<td>4.8 d</td>
<td>0.4 d</td>
<td>8.6 a</td>
</tr>
</tbody>
</table>

Because of its effect on perceived sweetness the ratio between sugar and acids in strawberries and other berries can act as an important indicator of fruit taste (Terry et al. 2005, Giné Bordonaba & Terry 2008), fruit ripeness (Perez et al. 1997) or even as an index of consumer acceptability (Keutgen & Pawelzik 2007). Values from this study were comparable to those found in the literature (Davik et al. 2006, Terry et al. 2007). This said, the sugar:acid ratios reported by Davik et al. (2006) were slightly lower than those reported in this study (between 5.4 and 6.5) which was mainly due to higher acid contents founds in their cultivars (viz. Polka Korona, Aurora, Babette, Carmen, Hanibal and four Norwegian advanced selections) or maybe related to different growing conditions.

In this study, the vitamin C content in frozen fruit samples was determined after reducing DHAA into AsA. Strong variations were found in the vitamin C content of the different cultivars (Table 3). The
vitamin C content in fruits results from a balance between synthesis and degradation. The in situ synthesis occurs in strawberries from D-galacturonic acid, a component of cell wall pectins during fruit ripening and the final concentration in the fruits depends on the expression of the gene GalUR as well as the availability of the substrate D-galacturonic acid (Agius et al. 2003). The first step of vitamin C degradation is the oxidation of AsA into DHAA, however, both forms of vitamin C are nutritionally available for utilisation as ascorbate (Welch et al. 1995).

Strawberry fruits are important sources of health-related compounds, from which, in the present work, special attention was given to anthocyanins and vitamin C concentrations. The HPLC method described herein allowed separation of five anthocyanin compounds (Fig. 1). Cya-3-gluc and pg-3-gluc were identified and quantified by comparing retention time and UV/VIS spectra (240-640 nm) with external standards. Other unknown peaks, mainly peaks 1, 2 and 3 were quantified as pg-derivatives using pg-3-gluc as standard and given the similarities of their UV/VIS-spectra with that of pg-3-gluc standard (data not shown). Differences existed not only in the actual amounts of anthocyanins detected among different genotypes but also in their anthocyanin profile (Table 4; Fig. 1). Pg-3-gluc remained quantitatively the main anthocyanin found in the strawberry extracts. Its concentration varied from 129 $\mu$g/g FW (Antea) to 181.8 $\mu$g/g FW (Asia) and represented between 75% and 93.8% of the total anthocyanin content. Pg-derivative 2 concentration in Matis and Clery reached 23.7 $\mu$g/g FW and 54.8 $\mu$g/g FW respectively (13.7% and 23.1% of the total anthocyanin content). This said, this specific anthocyanin was not detected in Antea and Asia. Similarly, pg-derivative 3 was only detected in Asia but not in the others. Pg-derivative 1 was ubiquitously present in all the cultivars studied representing a marginal 3% of the total anthocyanin concentration. Clery showed the highest total anthocyanin content among the cultivars investigated with 1.7-fold greater concentrations than that of Antea which, indeed, showed the lowest total content (Table 4).

Reported anthocyanin concentrations for strawberry fruits differ markedly (Lopez da Silva et al. 2007, Hernanz et al. 2007, Terry et al. 2007). The total anthocyanin contents found in this study were lower than the contents reported in five strawberry cultivars by Lopez da Silva et al. (2007), but slightly higher than the values reported by Hernanz et al. (2007). Despite the fact that different genotypes were used in each study, the higher values obtained by Lopez da Silva et al. (2007) may be partially explained by the different extraction procedure used as the extraction was repeated several times until complete removal of the colour was achieved. In contrast, in the present study, and as earlier reported by Hernanz et al. (2007), only one extraction step was performed despite still achieving total removal of the colour from the extracts. Further pelargonidin derivatives such as pg-3-rut, pg-3-malonylglicoside and pg-3-acetylglucoside have been identified in strawberries by other authors (Aaby et al. 2007, Lopez da Silva et al. 2007, Yoshida et al. 2002, Hernanz et al. 2007). The elution order of the pelargonidin derivatives and their occurrence in strawberry cultivars reported by others
Characterisation of major taste and health related compounds of four strawberry genotypes grown at two different Swiss production sites

strongly suggest pg derivative 1 to be pg-3-rut. However, to further corroborate this assumption the identification of the substituting sugar by mass spectra analysis would be necessary.

**Fig. 1:** Detected anthocyanins in four strawberry cultivars by means of HPLC-DAD (520 nm). Unknown peaks [1], [2] and [3] were identified as three pg-derivatives according to their UV/VIS spectra.
## Characterisation of major taste and health related compounds of four strawberry genotypes grown at two different Swiss production sites

Table 4: Anthocyanins contents and antioxidant capacity of strawberry fruits on a fresh weight (FW) and dry matter (DM) basis from different cultivars and production sites.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Site</th>
<th>Cya-3-gluc</th>
<th>Pg-3-gluc</th>
<th>Pg derivative 1</th>
<th>Pg derivative 2</th>
<th>Pg derivative 3</th>
<th>Total anthocyanin</th>
<th>Antioxidant capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/g FW</td>
<td>µmol TE/g FW</td>
<td>µg/g DM</td>
<td>µmol TE/g DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antea</td>
<td>Conthey</td>
<td>7.1 a</td>
<td>145.7 abc</td>
<td>5.1 b</td>
<td>0</td>
<td>0</td>
<td>157.9 cd</td>
<td>15.2 a</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>2.1 cd</td>
<td>112.3 c</td>
<td>3.2 c</td>
<td>0</td>
<td>0</td>
<td>117.7 d</td>
<td>11.4 b</td>
</tr>
<tr>
<td>Asia</td>
<td>Conthey</td>
<td>0.0 d</td>
<td>176.5 ab</td>
<td>8.4 a</td>
<td>0</td>
<td>3.8 a</td>
<td>188.7 bc</td>
<td>9.8 bcd</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>0.9 d</td>
<td>187.2 a</td>
<td>8.2 a</td>
<td>0</td>
<td>4.9 a</td>
<td>201.3 abc</td>
<td>9.8 bcd</td>
</tr>
<tr>
<td>Clery</td>
<td>Conthey</td>
<td>0.0 d</td>
<td>167.1 ab</td>
<td>5.1 bc</td>
<td>50.0 a</td>
<td>0</td>
<td>222.2 ab</td>
<td>10.6 bc</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>0.0 d</td>
<td>187.2 a</td>
<td>3.8 bc</td>
<td>59.6 a</td>
<td>0</td>
<td>250.6 a</td>
<td>8.9 cde</td>
</tr>
<tr>
<td>Matis</td>
<td>Conthey</td>
<td>4.9 ab</td>
<td>140.3 bc</td>
<td>4.1 bc</td>
<td>22.5 b</td>
<td>0</td>
<td>171.8 bcd</td>
<td>8.3 de</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>3.7 bc</td>
<td>141.1 bc</td>
<td>4.1 bc</td>
<td>24.9 b</td>
<td>0</td>
<td>173.9 bc</td>
<td>6.9 e</td>
</tr>
</tbody>
</table>

Different letters in a same column indicate significant differences. Significant parameters are indicated as follow:

**p<0.001, *p<0.01, **p<0.05, ns not significant. Antioxidant capacity is expressed in µmol Trolox Equivalent (TE) per g fresh weight or dry matter.

The total antioxidant capacity measured in fruits depends on the presence of oxygen radical scavengers such as phenolic compounds and vitamin C presents in the fruit tissues. Antioxidant capacity was highest in the Antea (13.3 µmol TE/g FW) followed by Asia and Clery (both 9.8 µmol TE/g FW) and lowest in Matis (7.6 µmol TE/g) (Table 4). The antioxidant capacity correlated strongly with the total vitamin C content of the samples ($r^2 = 0.84$) but not with their total or individual anthocyanin content ($r^2<0.1$).
3.2 Effect of production sites on agronomical traits and fruit taste and health related compounds

Agronomical traits such as yield, harvest duration and leaf area per plant were significantly affected by the production site (Table 5). Generally plants grown in the mountain region of Bruson had a higher mean yield (133 g more per plant), a shorter harvest period (4.7 days less) and a larger leaf area (525 cm² more per plant) compared to the plants grown in Conthey. Furthermore, Antea, Clery and Matis showed a higher leaf area/yield ratio when cultivated in Bruson. However, the higher leaf area/yield ratio in Bruson did not increase the content of sugars in the fruits compared to Conthey (Table 2). Despite of soluble solid content not always being well correlated with actual sugar concentrations in strawberry (Giné Bordonaba & Terry 2009) or other berry fruits (Giné Bordonaba & Terry 2008), this finding was in contrast with earlier findings by Carlen et al. (2007) where leaf area/yield ratio of strawberry cultivars was positively related to their fruit soluble solid content. Cloudier weather with more rain and less sunshine during the ripening period (Table 1) or the higher yield per day (Table 5) observed in Bruson, may also account for the discrepancies between this and earlier works (Carlen et al. 2007). These trends were consistent for all the cultivars except for Asia whereby high leaf area/yield ratio was recorded in both production sites probably due to the low yield of this cultivar and the late plantation of plug plants.

Table 5: Strawberry yield, harvest duration, yield per day, leaf area, leaf area/yield ratio of different cultivars and production sites.

<table>
<thead>
<tr>
<th>cultivar</th>
<th>site</th>
<th>yield</th>
<th>harvest duration</th>
<th>yield per day</th>
<th>leaf area per plant</th>
<th>leaf area/yield ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/plant</td>
<td>days</td>
<td>g/day</td>
<td>cm²</td>
<td>cm²/g</td>
<td></td>
</tr>
<tr>
<td>Antea</td>
<td>Conthey</td>
<td>309.2 b c d</td>
<td>24 b c</td>
<td>13.2 b c</td>
<td>2056 c</td>
<td>6.6 c</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>545.8 a b</td>
<td>20 c</td>
<td>27.9 a</td>
<td>7086 a</td>
<td>13.1 a b</td>
</tr>
<tr>
<td>Asia</td>
<td>Conthey</td>
<td>172.5 d</td>
<td>22 c</td>
<td>8.1 c</td>
<td>3238 c</td>
<td>18.9 a</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>248.9 c d</td>
<td>20 c</td>
<td>12.7 b c</td>
<td>4119 b c</td>
<td>17.0 a</td>
</tr>
<tr>
<td>Clery</td>
<td>Conthey</td>
<td>487.2 a b c</td>
<td>29 a b</td>
<td>16.9 a b c</td>
<td>4209 b c</td>
<td>9.3 b c</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>436.3 a b c d</td>
<td>22 c</td>
<td>20.2 a b</td>
<td>7035 a</td>
<td>16.3 a</td>
</tr>
<tr>
<td>Matis</td>
<td>Conthey</td>
<td>385.5 b c d</td>
<td>32 a</td>
<td>11.9 b c</td>
<td>1927 c</td>
<td>5.2 c</td>
</tr>
<tr>
<td></td>
<td>Bruson</td>
<td>660.5 a</td>
<td>26 b c</td>
<td>26.0 a</td>
<td>6222 a b</td>
<td>9.6 b c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cultivar (C)</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>*</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>site (S)</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>C x S</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Different letters in a same column indicate significant differences. Significant parameters are indicated as follow:

***p<0.001, **p<0.01, *p<0.05, ns not significant.

The production site had a significant effect on the content of monossaccharides of the different cultivars investigated. This said, such an effect was observed to be genotype specific (Table 2) with
greater differences between production sites encountered in Antea. Specifically, fruits from this showed significantly lower glucose and fructose content when plants were grown in the mountain region and hence potentially could have resulted in lower sweetness of the fruits (Table 2). Similarly, other studies (Terry et al. 2007, Giné Bordonaba & Terry 2009) have shown that preharvest factors generally resulted in changes in the monosaccharide, but not of sucrose concentration of the fruit. Thus, such changes may indicate the impact of preharvest conditions on respiratory metabolism in which sugars, especially glucose and fructose, are the main substrates.

Organic acid content of the fruits was generally affected by production site however no genotype x production site interaction was found and the observed differences regarded mainly the results in the fresh matter (Table 3). Significant differences among production sites were found for citric acid, in which for all cultivars lower values (4.1 mg/g FW vs. 4.9 mg/g FW, mean values for all cultivars) were observed in fruits from plants grown at the region of Conthey. There was a clear trend towards greater malic acid content, on a DM basis, in fruits obtained in the mountain region except for Matis, where the opposite was observed (Table 3). Similarly, Zheng et al. (2009) found significant differences in both malic and citric acid but not for ascorbic and total acid content in blackcurrant berries grown at two different latitudes, besides the observed effects were also cultivar dependent. In addition the same authors found citric and total acid concentrations to be negatively correlated with high humidity. Davik et al. (2006) found similar results with the acid content of strawberries being positively influenced by the radiation and the number of sunshine hours the day before the harvest.

Vitamin C is often regarded as one of the main health-related compounds present in strawberry fruits and is mainly responsible for the antioxidant capacity of strawberry fruits as showed above. Its content was detrimentally affected when plants were grown at in the mountain region of Bruson with values ca. 1.2-fold lower than those obtained in the lower region (Table 3). Furthermore in Bruson, the plants produced a higher yield during a shorter period (Table 5). Thus, a negative correlation was found between strawberry yield per day (g/day) and the total vitamin C in the fresh weight for each cultivar (Antea $r^2 = 0.92$, Asia $r^2 = 0.57$, Clery $r^2 = 0.74$, Matis $r^2 = 0.69$). The differences between production sites levelled off when considering vitamin C content on a dry matter basis (Table 3) suggesting a dilution of the vitamin C in the fruits.

The relative distribution of the anthocyanin compounds presents in each cultivar was consistent with the different production region. This result suggests that specific anthocyanin profile is mainly genetically inherited rather than being affected by external environmental factors. Similar results were found by Carbone et al. (2009) when the flavonoid composition of different strawberry genotypes grown at different locations within Italy was compared. The authors found that the general variation for anthocyanins in strawberry ripe fruits was affected largely by the genetic background than by environmental factors.
The concentration of minor anthocyanins on a fresh weight basis such as cya-3-gluc, pg-derivative 1 and pg-derivative 2 was significantly affected by the production site (Table 4). The differences were still significant when considering the concentration in the dry matter for cya-3-gluc and pg-derivative 2 but not for pg-derivative 1. Antea showed lower cya-3-gluc contents in both fresh and dry matter when cultivated in Bruson, while higher contents of pg-derivative 2 in the dry matter were observed in Clery. In contrast, the content of the main strawberry anthocyanin, pg-3-gluc and accordingly the total anthocyanin content was not particularly affected by the location where the plants were grown. To date no other studies have analysed the impact that production sites, principally differing in altitude, have on strawberry anthocyanins. Nevertheless, a similar study was conducted on berries of Vaccinium myrtillus (Rieger et al. 2008) in which lower amounts of anthocyanins were found in the berries collected from regions at higher altitude.

4 Conclusions

The variability in the composition of fruits from different cultivars grown in different regions strongly suggests that cultivars should be carefully selected for each production site. Based on one year data, the results presented in this work suggested that the newly released cultivar Clery may be a suitable cultivar for its high anthocyanin levels as well as for the great stability of its chemical composition regardless of the cultivation site. In this context, the selection of environmentally-adaptable cultivars such as Clery, may be of crucial importance given the expected effects that general climate changes and yearly climatic variation may have on berry production within Europe or elsewhere. Similarly, the high ascorbic acid content of Antea could be a promising property; however its potential sensitivity to production site has to be taken into consideration when selecting this cultivar. Finally, the results presented in this work corroborate the dominant role of strawberry genotype over environmental factors. Further work should address the impact that other environmental factors as well as the year-to-year variation may have on the final quality of strawberry fruits.
Evolution of fruit quality during the harvest: influence of the fruit order and time from flowering to harvest
1 Introduction

Strawberry fruit chemical composition is an important aspect determining taste and health value of strawberry fruits. In particular sugars, acids and antioxidants such as vitamin C and phenolic compounds have been shown to be important taste and health related parameters (Kallio et al. 2000, Azodanlou et al. 2003, Hannum 2004). However, according to a large Swiss food retailer 26% of the consumers are often disappointed and 33% are sometimes disappointed by strawberry quality (Azodanlou et al. 2003) revealing a high fluctuation of the fruit quality.

To improve the consistency of the fruit quality, there is a need to explore the cause of heterogeneity of the inner quality traits of strawberry fruits. One source for variation in taste and health related compounds are genotypic and environmental effects which have already been studied in the previous chapters. However variations in quality within a same genotype are also being reported and they can be responsible for the inconsistencies in genotype evaluation (Ford et al. 1997, Lopez da Silva et al. 2007). Therefore other factors have to be elucidated which could affect strawberry quality at harvest. It has been shown that fruit maturity at harvest can be a possible explanation for the variability in taste related compounds within a same cultivar (Sturm et al. 2003). Further plant physiological factors such as source-sink relations, which reflect assimilates availability for the developing fruit, may also play a role in the chemical composition of the fruits.

June bearing cultivars are harvested during three to six weeks and the fruit load on the plant strongly changes during this harvest period. As a consequence the source-sink relation varies during the harvest and may affect the fruits composition. Fruits of higher rank numbers (secondary and tertiary fruits) ripen later than primary fruits and differ in their single fruit weight and achenes number per fruit (Hansen 1989). Additionally Hansen observed that fruits of higher rank number have lower dry matter concentration than primary fruits. These previous findings suggest that fruit quality is affected by the harvest period but little is known about the changes in the chemical composition of the fruits.

The aim of this study was first to evaluate the plant to plant variability of taste and health related fruit quality traits in a standard cultivar (Clery). The second aim was to assess the evolution of those quality traits during the harvest period. The third aim was to elucidate whether the variation in quality during the harvest can be explained by the changing proportion of primary secondary and tertiary fruits, by the decreasing fruit size or by the time from flowering to full ripeness.
2 Material and methods

2.1 Plant material and sampling

2.1.1 Plant to plant variability assessment

Plant to plant variability was assessed by growing A+ frigo plants of cultivar Clery in an open field without tunnel covering in Conthey during the 2009 season. Thirty plants were planted at the same time (in June 2008) on raised beds covered with black plastic mulch in one row at a density of four plants per m². Each plant was marked and harvested individually. The fruits for analysis were collected at mid harvest from each plant independently and immediately snap-frozen in liquid N₂. The frozen fruit samples were milled wet with a laboratory blender (IKA® A 11 basic, Staufen, D). The obtained powder was transferred to plastic bottles (transparent PE-LD with screw cap, Semadeni, Switzerland) and stored in the dark at -80 °C until analysis. Soluble solid contents (SSC), titratable acidity, total anthocyanin contents and antioxidant capacity were measured as described below to assess the range of plant individual variation. Additionally the foliar surface of each plant was measured at harvest end a leaf area meter (Area meter 300, LiCor).

2.1.2 Harvest period - fruit order experiment

In this experiment the weekly evolution of the fruit quality during strawberry harvest and the role of the fruit order were assessed simultaneously. The experiment was conducted under plastic tunnel during two years in 2008 and 2009. The quality assessment focussed on soluble solid contents (SSC), titratable acidity, antioxidant capacity and total anthocyanin contents.

Plug plants of cultivar Clery, a standard cultivar widely grown in the Valais region, were planted each year on raised beds covered with black plastic mulch in one row plots each containing 35 to 37 plants at a density of 4 plants/m². Rows were separated by 1.25 m and the raised bed had an inner width and height of 30 and 15 cm respectively. The plants were arranged in 4 repetitions. The tunnel were 5 m wide, 3 m high and 52 m long, covered with new polyethylene plastic sheet (200 mu, 7.25 EVA Patilux, Italy) each year at beginning of March. The tunnel ends and the sides remained open during the day when temperature inside reached 20 °C.

Water and nutrients were given by fertigation (drip irrigation, T-Tape with emitters spaced 0.3 m apart) based on the recommendations for strawberries with a fruit yield of 2 kg/m²: 100 N, 15 P 50 K and 20 Mg kg/ha. Phytosanitary treatments were made according the Integrated Production System (Steffek et al. 2003)
Evolution of fruit quality during the harvest: influence of the fruit order and time from flowering to harvest

Three times per week, fully ripe strawberry primary secondary and tertiary fruits were harvested separately according to their position on the plant (Fig. 1).

![Fig. 1: Strawberry fruting cluster. 1 = primary fruit, 2 = secondary fruits, 3 = tertiary fruits.](image)

At each harvest the yield and single fruit weight of each fruit type was recorded and the whole fruits were frozen separately at -20 °C within three hours following harvesting. At the end of each week the fruits of the three harvests were pooled according to their order as presented in Table 1.

**Table 1: Sampling of the fruit from the different harvests.**

<table>
<thead>
<tr>
<th>week</th>
<th>primary fruits</th>
<th>secondary fruits</th>
<th>tertiary fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 1</td>
<td>P 1</td>
<td>S 1</td>
<td>not available</td>
</tr>
<tr>
<td>week 2</td>
<td>P 2</td>
<td>S 2</td>
<td>T 2</td>
</tr>
<tr>
<td>week 3</td>
<td>P 3</td>
<td>S 3</td>
<td>T 3</td>
</tr>
<tr>
<td>week 4</td>
<td>P 4</td>
<td>S 4</td>
<td>T 4</td>
</tr>
<tr>
<td>week 5</td>
<td>P 5</td>
<td>S 5</td>
<td>T 5</td>
</tr>
<tr>
<td>week 6</td>
<td>P 6</td>
<td>S 6</td>
<td>T 6</td>
</tr>
</tbody>
</table>

In 2008 the frozen fruit samples were milled wet with a laboratory blender (IKA® A 11 basic, Staufen, D). The obtained powder was transferred to plastic bottles (transparent PE-LD with screw cap, Semadeni, Switzerland) and stored in the dark at -80 °C until analysis.
In 2009 the frozen fruit samples were freeze-dried and the dry powder was stored vacuum packed in sealed plastic bags at -20 °C until analysis.

In order to evaluate the influence of the different methods used between both years specifically the effect of freeze drying, three samples randomly chosen were analysed in duplicate wet frozen and after freeze drying. Anthocyanin contents and antioxidant capacity were related to the fresh weight for both methods and differences were assessed with ANOVA. For the total anthocyanin contents no significant differences were observed between the freeze-dried and the wet frozen samples. For the antioxidant capacity however significant losses in DPPH radical scavenging capacity (-16%) were observed in the freeze dried samples probably due to the degradation of ascorbic acid. For this reason, the results of each trial year were considered separately.

2.1.3 Development and ripening time experiment

In this experiment the effect of fruit development and ripening time, defined as the number of days from flowering to full ripeness, on total anthocyanin contents and antioxidant capacity was assessed. The experiment was conducted in 2009 on open field in Conthey without tunnel covering.

A+ frigo plants of cv. Clery were planted on raised beds covered with black plastic mulch and cultivated as described above except from the tunnel coverage.

Each flower of 15 plants distributed in three replications of five consecutive plants was tagged as soon as white petals were visible and the date was recorded as the flowering date. The fruits were carefully harvested at their individual fully ripe stage (when 100% red) and the date of harvest was recorded. The number of days from the flowering date to the harvest date was calculated for each fruit and defined as the development and ripening time. At each harvest date, fruits with the same development and ripening time were pooled from the five consecutive plants in order to have enough fruit material for analysis. The fruits were immediately snap-frozen in liquid N₂ and freeze dried prior to analysis. Samples consisting of less of four fruits were excluded from the analysis.

2.2 Analysis of taste and health related quality parameters

2.2.1 Soluble solids content and titratable acidity

SSC and titratable acidity were measured on the thawed fruit samples after extracting the juice with a commercial Juice Master (Hapag, Switzerland). SSC expressed as % Brix was analysed with a refractometer (Atago, PR-1, Kunzmann, Switzerland) and titratable acidity by titration of 10 g of clear fruit juice with NaOH 0.1 N until a pH of 8.2 with an automated titrator (Titino DMP 785, Metrohm AG, Schweiz). The result was expressed in g citric acid equivalent per 100 g juice.
2.2.2 Extraction and quantification of the antioxidant capacity and total anthocyanin contents

Antioxidants were extracted according to the method of Tulipani et al. (2008) with slight modifications. Briefly, 5 g of frozen strawberry powder were weighed in 25 g of the extraction solution containing methanol, water and formic acid (80:19:1, v:v:v). The obtained slurry was sonicated in a with ice-cooled (0-4 °C) water bath for 15 minutes then centrifuged 5 minutes at 9000 rpm. The supernatant was filtered through a LS 14 ½ filter (Schleicher and Schuell, Germany). The extracts were stored up two 3 days at -80 °C and were used for the determination of the antioxidant capacity and the total anthocyanin contents.

The determination of antioxidant capacity with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is based on the properties of DPPH, which in its radical form has an absorption band at 517 nm and disappears upon reduction by an radical scavenging compound. The quantification of AC was performed according to the method described by Brandwilliams et al. (1995). All chemicals used in this section were purchased by Sigma-Aldrich (Buchs, Switzerland). Briefly, 100 μl extracts were added to 10 ml of a 0.1 mM DPPH solution stirred well and left to react at room temperature. The absorption at 517 nm was measured after 30 minutes against the methanol/water extraction mixture (blank). Quantification was performed with a Trolox standard calibration curve (0-2.4 μmol/ml) and the results were calculated in μmol Trolox Equivalent (TE) per g DM.

Total anthocyanin contents were measured according to the method described by Lee et al. (2005). The method is based on the structural change of anthocyanin chromophore between pH 1 and 4.5. pH 1 buffer was a 0.025 M potassium chloride solution. pH 4.5 buffer was a 0.4 M sodium acetate solution. 800 μl of the strawberry extract was diluted with 3200 μl of each buffer solution. Absorbance was measured at 520 and 700 nm between 20 to 50 minutes following the preparation against distilled water as blank. Anthocyanin pigments concentration was calculated as pg-3-gluc using the molar absorptivity of 22400 L/mol cm. According to the sample procedure used (freeze dried samples or wet) results were expressed per 100 g FW or per g DW. When results had to be compared, all results were converted in FW according to the DM of the sample.

2.3 Statistical methods

All statistical analyses were carried out using XLSTAT Version 2007.5 (Addinsoft, Paris, France). Data were subjected to analysis of variance and the differences between the means were assessed with Fishers Least Significant Difference (LSD) at P<0.05. Relationships between factors were analysed by the Simple Linear Regression and by the Coefficient of Determination (\(r^2\)), calculated from the Pearson Product Moment Correlation Coefficient (r).
3 Results

3.1 Plant to plant variability

The range of variation in the quality of plants from the same cultivar (Clery) and grown next to each other under identical condition were important. SSC varied from 7.6% to 11.2% Brix representing a coefficient of variation (CV) of 8.6%. TA values ranged from 0.60 to 0.87 g/100 g (CV = 9.2%). No correlation was found between foliar surface of the plant or between number of leaf and SSC or TA.

Concerning the health related compounds, total anthocyanin contents had a CV of 9.6% with values spread between 16.8 and 23.7 mg/100 g FW. Lower variations were found in antioxidant capacity with values ranging from 1018 to 1360 μmol TE/100 g FW and representing a CV of only 6.5%.

3.2 Weekly evolution of the fruit quality during the harvest

The whole strawberry harvest period lasted five weeks both years (Fig. 2). In 2009 the plants reached their peak of fruit production during the second harvest week with an average yield of 235 g/plant. In 2008 the production peak was reached during the third harvest week with a maximum yield of 182 g/plant. The single fruit weight generally decreased during the harvest; however the strong decrease during the first three harvest weeks of 2009 contrasted with the more stable fruit weight in the first weeks of the 2008 harvest. Towards the end of the harvest (week 5) the single fruit weight was around 9 g/fruit in 2008 and 7 g/fruit in 2009 representing respectively 48% and 30% of the initial fruit weight (week 1).

Significant differences were found between the harvest weeks concerning the quality parameters SSC, titratable acidity and total anthocyanins. The first week of harvest, these three parameter showed similar values in both years (SSC: 8.9% Brix, titratable acidity: 0.73 g/100 g FW, total anthocyanin contents: 24.8 mg/100 g FW), but the subsequent evolution of the quality differed both years especially the anthocyanin content. The weekly variation was generally less marked in 2008 compared to 2009 for all the measured parameters. In 2009, the weeks from 3 to 5 were characterised by a significant increase in SSC from 9.3% to 12.6% Brix and titratable acidity from 0.83 to 1.01 g/100 g contrasting with a significant decrease in anthocyanin contents from 29.7 to 19.7 mg/100 g FW. The increase in SSC was less marked in 2008, while titratable acidity and total anthocyanin contents remained stable during the last three weeks of harvest. The total anthocyanin content was generally higher in 2008 than in 2009, especially towards the end of the harvest.

Significant inverse relations were found between SSC and total yield (p=0.001, r²=2.981), between SSC and single fruit weight (p<0.0001, r²=0.4882) and between SSC and the fruit charge remaining on the plant (calculate as the weekly difference between total fruit yield and harvested yield). However,
this last correlation was the strongest with $r^2 = 0.552$ (p<0.0001). Although titratable acidity followed similar trends as SSC, no correlation was found between acidity and fruit charge remaining on the plant neither with the single fruit weight.

The total antioxidant capacity remained stable over the five harvest weeks with average values of 1113 μmol TE/100 g FW in 2008 and 792 μmol TE/100 g FW in 2009. The antioxidant capacity of the fruits collected in 2009 represented 29% less than 2008. The differences were partially due to the use of freeze drying in the sample preparation process in 2009.
Evolution of fruit quality during the harvest: influence of the fruit order and time from flowering to harvest

Fig. 2: Evolution of the yield, single fruit weight, SSC, titratable acidity, anthocyanin contents and antioxidant capacity of the fruits during the harvest 2008 and 2009. Vertical bars at each data point indicate standard deviation (absolute values).
3.3 Influence of the fruit order on taste and health related parameter

The proportions of the different type of fruits (primary, secondary and tertiary fruits) changed drastically during the harvest (Fig. 3). The proportion of primary fruits decreased continuously during the five harvest weeks representing over 80% of the total harvested fruits the first week and less than 20% at harvest end. Tertiary fruits start to be ripe during the second harvest week and their proportion in the harvested fruits increased from the week 2 to 4. The proportion of secondary and tertiary fruits increased more rapidly in 2009 than in 2008, following the trend observed with the weekly yield (peak of production arriving earlier in 2009). In the last harvest week, the proportion of the different fruit type remained similar for both harvest years.

Fig. 3: Changes in the proportion of primary, secondary and tertiary fruits during the harvest. Vertical bars indicate the standard deviation.
The fruits ranking had a significant effect on the single fruit weight with a decreasing fruit weight from primary to tertiary fruits (Table 2). In addition the single fruit weight of each fruit type decreased during the harvest and no significant interaction between fruit order and harvest week was observed. However, because tertiary fruits are the smallest fruits and their proportion increase at the end of the harvest, the overall decrease in single fruits weight was more drastic than the decrease in fruit weight of each individual fruit type (result not shown).

In 2009 the dry matter of the fruits was quantified in order to convert all the results to FW values and to have comparable results with 2008 when fresh fruits were analysed. The dry matter represented 11% of the fresh weight and no significant differences (p=0.905) was found in the dry matter content of primary, secondary and tertiary fruits.

As far as the taste related parameters SSC and titratable acidity were concerned, no significant differences were observed between the different fruit order and this result was consistent over the two trial years (Table 2).

Table 2: Differences in single fruit weight, Soluble Solid Contents SSC and titratable acidity (TA) between primary secondary and tertiary fruits. Different letter in a column indicate significant differences at p<0.05 (Tukey test).

<table>
<thead>
<tr>
<th></th>
<th>single fruit weight (g)</th>
<th>SSC (% Brix)</th>
<th>TA (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
<td>2009</td>
<td>2008</td>
</tr>
<tr>
<td>Primary fruits</td>
<td>21.3 a</td>
<td>18.5 a</td>
<td>9.3 a</td>
</tr>
<tr>
<td>Secondary fruits</td>
<td>13.1 b</td>
<td>10.7 b</td>
<td>9.0 a</td>
</tr>
<tr>
<td>Tertiary fruits</td>
<td>6.7 c</td>
<td>6.7 c</td>
<td>9.5 a</td>
</tr>
</tbody>
</table>

The $r^2$ of the correlation between SSC and single fruit weight dropped from 0.4882 to 0.128 (p=0.001) when considering all the individual fruit types values rather than the weekly average.

Health related parameters showed contrasting trends. While the anthocyanin content remained unaffected by the fruit order, the antioxidant capacity was higher in tertiary fruits (Table 3). Despite the yearly variation due to the sample preparation discussed above, tertiary fruits showed both years a higher antioxidant capacity (+16% in 2008 and +9% in 2009) compared to the primary and secondary fruits, which did not differ significantly.
Table 3: Differences in health promoting quality traits between primary secondary and tertiary fruits. Different letter in a column indicate significant differences at p<0.05 (Tukey test).

<table>
<thead>
<tr>
<th></th>
<th>total anthocyanins (mg/100 g FW)</th>
<th>antioxidant capacity (μmol TE/100 g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
<td>2009</td>
</tr>
<tr>
<td>Primary fruits</td>
<td>35.2 a</td>
<td>24.7 a</td>
</tr>
<tr>
<td>Secondary fruits</td>
<td>35.0 a</td>
<td>25.6 a</td>
</tr>
<tr>
<td>Tertiary fruits</td>
<td>35.9 a</td>
<td>24.5 a</td>
</tr>
</tbody>
</table>

3.4 Effects of the fruit development and ripening time on health related parameter

In the second experiment, the tagging of the flowers allowed a precise determination of the time from flowering to harvesting (full ripeness) of each individual fruit independently of the fruit order. The duration from flower to fully ripe fruit varied from 32 to 42 days. Single fruits with a ripening time of 29, 43 and 44 days were also found but were not considered in the analysis because of the rare occurrence (less than three fruits on five plants). At each harvest date, fruits with different fruit development and ripening time were collected.

Biochemical analyses were performed on freeze dried fruits and results were therefore expressed in dry matter. A great variation in anthocyanin contents ranging from 1.68 mg/g DM (16.55 mg/100 g FW) to 3.78 mg/g DM (29.65 mg/100 g FW) was found among the fruit samples collected at different harvest dates and with different fruit development and ripening time. The antioxidant capacity varied between 83 μmol TE/g DM (822 μmol TE/100 g FW) and 117 μmol TE/g DM (1095 μmol TE/100 g FW) showing a lower variation among the samples.

The total anthocyanin contents appeared to be positively correlated with the fruit development and ripening time ($r^2 = 0.489$, Fig. 4) but it was not the case for the antioxidant capacity ($r^2 = 0.013$).

Harvest date correlated neither with the total antioxidant capacity nor with the total anthocyanin contents.
4 Discussion

4.1 Variation in SSC and acidity during harvest

Despite the large plant to plant inhomogeneity detected in fruit quality, general trends were detected concerning its evolution during the harvest period. The increase in SSC towards the end of the harvest was related to the fruit charge remaining on the plant rather than with the decreasing fruit weight. An additional result confirming this hypothesis is the absence of significant differences between the tertiary and primary fruit’s SSC despite the great difference in their single fruit weight. This fact contradicts a popular claim suggesting that smaller berries are sweeter.

High fruit loads at harvest begin requires an important assimilate flow from other plant organs, also called source, to the developing fruits representing the sink. This important sink activity may create an increased competition for nutrients between the fruits as the source may be limited. These facts could explain the lower SSC during the first weeks of the harvest. Toward the harvest end, the fruit number on the plant decreases and the balance between source organs and sink changes allowing more assimilates to be available for the remaining fruits. Previous experiments with cultivar Marmolada showed that when fruit number was reduced trough flower removal, an enhanced SSC was observed in the fruits (Carlen et al. 2007) supporting the source sink hypothesis. The authors identified the leaf
Evolution of fruit quality during the harvest: influence of the fruit order and time from flowering to harvest

area to fruit ratio as the factor responsible for SSC accumulation. However in our plant to plant experiment no correlation was found with the leaf area nor with the leaf to fruit ratio and SSC. These findings suggest further source organ to be involved in the accumulation of sugars in the fruits. Crown and roots represent carbohydrate reserves of the plant. The carbohydrate reserve in the roots seems to be used primarily to support the growth of inflorescence and developing leaves (Nishizawa & Shishido 1998). Macias-Rodriguez et al. (2002) observed a high metabolic activity in crown upper section during strawberry fruit development. The authors suggested strawberry crown to be an important source of carbohydrates for the forming fruits.

4.2 Variation in anthocyanin contents

Anthocyanins are mostly responsible for the red colour of strawberry fruits. In this context, anthocyanin contents increase significantly during fruit ripening especially during 14 to 35 days from fruit set (Montero et al. 1996) or between the ripening stages “5% red” to “purple red” (Ferreyra et al. 2007). Differences in fruit maturity may have therefore a strong influence on the anthocyanin contents. However, in our study, all fruit were harvested in the “100% red” stage and the plants were checked for ripe fruits every second day. Therefore no fruit was allowed to reach the “purple red” stage. Thus, similar anthocyanin contents could have been expected. But anthocyanin contents varied during the harvest period and its variation was not consistent over the years (Fig. 3) suggesting year specific factors also to be determinant. At the same time the variation in anthocyanin contents was not related to the fruit order as primary, secondary and tertiary fruits did not differ significantly in their anthocyanin contents. Anttonen et al. (2006) found higher anthocyanin contents in tertiary fruits, but their results were not consistent over different planting dates.

The fruit development and ripening time experiment showed that the slower the fruits developed and ripened the higher was the observed anthocyanin content (Fig. 4). The different anthocyanin levels observed in fruits of apparent similar maturity stage could relies on a different accumulation of anthocyanins in the fruit inner tissues. Accelerate ripening through higher temperatures may lead to a rapid colour change in the external fruit tissues thus allowing the picking of fully red fruits earlier. However, when fruits have a longer ripening time, the delay in external colour change seemed to favour the anthocyanins accumulation in the inner fruit tissues. Longer ripening times may be due to lower temperatures or other factors such as the position of the fruit on the plant, for example with a leaf shading the fruit. Yoshida et al. (2002) observed the evolution of colour parameter during fruit ripening and noted that the colour value changed significantly as total anthocyanin contents increased from 0 to 0.1 μmol/g FW. Above this anthocyanin level the colour values remained stable while anthocyanin concentration continued to increase. These observations support the hypothesis that anthocyanins accumulate first in the cells near the surface at earlier stage of colour development and
only later in the cells of the inner flesh. Our results suggest the accumulation of anthocyanins in the inner fruit tissues to be less influenced by the temperature or radiation and thus, when ripening of the outer tissues is delayed, anthocyanins continue to accumulate in the inner tissues leading to fruits with overall higher anthocyanin contents. Another observation seems to strengthen this hypothesis: in the fruit order trial, the fruit production was delayed in 2008 compared to 2009 with the production peak arriving one week later and a slower ripening of the fruits of the different types. This slower ripening was linked with higher anthocyanin contents in the fruit from the second harvest week on. In order to confirm this hypothesis, it would be necessary to analyse separately the anthocyanin contents in the internal and external tissues of the fruits with different ripening stages. Such hypothesis could be an explanation for the important variability observed in anthocyanin contents of fruits collected from the 30 replications.

4.3 Variation in antioxidant capacity

The antioxidant capacity followed different trends than the anthocyanin content suggesting other compounds being behind its variation such as ascorbic acid or other phenolics. Ferreyra et al. (2007) showed that the antioxidant capacity of strawberries decreased by 90% from the initial value during the first stages of fruit development from small green fruits to white fruits and remained unchanged until fully ripe despite the accumulation of anthocyanins during the last stages of fruit development.

The higher antioxidant capacity observed both years in tertiary fruits compared to primary and secondary fruits confirm results reported by Anttonen et al. (2006), who found that the antioxidant activity was 11% higher in tertiary fruits compared to primary fruits. Despite their small proportion in the fresh weight (around 1%), the achenes represent 14% of the antioxidant capacity of the fruit (Aaby et al. 2005). These results seem to be related to the higher proportion of ellagic acid and ellagitannins present in achenes (Williner et al. 2003, Aaby et al. 2005) compared to strawberry flesh. Tertiary fruits having a higher achene number per gram (Hansen 1989). This fact can explain the higher antioxidant capacity of tertiary fruits compared to primary fruits even though anthocyanin contents remain stable.

On the other hand, antioxidant capacity does not seem to be directly related to the fruit size as no correlation between single fruit weight and antioxidant capacity was observed. Anthocyanin are known to be strong antioxidants (Wang et al. 1997), however the lack of relationship between anthocyanin contents and antioxidant capacity has been described previously (Tulipani et al. 2008, Crespo et al. 2010).

5 Conclusions

This work confirms the high fluctuation of the strawberry quality criticised by the consumers. Furthermore it indicates the harvest period as a possible source of inconsistencies observed in
Evolution of fruit quality during the harvest: influence of the fruit order and time from flowering to harvest

strawberry quality, especially concerning the SSC and the anthocyanin contents. Another source of variation for the anthocyanin contents of fruits at same maturity was related to their individual development and ripening time. With slow ripening more anthocyanin seems to accumulate in the inner fruit tissues without affecting fruit external colour. This hypothesis needs to be confirmed with the separate analysis of the inner and outer fruit tissues of fruits with different ripening time. Further work is needed to understand the plant physiological aspects behind the high variability observed in fruits quality traits of cultivar Clery.

Additionally the results of this work contradict a popular claim saying that smaller fruits are a concentrate of taste and health related compounds, since no correlation was found between single fruit weight and the different quality parameters measured. On the other hand, the results confirm the higher antioxidant capacity of tertiary fruits which could be related to their higher proportion of achenes in relation to the fruit weight.
CHAPTER 5

General conclusions
General conclusions

In Switzerland, inconsistencies in strawberry quality are often criticized by the consumer. The purpose of this study was to analyse possible sources of variation of taste and health related composition of strawberry with the objective to improve the fruit quality and to reduce its fluctuation.

To achieve this aim, the influence of newly released cultivars and standard cultivars on specific health and taste related target parameter as well as their stability when grown in different environments such as under plastic tunnel or in mountain regions was analysed over two consecutive years. Furthermore other factors responsible for the observed inconsistencies in the fruit quality within cultivars, such as harvest period, fruit order and time from flowering to harvest were evaluated.

1 Taste related compounds

SSC and titratable acidity were the two main parameters retained throughout the study to assess taste-related potential of strawberry fruits, as they belong to the standard quality control parameter for soft fruits. Additionally individual sugars (viz. fructose, glucose and sucrose) and acids (viz. ascorbic, citric and malic acid) were identified and quantified during one season in four cultivars grown in two different production sites (Chapter 3).

Genotypic variation in SSC and TA were considered as low (CV 4%) compared to the other quality parameter. The differences between the genotypes ranged from 8.3%- 9.5% Brix. Only two genotypes (Matis and Yamaska) differed significantly from the others by showing lower SSC (8.3% and 8.4% Brix), but the SSC of all the cultivars studied were far above the limit of 7% Brix considered as sufficient for strawberry quality. Furthermore for most of the cultivars the variation in SSC observed between environments and years was low suggesting SSC to be a stable property. The experiments were conducted in a region where precipitations are limited to ca. 600 mm per year and the climatic variation between the environments chosen were low. On the other hand wet years, as observed in Bruson in 2008, did have a negative impact on the sugars content. In Antea fruits, the content of monosaccharides (viz. glucose and fructose) was lower in Bruson in 2008 compared to Conthey, while sucrose levels remained constant (Chapter 3). These results suggest a possible reduced invertase activity in the fruits cultivated under humid or cloudy condition. In 2009, weather in Bruson was similar to Conthey and the differences in SSC were less important but a tendency towards lower sugar content in Bruson remained, suggesting further factors to be involved.

Improving strawberry fruit taste through the use of plastic tunnel or production in mountain regions under dry and sunny climatic condition seems difficult due to the strong stability of the sugars and acids components. Controlling the taste of strawberry fruits by deficit irrigation and/or tunnel protection could be an approach to produce fruits with increased sugars and acid concentration and hence better taste. However, further factors may also play a role in carbohydrate metabolism in the plant as weather independent differences remained.
The above mentioned low variation in SSC between genotype and environment contrasted with the important plant to plant variability reported for the SSC and titratable acidity in Clery fruits (7.6%-11.2% Brix and 0.6-0.87 g/100 g, Chapter 4). There is an important need to investigate the source of individual plant to plant variation in order to improve the consistency of strawberry taste. Understanding the plant physiological parameter behind those differences could provide new tools to select plants with greater taste homogeneity. It is known that sugars and acids originate from photosynthesis accumulates. Additionally, SSC varied significantly during the harvest period with higher SSC towards the end of the harvest indicating the final level in the plant to be affected by the fruit load on the plant with enough assimilates still produced at the end of the fruiting season (Chapter 4). But an increase of leaf area at the main fruiting period is not easily feasible as a stronger vegetative growth may also increase its sink strength per se and reduce the sugar level available for the fruits. Strawberry leaves are important for photosynthesis and their role in accumulation of sugar is well known but they also may represent a sink for the plant or shade each other if too abundant. In Chapter 3 it was observed that plant produced in Bruson had both years a greater leaf area (1.9 times larger) than plant produced in Conthey and sugar content in the fruit were lower (7% and 4% less in 2008 resp. 2009). This may reflect plant vegetative growth as a possible competitor for sugars during fruit development or a combined effect with the cloudier weather mentioned above.

Fruit thinning combined with a controlled leaf area could be considered to successfully increase SSC and hence improve strawberry sweetness which is known to be related with consumer acceptability. However the cost of this technique accompanied by the yield reduction may considerably limit the acceptance of this approach on a commercial basis. Agronomical practices which allow controlling vegetative growth while favouring the accumulation of the carbohydrate in the plant should be developed. In this context planting date could be a key factor as it is known to influence the vegetative growth and generative plant development.

2 Health related compounds

Despite bioactive compounds being produced by the plant as a protection against environmental damage or pests, the genetic remains the major factor determining their level in the fruit. This fact reflects the advantage in the evolution created by the protective effect of such phytochemicals in the strawberry plant. As a consequence, fruit nutritional quality seems to be an inheritable trait in strawberries.

In our study, significant differences in the content of health promoting compounds such as vitamin C, anthocyanin contents, total phenolics and antioxidant capacity has been observed in ten strawberry cultivars. Furthermore, the role of the genotype appeared to be dominant over environmental variation, as reported in Chapter 3. These facts have important implications for strawberry breeding programs to increase health value of the fruit. The detailed characterisation of ten cultivars reported in chapter 2
provides useful information for the breeders to select appropriate parents. In this context Antea and Clery appeared to be promising parents for high vitamin C resp. high anthocyanin content.

Antioxidant capacity, total phenolic compounds and vitamin C were more affected by the year than by the production site, suggesting meteorological condition as being more important for the formation of antioxidant compounds than fixed site related factors. Since cultivars responded differently to environmental variation, genotype stability seems the most effective way to reduce the variation due to unpredictable year specific conditions. In this study the cultivars Clery, Manille and Asia showed the greatest stability over year and environment in their antioxidant capacity.

Antioxidant capacity remained stable over the harvest and the plant to plant variability was low (CV 6.5%) in contrary to the SSC variation. Antioxidant capacity did not depend on the fruit size. However, higher antioxidant capacity was observed in tertiary fruits while the anthocyanin content remained constant among all fruit types. Ellagic acid and ellagitannins present in higher concentration in the achene seems to have explained the differences observed in antioxidant capacity, as tertiary fruits have a higher proportion of achenes. Availability of nutrients from achenes should be rare for humans by fresh consumption though it has not been investigated yet. Therefore it is could be a hazardous artefact due to the total analysis of all fruit parts to claim tertiary fruit to be healthier for the consumer.

The results presented in Chapter 4 allowed identifying also the harvest period as a possible source of inconsistencies observed in the anthocyanin contents. Additionally large plant to plant variability in anthocyanin contents ranging from 16.8 to 23.7 mg/100 g FW was found in Clery fruits collected at the same time. Finally, with slow ripening more anthocyanin seemed to accumulate in the inner fruit tissues without affecting fruit external colour.

Anthocyanin accumulation occurs in the last stage of fruit development between white and full red stage, anthocyanins being mainly responsible for strawberry colour. Differences in concentrations observed during the harvest period may have been due to changing environmental condition during the last ripening phase when anthocyanins accumulate in the fruit. PAL activity, the enzyme responsible for anthocyanin synthesis, has been shown to be influenced by environment, ripening stage and injuries. However, under condition of sufficient precursors, anthocyanin synthesis can also occur independently of changes in PAL activity. Independent accumulation in the inner and outer strawberry fruit tissues may reflect different condition for anthocyanin synthesis. Increasing the health value of strawberry fruits by impacting on fruit ripening time should therefore be investigated.

Finally, the great genotype variability observed in the health related compounds underlines cultivar choice being the most efficient approach to increase the nutritional quality of strawberry fruits. Furthermore, the possibilities are promising for breeding new cultivars with high level of bioactive compounds because high variability exists in health promoting compounds of existing cultivars.
Further research is needed to control health related components by manipulating plant physiological aspects in order to stimulate biosynthetic pathways.


strawberry, raspberry, and blueberry crops cultivated under plastic films varying in ultraviolet transparency. *Food Chemistry* 119(3), 1224-1227.


