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TITOLO TESI

Comparison of apple cultivars based on volatile organic compounds (VOC) release determined by Proton Transfer Reaction Mass Spectrometry.

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1 Introduction

1.1 Odour and flavour

A volatilized chemical compound, in general at a rather low concentration, that living organisms can perceive by the olfactory sense, is called odour. Our world is full of odours which are the result of volatile compounds produced and emitted by plants. Every plant produces a characteristic volatile compound profile which results in a unique odour complex. The volatile compounds have a large range of biological functions including attraction of pollinators and seed dispersers, repelling herbivorous insects, recruiting predators of insect herbivores etc. (Gan05; Pic02).

A similar term to odour is smell, which can be used for both unpleasant and pleasant odours. The food and cosmetic industry uses the terms aroma, fragrance and scent to characterize a pleasant odour. They are sometimes used to refer to perfumes. On the other hand, malodorous, raunchy, stench, reek and stink specifically describe unpleasant odours.

The perception of odour is a complicated process which is not explainable by simple models. In general, a two-step process occurs when we are perceiving odours: a physiological part is followed by a psychological phase. First of all, in the physiological part, there is the sense of stimulus by nose receptors. Then, in the psychological part those stimuli are processed by a specific region of the human brain that is responsible for smelling. Resuming this, odour perception is the result of the processing of the stimulation of olfactory receptors by volatile compounds. Hundreds of compounds simultaneously influence the human olfactory receptors and the physiological response is far from linear, the overall effects not just being the overlapping of the effect of single stimuli (Law99). As a conclusion, odour feelings are strictly individual perceptions: personal reactions are amongst many others definitely related to age, gender, state

of health and private affectations. Further complications arise from the interplay of environmental and subjective effects.

In fact, the odour consisting of the volatile compounds in the headspace above the food gives a first flavour impression and highly influences the acceptability and judgment of any food. The whole sensory impression of food or other substance is called flavour. It is mainly determined by the combination and interaction of the chemical senses of taste and smell. Flavour perception in humans involves three distinct sensory systems (olfactory, gustatory and trigeminal) that respond to food components. These three sensory systems, however, are influenced greatly by many other properties of the foods in which these flavour stimuli are released. Texture and many others contribute indirectly to flavour perception. Volatile compounds stimulate the olfactory sensory organs in the roof of the nasal cavity. On the other hand, the trigeminal sense, a system that is dependent on free nerve endings in the eyes, nose and mouth, is stimulated by the presence of coolness, heat, astringency, acridness and pungency. Of the three chemical senses, smell is the main determinant of a food's flavour. While the taste of food is limited to sweet, sour, bitter, salty, and savoury – the basic tastes – the smells of a food are potentially limitless. A food's flavour, therefore, can be easily altered by changing its smell while keeping its taste similar.

The flavour in plants is a complex mixture of volatile compounds, to which a wide range of biochemical pathways in the plant contribute. The bouquet of flavour compounds is very sensitive to breeding, cultivation and storage.

1.2 Measuring odour and flavour

Nowadays, traits such as flavour and aroma are of increasing importance for the consumer. Along with food appearance and texture, food aroma appreciation is one of the first evaluation signals encountered by consumers during eating of food. Therefore there is an increasing need to

develop tools to quantify those traits in an objective and reproducible way. Having said this, we still have to keep in mind that it is impossible to have a fully objective and representative analytical measure of odour and flavour.

A quite useful tool to measure in a rather reproducible and reliable way the components of odour and flavour of food is the sensory descriptive analysis, in particular Quantitative Descriptive Analysis (QDA) (Sto98). Correlating descriptive data and the instrumental determination of volatile compounds is difficult, because there is a difference between the complexity of the perception mechanisms and the few attributes usually addressed by descriptive analysis. It is definitely questionable if the ensemble of single attributes measured by descriptive techniques can provide a valid description of the overall sensory experience (Law98).

The situation outlined above has been significantly improved by the emergence of two important new approaches: first, innovative instrumental techniques are available nowadays, which seem to be able to compete with the sensitivity of the human receptors and at the same time, to analyse directly the genuine mixture interacting with our senses, without any extraction/concentration procedure. Secondly, new statistical multivariate methods (both for sensory and instrumental data) enable to work with high dimensional and high collinear data. Descriptive sensory methods are indeed expensive and time-consuming. The chance and possibility to calibrate their results with instrumental data has an important applicative relevance. These tools could revolutionize research on consumer preference and acceptance. It is well known that food characteristics like flavour and aroma strongly influence consumer acceptability and preference judgement.

1.3 Volatile compounds in food

Some inorganic substances like ammonia and hydrogen sulphide are odorants, although most of the odours consist of organic compounds. More

than 6900 volatiles have been identified in foods and beverages (Mis96). The progress in instrumental analysis, especially but not only concerning gas chromatography-olfactometry methods, is continuously enlarging this list of volatiles (Rut01).

Only a limited number of the volatile compounds in food shows characteristics of odours, as defined above, and is as a consequence sensorially relevant. These restricted numbers of volatile compounds play a crucial role in the specific odour of a food product, commonly defined as ‘‘odour active compounds’’. The distinction between odour active compounds and whole range of volatiles in a food product was suggested by flavour chemists to be a very important task in flavour analysis (Gro93).

1.4 Aroma of apples

For more than 50 years the volatile flavour constituents of apples have been studied. Those studies have been extensively reviewed (Dim83). In the first phase, until the late 1970s, most research on aroma and flavours of apple fruit focused on identifying volatiles produced by ripening fruit (Tre75). Analytical investigations were performed under the assumption that all the volatiles occurring in apple contribute to its aroma.

Recent reviews have discussed metabolic aspects, since part of the research later on has been concentrating on the biochemical origin of aroma volatiles. Lots of improvements occurred in the development of methods for separation and identification of volatile compounds, often in trace amounts of a few parts per million. Researchers have been using these new methods to examine in more detail biosynthetic pathways and control mechanisms in the synthesis and subsequent accumulation and release of volatiles from apples.

It is known apple aroma is due to a complex mixture of volatile compounds. Only a few of the aroma compounds present in volatile emissions from apples influence and define characteristic apple aroma or taste (Cun86). A

relatively small amount of the compounds emanating from apples have been determined to have a decisive impact on the sensory quality and are, therefore, designated as impact compounds in apple fruits. The biggest challenge has been to separate the few most important flavour compounds, which might be trace chemicals, from the vast amount of inactive compounds.

The typical apple aroma is known to be the result of more than 300 volatile compounds including alcohols, aldehydes, esters, ketones and ethers (Dim83, Pai90). On average it is estimated that 78-92% are esters and 6-16% alcohols. In fact, the majority of apple aroma compounds are volatile esters. Apple fruit volatile esters contribute to fruit quality and influence consumer acceptance (Dai96; Dev95; Dix00; Har03). However, apples for example also produce a relatively large amount of α-farnesene (Bra93; Gir95; Oli92).

The apple-like or fruity smell in various cultivars of apple is due to esters (Fel93). The most commonly reported impact compounds in apples are ethyl acetate, ethyl butyrate and methyl anthranilate (Kak86). Compounds known to possess green apple-like odours are hexanal and trans-2-hexenal (Fla67, Fuh02). These compounds are not present in significant amounts in whole apples; they are formed after disruption of the cells during processing or chewing. Other compounds, such as butan-1-ol (which possesses a sweet aroma), or ethyl butanoate and ethyl 2-methylbutanoate (which are responsible for a fruity, estery aroma), contribute to apple aroma characteristics as well as to aroma intensity (Dix00).

Resuming this, about twenty chemicals have been reported to be "character impact" compounds (Table 1).

Table 1: Important apple volatile compounds and their sensory descriptions

Compound	Sensory description	Cultivar	Reference
Aldehydes			
acetaldehyde	green/sharp	Golden Delicious	Riz89
trans-2-hexenal	green/sharp	Golden Delicious	Riz89
	overall intensity	McIntosh	Pan80
	green apple	Delicious	Fla69
	harmonious, fruity	many	Due79
hexanal	green/sharp, earthy	Golden Delicious	Riz89
	overall intensity	McIntosh	Pan80
	good, green apple	Delicious	Fla69
	grass like	many	Due79
Alcohols			
butan-1-ol	overall flavour, aroma,	Royal Gala, Golden	You96
	sweet aroma	Delicious	Riz89
hexan-1-ol	earthy, unpleasant	Golden Delicious	Riz89
trans-2-hexenol	harmonious, fruity	many	Due79
Esters			
butyl acetate	red apple aroma	Royal Gala	You96
	Cox-like aroma	Cox's Orange Pippin	Wil77c
	harmonious	many	Due79
	nail polish	Gala	Plo98
pentyl acetate	banana like	Cox's Orange Pippin	Wil77c
	apple, fruity	Golden Delicious	Riz89
	Gala	Gala	Plo98
hexyl acetate	red apple aroma	Royal Gala	You96
	characteristic apple		
	Cox-like aroma	Cox's Orange Pippin	Wil77c
	ripe Golden Delicious	Golden Delicious	Riz89
	sweet fruity, apple		
	Gala, ripe, pear		Plo98

2 methyl butyl acetate	overall aroma, characteristic apple	Royal Gala	You96
	solvent	Gala	Plo98
	banana like	Cox's Orange Pippin	Wil77c
ethyl butanoate	fruity, estery	Golden Delicious	Riz89
	harmonious, fruity	many	Due79
ethyl-2-methyl butanoate	fruity	Golden Delicious	Riz89
	apple like	Delicious	Fla67
	sweet strawberry	Gala	Plo98
4-methoxyallyl benzene	spicy, aniseed	many	Wil77c
methyl-2-methyl butanoate	sweet fruity	Gala	Plo98
propyl-2-methyl butanoate	very sweet, strawberry	Gala	Plo98
butyl-2-methyl butanoate	fruity, apple	Gala	Plo98
hexyl-2-methyl butanoate	apple, grapefruit	Gala	Plo98
butyl hexanoate	green apple	Gala	Plo98
hexyl propanoate	apple	Gala	Plo98
butyl butanoate	rotten apple, cheesy	Gala	Plo98
butyl propanoate	fruity, apple	Gala	Plo98
hexyl butanoate	apple	Gala	Plo98
hexyl hexanoate	apple	Gala	Plo98

The different volatile compounds have a diverse organoleptic contribution, showing a range of aroma thresholds. Although some of them are present in very low concentrations they contribute to potent aroma characteristics typical of apple aroma/flavour (for example ethyl-2-methyl butanoate, Fla67). Others are related to aroma quality (e.g. ethanol, Due81) or contribute to aroma intensity (e.g. trans-2-hexenol).

The alcohols butanol and hexanol give a sensation of sweetness, while acetaldehyde and trans-2-hexenol are associated with the stinging acidity.

Furthermore the ethyl butyrate and ethyl 2-methylbutyrate correlate with the fruity flavour and the hexyl acetate with a sweet-fruity odour. Interestingly, others such as propyl, butyl, pentyl acetate or butyl butyrate do not really contribute with any specific character but their absence has been reported to have negative effects on the overall aroma (Vis93).

In a recent work (Meh06) characterization of volatile compounds contributing to apple aroma extracted by vacuum hydrodistillation was done by GC-O on commercialized fruits of Golden Delicious, Fuji, and Braeburn. It was possible to detect thirty-six odorant compounds, twenty-four of which were common to all extracts. Among these thirty-six compounds, two were tentatively identified with two different methods and twenty-five were identified with at least three identification methods. These compounds corresponded to sixteen esters, six alcohols, three aldehydes, one ketone, and one terpenoid.

Generally higher molecular weight volatiles, often having one or two hydrophobic aliphatic chains, are not found in the headspace. They are most probably trapped by waxes on the apple skin.

Apple volatile production has been categorised in many different ways, the most used categorizations have been according to aroma production pattern (Dir89), type and quantity of esters or alcohols (Dir89; Pai90), skin colour (Pai79) or C6 aldehydes (Pai90).

1.5 Metabolism of apple aroma compounds

During the ripening process the production of aroma volatiles increases. One peak is reported to be at the climacteric maximum (Son96). In general, aroma compounds are synthesised from amino acids, membrane lipids and carbohydrates (Sal76, San97, Figure 1).

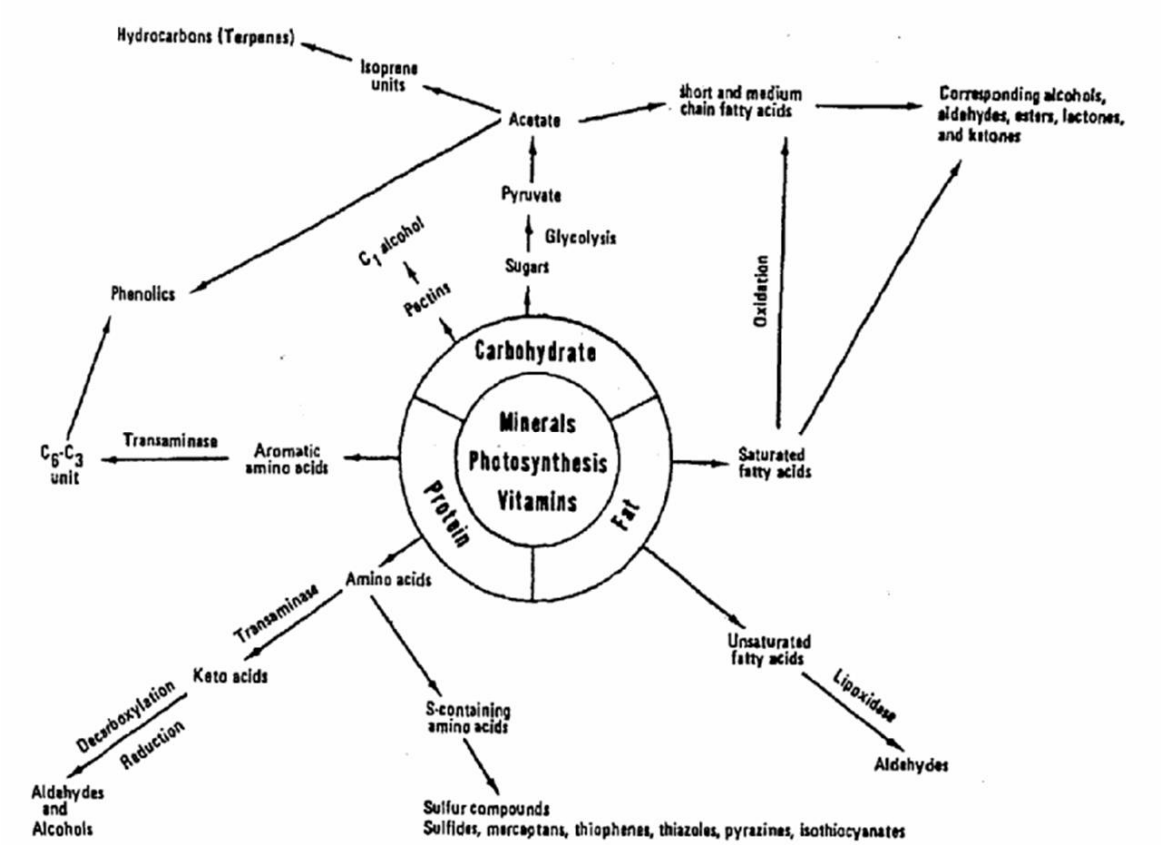


Figure 1: Formation of volatile aroma in fruits and vegetables (from Sal76)

They are produced from those primary metabolites via at least four pathways. Lipids which are broken down initially through ß-oxidation, then by lipoxygenase activity produce straight chain esters (Row99). The resulting hydroperoxides are then converted first to aldehydes, then to alcohols, and finally to esters. Branched chain esters, on the other hand, are produced from the breakdown of Ile (Row96).

Fatty acids supply straight chain alcohols and acyl CoAs through ß - oxidation. Branched-chain volatiles are formed by metabolism of amino acids, in particular isoleucine, leucine and valine (Hea86). Acetyl CoA is probably synthesised mainly from pyruvate, as it is the substrate of the tricarboxylic acid cycle (Mat96). Formation of alcohols from fatty acids and amino acids is by reduction of aldehydes to alcohols catalysed by alcohol

dehydrogenase. Esters are produced by combining alcohols and CoA derivatives of carboxylic acids in an oxygen-dependent reaction catalysed by alcohol acyl CoA transferase (Har85). Since acetyl CoA is the most abundant CoA present in fruit tissue, the majority of esters are acetate esters.

Several authors have indicated 3-methyl-branched volatiles as apple constituents (Maa89). On the other hand, other publications were contradictory, and only small amounts of 3-methylbutanal and 2-methyl-branched acid, esters, and alcohol were identified (Kar94). Later on it has been proved that these compounds are not genuine apple compounds (Sch98). It was proved that those compounds derive from the metabolism of microorganisms that infect apples naturally or by purposeful introduction.

1.6 Aroma differences between cultivars

The volatile compounds are indicative of the stage of fruit development and are dependent on several factors including cultivar, cultural conditions, storage time and conditions (Dim82; Ber91; Han92a,b; Boy94, Aab02). The concentration and composition for example of volatile esters in fresh and stored apples can be affected by several factors including the ripening stage at harvest (Sap77; Yah90b) and the type of storage regime (Pat74; Str88). When apples are dried, big changes occur in the fruit. Gas chromatograms of dehydrated apples showed a marked decrease in volatiles compared to fresh and frozen samples (Lee67).

The qualitative and quantitative differences in volatile ester production are highly significant among apple cultivars (Fel00; Hol05; Lòp98). That means that phenotypic differences in volatile production are apparently caused by the plant genotype, there is in fact a great variation in apple flavour chemistry due to cultivar alone. Differences in apple aroma are apparent to sensory panellists, where cultivars with strong `typical' apple aroma are

preferred (Pol81). Much effort has been made to identify the most important contributors to apple flavour in different apple varieties.

It is possible to typify the apple cultivars according to their specific volatile compounds (Dou90). Apple cultivars may be either “acetate-ester producing” such as Golden Delicious, Red Delicious, Fuji (Dix97) and Jonagored Jonagold or “non-acetate-ester producing” such as Cortland and McIntosh (Yah90a). Whereas esters in apple impart a generic fruity aroma, the spicy flavour of Jonagored Jonagold apples could be related to 4-methoxy-propenylbenzene (Wil77a).

Ester type cultivars can be categorised in a more detailed manner according to the types of esters which prevale in their aroma profile: acetate ester types (Calville Blanc, Golden Delicious), butanoate ester types (Belle de Boskoop, Canada Blanc, Richared), propanoate ester types (Reinette du Mans, Richared, Starking), and ethanolic ester types (Starking).

Yellow-skinned cultivars have been reported to produce mainly acetic acid esters and red-skinned cultivars mostly butyric acid esters. High concentrations of hexyl acetate and butyl acetate were considered to characterise Cox's Orange Pippin, Elstar, Golden Delicious, Jonagold and Jubliè Delbar, with Granny Smith, Nico, Paulared, and Summerred being characterised by high concentrations of ethyl butanoate and hexan-1-ol and Boskoop and Jacques Lebel characterised by α-farnesene and hexyl 2-methyl butanoate. Concentration of C6 aldehydes for Cox's Orange Pippin and Jonathan apples was four to five times that of Golden Delicious for hexanal and 100-fold more for trans-2-hexenol. Apple cultivars also differ in concentrations of other volatiles such as 4-methoxyllylbenzene (a spice-like aroma compound, according to an English sensory panel) which can constitute up to 0.27% of headspace volatiles in some cultivars.

The compound that had the most effect on those sensory attributes which are particularly important for the typicity of apples of the cultivar Royal Gala is 2-methylbutyl acetate (You96).

In Golden Delicious, butyl acetate and hexyl acetate together represent 60% of the total aromatic components in ripened fruit (Bra93).

1.7 Sensorial evaluation

Sensory quality of apple fruits is dependent on a number of factors including size and shape, colour, texture, taste (sweet, sour, salt and bitter sensations) and aroma (volatile compounds). Among these attributes, texture and aroma appear to be the most important for the consumer (Acr88). Since the flavour profile method was set up (Cai50), most food flavour sensory evaluation has been carried out by judge panel analysis. When correct panel selection and training are performed, sensory analysis can provide valuable information in terms of consumer response forecast and quality control (Nei88). One of the limits of this technique is represented by the difficulty of using, as an instrument, people who are often expensive, unavailable and easily influenced by external stimuli. This aspect leads to the need for rigorous and time consuming training in order to produce good-quality data. Owing to this state of the art, in the last decades there has been much research activity to find alternative instrumental techniques to classical sensory analysis. The aim is to correlate consumer or judge sensory data with chemical analysis of food aroma compounds, obtained mainly via gas chromatography - mass spectrometry (GC-MS), in order to be able to replace the former by the latter as a flavour profile evaluation technique. At the moment this goal is far from being reached, because of the fact that the GC- MS technique, until a few years ago, was 100-1000 times less sensitive than mouth and nose receptors (Mei90). Now, even though some GC-MS techniques, coupled to sample concentration procedures, have greatly enhanced the

analysis sensitivity, several limits remain and the possibility to replace the panel group by flavour profile evaluation is still unavailable.

A vocabulary for sensory profiling of fresh apples was reported in several studies (Wil77b, Wat80). Others have evaluated single attributes such as firmness, juiciness or sweetness, and correlated these to instrumental measurements (Pao93; Plo97; Wat81; You96).

1.8 Instrumental techniques for VOC detection

The measurement of volatile organic compounds (VOCs) provides an interesting way to check apples and food in general, since the amount of VOCs is often connected both with their intrinsic properties (e.g. ripening degree, defects, shelf life evolution, effect of treatments) and with the quality perceived by the consumer (Rob00).

Aroma is indeed one of the most relevant quality criteria of fruit and vegetable products, and a considerable amount of research has been devoted to developing analytical techniques for characterizing quantitatively and qualitatively aroma-producing compounds. Both qualitative and quantitative information is desired in order to monitor product flavour quality and ripeness and to provide quality control for fresh and processed products.

Rapid progress in aroma research was impeded primarily by slow sampling, separation, and detection methodologies. In fact, fruit aroma research is subject to a number of constraints that make more rapid analysis desirable. The seasonal nature of fruit harvest and production requires that new protocols be worked out quickly and efficiently in order to meet research ‘time windows’ established by the commodity. Typical horticultural and biochemical research requires multiple experimental treatments as part of a statistically relevant design. As a consequence sample numbers for most experiments are relatively large (tens to

hundreds). Additionally in many cases an entire study must be completed in a limited time.

Ideally, analytical methods for flavour research on horticultural products should be inexpensive, fast, solventless, relatively independent of the instrument design and adaptable to automation. They should also be applicable to gaseous and liquid samples and have a large linear dynamic range while retaining low detection limits. Furthermore, they should be able to function as a screening technique or be used in the quantitative analysis of selected volatile compounds or classes of compounds for monitoring physiological processes that ideally could be directly related to biochemical changes.

To be successful, instrumental methods are required to provide rapid, reproducible results, with continuous operation, and be cost effective. To date, the instrumental methods have lacked the ability to consistently perceive all of the key sensory attributes of interest, and predictive relationships between sensory and instrumental measures have been relatively weak and unreliable.

1.8.1 Gas chromatography and Gas chromatography-olfactometry (GC-O)

In the 1960s, gas chromatography gained an increasing importance for the investigation of apple volatiles (Dra69). In general, chromatographic techniques are standardized and have sufficiently low detection limits for flavour analysis. However, it often takes more than one hour to analyse a single sample.

Gas chromatography-olfactometry (GC-O) is a commonly used technique for analysis of odour-active flavour compounds, introduced in 1964 (Ful64) and further developed in the following decades (Fri98). Combining the capability of a capillary column to separate compounds with high sensitivity comparable to that of the human nose as a detector, GC-O has shown to

be an optimal instrument to associate odour with eluting compounds. A description of the odour can be given for each retention time corresponding to an odour-active compound (Figure 2).

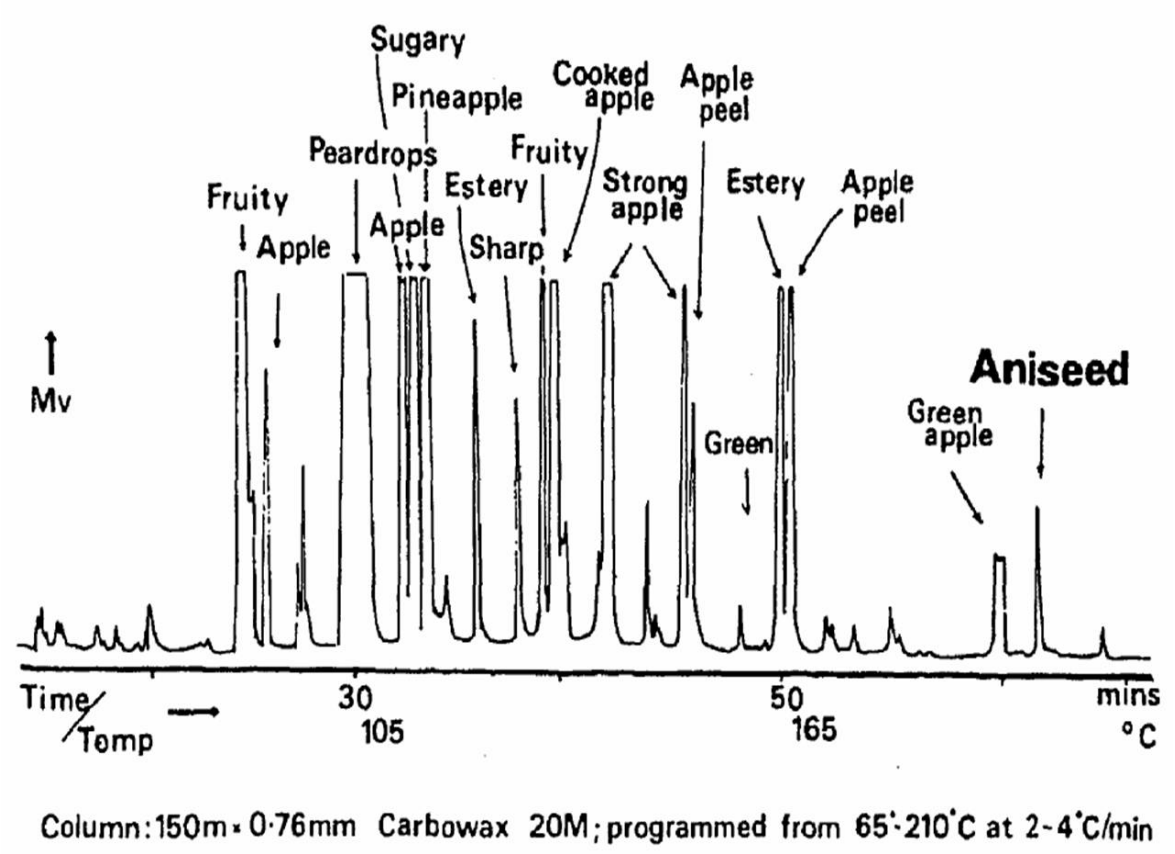


Figure 2: Chromatogram of volatiles from apples (cultivar Ellison's Orange) with aroma descriptions (from Wil77c)

Quantification can be carried out by a multitude of methods, one of which is the detection frequency method, utilizing a group of assessors (Pol97, van01). The number of assessors detecting a specific odour-active compound at the sniffing port at the same time (the frequency of detection) is used as a measure of the intensity of a compound.

1.8.2 Gas Chromatography–Mass Spectrometry (GC–MS)

In this technique, compounds are chromatographically separated before they will be detected by mass spectrometry. In GC/MS, analyte molecules are ionized by electron impact (EI) at a typical collision energy of 70 eV. This is a highly energetic ionization method, which results in extensive fragmentation of the molecules being studied. Detection and quantification of gas molecules that are present in low concentrations requires long pre-concentration steps, during which analyte molecules are collected on an adsorbant medium. In a second phase the adsorbed species are released by thermodesorption, separated by GC, ionized and mass analyzed. Because of this comlex and long lasting pre-concentration step, GC–MS does not have a good time resolution and this may be inconvenient for example when there is a need to monitor rapidly varying emissions of reactive trace compounds.

In practice, the sample preparation technique is tedious and needs a lot of time to single out only a few aroma compounds. Often multiple extraction is necessary to obtain a complete picture of the substances contributing to the flavour profile. Very often several GC techniques (e.g. different columns and conditions) are needed to analyse most of the compounds of interest at the sensitivity required. There is also a risk of introducing artefacts during the complex procedure of sample preparation, concentration and analysis.

However, gas chromatography coupled with various purge-and-trap or headspace sampling techniques (e.g. Solid Phase Micro Extraction, Paw97) and mass spectrometric devices are still the most used and accepted methods for VOCs detection and provide definitive compound identification and quantification. The main limitations of these techniques are that they require time-consuming sample preparations, difficult

chromatographic separation conditions and, in addition, the necessity of trapping and pre-concentrating the VOCs.

1.8.3 Electronic nose

Many scientists have been trying to build aroma sensors able to imitate the human one. In recent years, various systems have been proposed, including the so-called electronic nose based on solid state devices. The majority of electronic nose instruments that have been used for quality control purposes were developed using the following methodology. Typically, R&D, plant personnel and sensory staff have been responsible for determining sensory specifications based on bench-top screening and/or experience of technical staff, often with little input from consumers. Using the expertise of the sensory personnel, samples representing acceptable, borderline and unacceptable sensory quality specifications were selected. These selected samples were then used to develop models on the electronic nose.

Nowadays commercially available E-noses use an array of sensors combined with pattern recognition software. Several of the recently developed E-nose systems have helped in overcoming some of the problems of the past, but still show some limits (Hil95). Owing to the limited number of available sensors, of course only a limited number of aroma descriptors can be included. Basically there aren't still sensors available for all the interesting types of aroma. The machines often show low selectivity for different aromas present at one time. Complex aroma mixtures are often difficult to manage and discriminate. It is very difficult to identify unknown or unexpected compounds.

Little work has been done up to now specifically on apples, some promising results show that the electronic nose may offer a fast and non-destructive alternative to the measurement of volatile emission of apples in future (Sae04).

1.8.4 Proton Transfer Reaction Mass Spectrometry (PTR-MS)

Proton Transfer Reaction Mass Spectrometry (PTR-MS) belongs to the Chemical Ionization Mass Spectrometry (CIMS) techniques, in which ionization of the analysed molecules is achieved through fast exothermic ion/molecule reactions. Since there is less excess energy available for fragmentation, the chemical ionization (CI) is generally considered as a soft ionization method. PTR-MS is a relatively new technique which was introduced by Lindinger and co-workers (Lag94; Lin93). The idea was to create an instrument which should have all the prerogatives to allow a fast and accurate determination of the concentration of volatile organic compounds. The first PTR-MS instrument was finally built in 1994 in Prof. Werner Lindinger's laboratories at the Institute of Ion Physics of the University of Innsbruck in Austria. Further developments lead to more sophisticated and user friendly instruments, although the original structural principle has remained the same throughout the years (Figure 3).

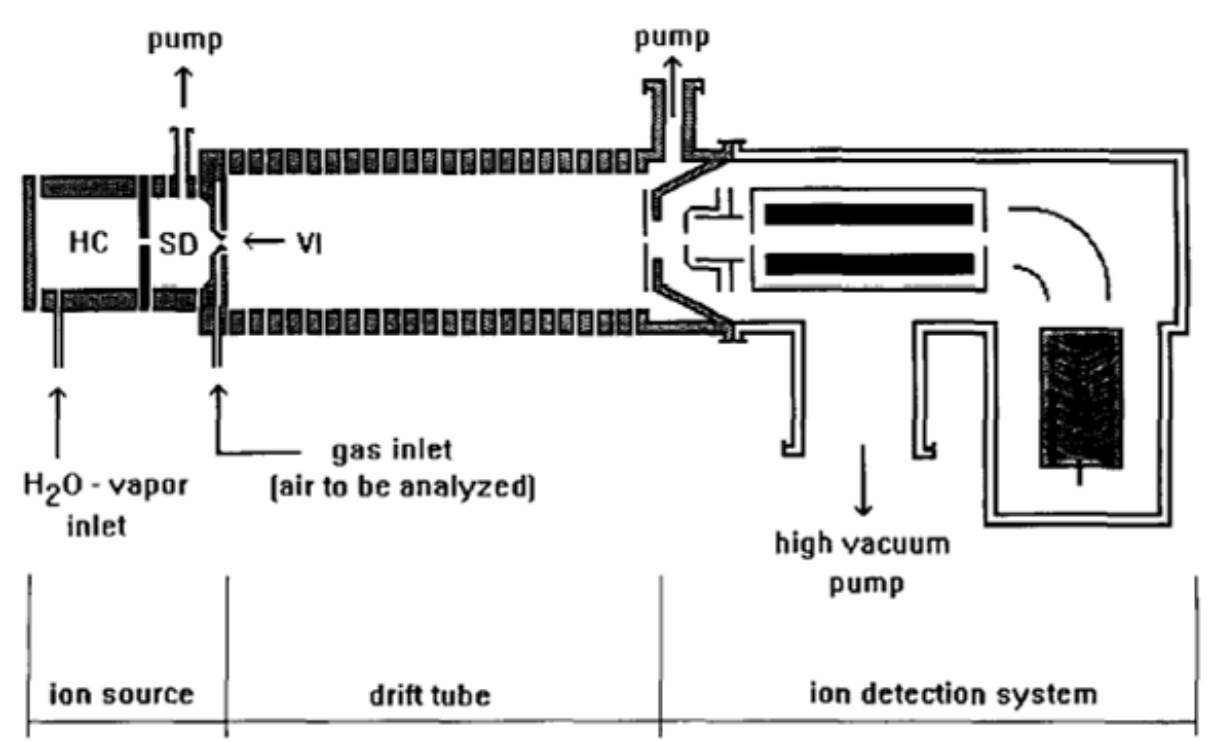


Figure 3: Schematic representation of the PTR-MS apparatus: HC, hollow cathode; SD, source drift region; VI, Venturi-type inlet.

In PTR-MS H_3O+ ions are used to ionise volatile organic compounds present in gaseous media, for example in air. An intense beam of H_3O+ ions is produced in a hollow cathode (HC) discharge source and interacts with the volatile organic compounds to be measured in a separate reaction chamber. Every molecule with a proton affinity bigger than water will eventually interact with H_3O+ exchanging the proton. The produced ions are then detected by a commercial quadrupole mass spectrometer. The major constituents of normal air are not detected due to their lower proton affinity and thus normal air can be used as carrier gas, avoiding dilution of samples. Due to these considerations no sample pre-treatment or pre-concentration is needed giving a genuine image of the volatile mixture.

This technique enables a variety of organic species in complex matrices to be monitored in real-time, with detection limits as low as a few parts per trillion volume (pptv). Compared to ordinary GCs, PTR-MS systems have the ability to monitor and quantify online and in real-time complex mixtures of VOC in the gas phase such as alkenes, alcohols, aldehydes, aromatics, ketones, nitriles, sulphides and many others. There is, as mentioned before, no need for specific sample preparations before injection into the PTR-MS inlet. Mass spectrometry based on traditional electron impact ionization usually has the disadvantage that the VOC molecules to be analyzed are strongly fragmented in the ionization process and thus the analysis, without additional measures (e.g., separation prior to measurements), is difficult in the case of complex mixtures. PTR-MS mass spectra are relatively simple because the ionization method of proton transfer is in comparison quite “soft”, resulting in relatively little fragmentation. The measured mass spectral profile closely reflects genuine headspace distribution and could be potentially used to assess the authenticity of a product, monitor deviations in production from a reference sample or classify products and raw materials.

1.9 Applications of PTR-MS

Over the last decade the PTR-MS technique has been used extensively in environmental sciences as a fast VOC sensor. Even if up to now the most successful applications of PTR-MS are probably in the monitoring of atmospheric gases and related issues (Hol99) there are several examples of the application of PTR-MS in other fields from breath analysis (Jor95; May03a; Rob03) to plant physiology (Fal01). Other potential applications are in the field of waste incineration, process monitoring, ambient air quality, medical and food and flavour science.

As mentioned above, there are strong efforts in the scientific community to develop fast, sensitive and non destructive techniques that can be used

routinely for real time evaluation and classification of food samples. In the past years, several laboratories have used PTR-MS to investigate different issues in food science (Bos03; Bos99; May03b; Pol03; van04) showing that PTR-MS spectra provide a useful tool to investigate the properties of agro-industrial products and processes. There is indication that the spectra can be related to the sensory characterisation of cheese (Gas01) and processed orange juices (Bia03a). It has been shown that for bread an objective understanding of the freshness can be derived by relating consumer freshness judgements to sensory descriptive analysis and volatile composition determined by PRT-MS (Hee04).

Analytical studies and sensory profiling have also been performed on different commercially available espresso coffee products. On-line analysis using PTR-MS is used to obtain chemical information about difference in composition of the coffee headspace characterizing the different coffee blends. In addition, expert panels trained for coffee tasting describe each sample by scoring 10 key flavour attributes on a 10-point scale. The overall sensory description of each sample is correlated with the analytically obtained differences in chemical composition in order to develop a statistical tool to predict the sensory profile based on analytical data. This system is routinely in use on a commercial level.

1.10 PTR-MS studies on fruits

The Proton Transfer Reaction Mass Spectrometer (PTR-MS) is a rather new technique to measure volatile compounds of flavour on fruits.

Up to date there are several publications dealing with an application of PTR-MS on research on volatile organic compounds (VOCs) release from fruits. PTR-MS spectra were successfully used for fingerprinting of single whole strawberry fruits of different cultivars (Bia03b). PTR-MS spectra can be used as a fingerprint to identify the cultivar of single whole strawberry fruits. Furthermore the head-space composition of the VOCs emitted by

apple fruit genotypes of the progeny Fiesta × Discovery was measured (Zin05). It was shown that it is possible to find quantitative trait loci (QTLs) related to PTR-MS characterisation of the head-space composition of single whole apple fruits indicating the presence of a link between molecular characterisation and PTR-MS data. PTR-MS analyses of VOCs of fruit head space (e.g. strawberries) showed a direct correlation between the emission of esters and differential expression of the alcohol acyl transferase gene involved in their synthesis (Car06). Experiments carried out on Red Delicious under Initial Low Oxygen Stress (ILOS) treatment show a strong dependence of the measured VOCs emissions from a large number of parameters that characterise a fruit (Bar05). PTR-MS can be used to qualify post-harvest preservation. The preservation of two varieties of apples (Renetta Canada and Golden Delicious) was investigated under different conditions and treatments over the whole storage period and during their shelf-life (Bos05). As shown, the emission of VOCs was clearly influenced by the storage conditions and treatments and correlated to final product state and quality. Principal Component Analysis (PCA) confirms the ability of PTR-MS to be a reliable and very sensitive way to discriminate the different procedures and conservation receipts by detecting VOCs. In a recent study PTR-MS measurements were performed on apples conserved under four different storage conditions: ULO (ultra low oxygen), DCA (dynamic controlled atmosphere), ILOS+ (initial low oxygen stress) and 1-MCP (1-methylcyclopropene) + ULO. The most important family of molecules, which are the major contributors to the characteristic apple-like aroma, were identified through PCA analysis and the essential differences during shelf-life regarding these family were evaluated (Cie09). Non-invasive experiments performed with a custom-built PTR-MS on monitoring the release of a number of aroma, flavour and fermentation related trace

gases by four apples cultivars (Elstar, Granny Smith, Jonagold and Pink Lady) under anaerobic and post-anaerobic conditions were reported .
The release of VOCs from apple fruits has been subject of detailed studies (Her03, Ech04, Lar07) but no studies have been performed on a wide range of cultivars.

1.11 The South Tyrolean fruit industry

South Tyrol's climate is strongly characterised by its openness to the Mediterranean in the south and its north side protected by the main ridge of the Alps. The southern floor of the Adige valley is more strongly influenced by the Mediterranean climate and makes for a longer growing season. The cultivation areas higher up, on the other hand, are characterized by the high alpine climate, which causes a noticeably later start and earlier end of the vegetative cycle. This is also the reason that the Laimburg Research Center carries out its variety testing at two different altitudes (Laimburg, 220 m NN, Tarsch, 670 m NN). The main differences in terms of climate are resumed in Table 2.

Table 2: Main climatic and phenological data of the 2 macroclimatic areas in South Tyrol

yearly sum/average (1965-2008)	Laimburg (220 m NN)	Tarsch (670 m NN)
rainfall (mm)	813	528
average temperature (°C)	11,5	9
difference minima-maxima in autumn (°C)	16	22
vegetative period (days)	240	215
sunshine hours (h)	1900	2280
full bloom of Golden Delicious	17-apr	27-apr
harvest of Golden Delicious	11-set	25-set

The Research Center Laimburg has worldwide connections with the most important breeding stations in the world; this enables it to get most of the new promising material for testing. In the sum around 400 apple hybrids and mutants are in Laimburg's collection, half of them replicated at the trial orchard in Tarsch. There are 4 to 6 trees per accession and site.

1.12 Storage of apples

Post-storage ripening of apples or 'shelf-life' depends on a great number of factors, such as growth, harvesting operations or storage conditions (Sol02). Optimisation of storage conditions has been the subject of many studies: in general, the aim of this process is to maintain and preserve fruit quality in accordance with consumer expectations (Hoe03). This is possible by limiting the production of ethylene using low temperature and an atmosphere with high carbon dioxide (CO_2) and low oxygen (O_2) concentration. This constitutes the basis of the so-called "controlled atmosphere" (CA) storage (Smo79). The state-of-the-art CA technique is "Ultra-Low O_2" (ULO) storage (Lau90). A modification of the ULO method is "Initial Low O_2 Stress" (ILOS) (Mat05). The main advantage of this technique is that it controls to a certain extent the physiological disorder scald which causes commercial losses.

An improvement of the CA-ULO technique consists in the "Dynamic Controlled Atmosphere" (DCA), which represents a dynamic adjustment of CA conditions to the physiological state of apples, without the need of post-harvest chemical treatment of fruit (Pra05). Online fluorescence measurements allow to detect the stress level of the fruits caused by low O_2 and to adapt the storage atmosphere to the physiological demands of apples to improve the storage process.

Another innovative fruit storage method is the use of specific molecules that are able to bind to ethylene receptors thus delaying the fruit's

maturation processes. 1-MCP (1-methylcyclopropene C_4H_6) formulated as SmartFresh® is largely used for this purpose (Bla03).

The choice of the storage method influences the parameters which determine the quality of the final product. It is necessary, therefore, to understand how the storage method affects the evolution of principal aromas in apples in order to obtain a high quality product.

One of the main problems regarding the storage techniques is that they not only delay ripening and ageing but also cause a suppression of the development of the principal volatile organic molecules (VOCs) of apples such as esters, aldehydes, alcohols and terpenes (Mat98; Val05) which makes the choice and evaluation of the suitable storage method difficult.

1.13 Objective of the study

The presented work is divided into two parts. Part 1, which represents the initial idea of the whole study, is dealing with comparisons of different cultivars. In a second step represented by Part 2 of the work, the effect of four different storage techniques on the dynamics of VOC emissions in apples during shelf-life were analysed.

1.13.1 Part 1

The aim of this part was to assess differences in VOCs between different apple genotypes and between two different environments. Furthermore the relation between instrumental analysis by PTR-MS and human perception of flavour and aroma should be investigated. This way the relationship between the PTR-MS fingerprint of the volatile mixture present in the apple head-space and its sensory characterisation can be investigated.

1.13.2 Part 2

The aim of this part was to investigate the dynamics of aroma production in apple fruits stored for seven months with four different storage techniques and during a subsequent period of shelf-life ripening of four weeks using PTR-MS analysis.

2 Part 1

2.1 Materials and Methods

2.1.1 Fruit materials

In year 2007, 59 apple cultivars have been picked as they reached a ripe harvestable stage, as described by King et al. (Kin01). 40 of these 59 cultivars have been harvested both at Laimburg (220 m a.s.l. in the valley area) and in Tarsch (670 m a.s.l. in the mountain area) both in South Tyrol, Italy.

Fruits which were asymmetrical, damaged from hail, insects or birds, were eliminated. In general fruits of medium size and colouring were collected, avoiding extraordinary small or big fruits.

2.1.2 Biometric, physical and chemical evaluations on fruits

The day of full flowering was registered. At harvest the crop load was assessed by counting and weighing the fruits per tree. Fruit size and weight of the single fruits harvested has been furthermore measured with the sorting machine Aweta at Laimburg, which additionally gives values of the percentage of overcolour.

Picture 1: Pimprenelle instrument at the Research Centre Laimburg

Quality analysis at harvest was performed with the semiautomatic Pimprenelle robotic machine (Picture 1, Setop Giraud Technology, France). The instrument consists of three measuring units: penetrometer, optical refractometer and titrator. It determines automatically and destructively weight, the soluble solid content, pulp firmness, titrable acidity and the juice content. Soluble solid content (°Brix), Acidity (malic acid, g/l) and firmness (kg/cm^2) was assessed and registered on a sample of 10 fruits.

The starch content was assessed on an equatorial slice of each of the 10 fruits. The slices were dipped in a potassium-iodine solution. The potassium-iodine solution was prepared using 10 g of potassium iodide and 3 g of iodine in 1 l of water. The residual starch in the fruit turns dark blue to black upon contact with the potassium-iodine solution. After approximately 1 minute, the blue colouring could be compared with the five-level scale colour chart. The five-level scale introduced by the Laimburg Experiment Station has proved itself in South Tyrol. In other areas of production, a 10-level scale is preferred.

Fruits were over-sampled in the field, to allow replacement for possible damage or decay in transit. All fruit samples have been stored in standard apple trays at 2°C normal cool storage at 90% of air humidity. After 3 months of storage (± 2 weeks) 10 fruits were analysed again with the semiautomatic Pimprenelle robotic machine for soluble solid content, firmness and acidity. Furthermore 6 fruits per cultivar and location were taken out of storage and stocked at 20°C for a period of 1 week of shelf life.

2.1.3 Sensorial evaluation

With the expertise of 3 people of the trained expert tasting panel at Laimburg sensory profiles of 3 out of the 6 fruits coming from shelf life were generated. The following fruit characteristics were considered and scored on a 1 to 9 scale for intensity (1 being very low intensity, 9 being very high intensity): odour intensity (detected by smelling), firmness, juiciness,

mealiness, taste sweet, taste sour, fruitiness intended as classical apple-like flavour, aroma in general (detected by tasting). Type of odour and type of aroma were described by adjectives if any outstanding perfume was noted.

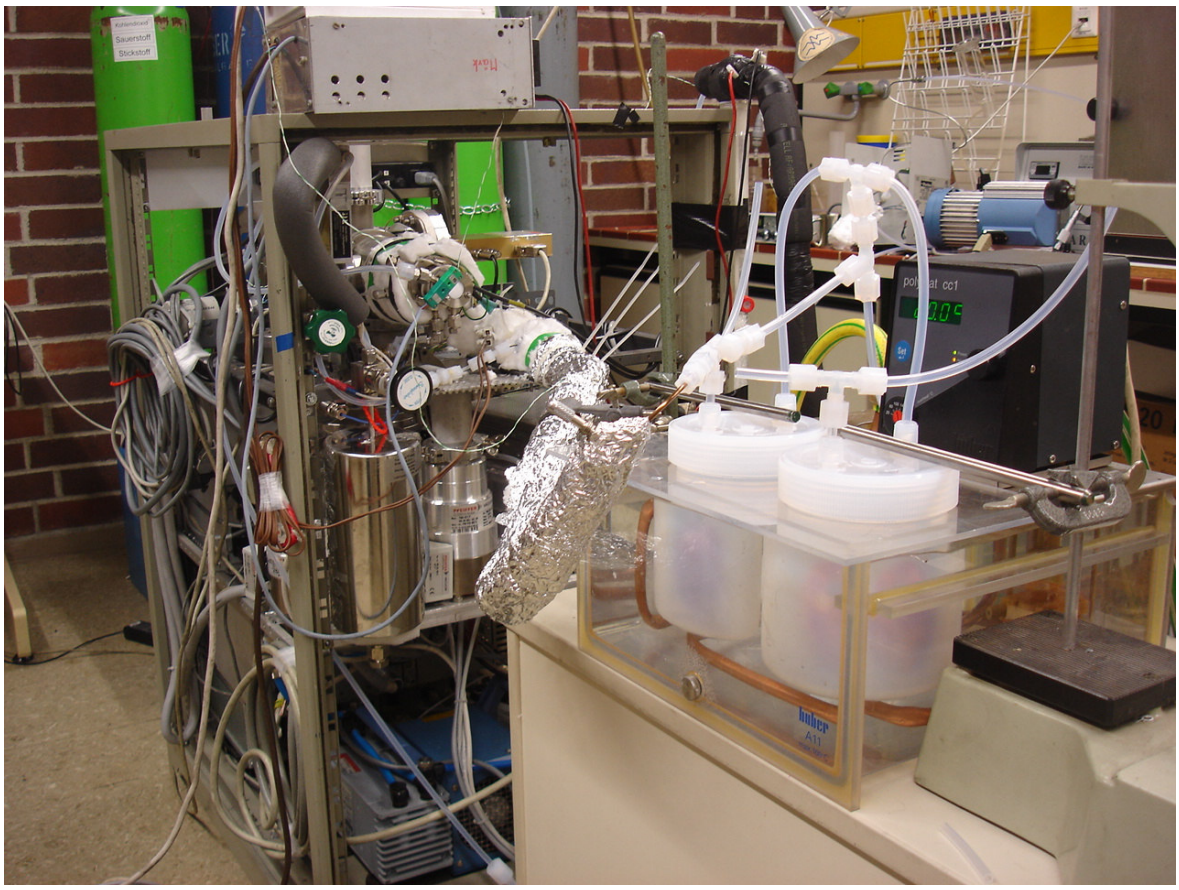
2.1.4 PTR-MS analysis

The remaining 3 fruits out of the 6 were analysed also after one week of shelf life with the PTR-MS apparatus (Picture 2) at the Institut für Ionenphysik und Angewandte Physik of the Leopold-Franzens Universität in Innsbruck Austria (responsible scientist Dr. Armin Wisthaler) in three periods:

12 – 15th of November 2007

10 – 12th of December 2007

10 – 11th of January 2008



Picture 2

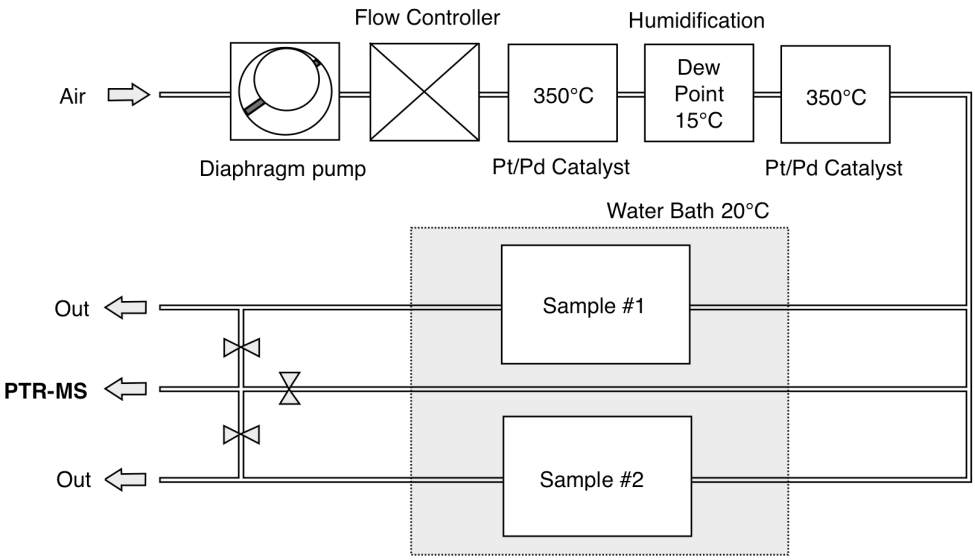


Figure. Experimental setup for apple measurements.

Picture 2, Figure 3: Experimental PTR-MS setup for apple measurements

A simplified scheme of the VOC measurement system is shown in Figure 3. For the analysis of VOCs emitted by apples, 1000-ml PFA jars were used, equipped with two-way PFA covers. An experimental set-up for simultaneous head-space VOCs analysis of two parallel apple samples was designed. The jars were connected to the carrier gas (zero air) supply. The used timing was 10 minutes measuring period of one sample with parallel conditioning (for apple head-space equilibrium) of another one to optimise experimental performance.

The head-space of each sampling jar was constantly flushed with zero-air (made out of lab air) that was first cleaned passing through a Pt/Pd catalyst operated at 350℃ in order to destroy any organic contaminants then humidified with set dew point to 15℃ and consequently passed through second Pt/Pd catalyst. A total carrier gas flow of 1000 ml/min was used with the flow being equally divided between the two sampling jars, i.e. the headspace of each bottle was flushed at 500 ml/min. If needed, a higher

headspace flow can be selected to increase the dynamic headspace dilution and to restrict headspace VOC mixing ratios to within the upper limit of linearity of the PTR-MS instrument. For analysis, 50 ml.min^{-1} of each jar was alternately fed to the PTR-MS instrument, with the overflow into the lab. Constant temperature in the jars during analysis was ensured with 20°C controlled water bath.

VOCs were measured by proton-transfer-reaction mass spectrometry (PTR-MS). PTR-MS (19) is a chemical ionisation technique. VOCs are ionised by way of proton transfer reactions from H_3O^+ ions and are mass spectrometrically detected one atomic mass unit (amu) higher (m+1) than the molecular weight (m) of the neutral compounds. Mass scans were performed from 18 to 200 amu using a dwell time of 1 s per amu. PTR-MS operating conditions were as follows: drift tube voltage, 470 V; drift tube pressure, 2.00 ± 0.05 mbar; drift tube temperature, 60°C; O_2^+/H_3O^+ ratio ≤ 2 %; inlet flow, 50 ml/min (STP); inlet temperature, 60°C. Memory effects (due to the sequential analysis of different samples) were reduced by the application of elevated inlet and drift tube temperatures (60°C), and the use of inert Silcosteel® material for the inlet tubing.

2.1.5 Statistical analysis

Intensities of the signals were normalised to the primary ion intensities ($[H_3O^+]$ + $[H_3O^+ \cdot H_2O]$). The mean values of the signal intensities were calculated for scan cycles five to nine for each mass in the range from 18 m/z to 200 m/z. Data normalisation and calculations of means were conducted using Microsoft Excel version 2002. Principal component analyses (PCA) tests were performed using SPSS 12.0 for Windows.

Both on the sensorial analysis and on the PTR-MS results a PCA was performed.

Pearson coefficient was calculated to estimate on one hand the correlation between Pimprenelle analysis and sensory evaluation and on the other hand the correlation between PTR-MS data and sensory analysis.

Subgrouping of the cultivars based on PTR-MS results was performed in two ways. Firstly, the values of the two main factors of the PCA were arbitrarily divided into three groups. Secondly, the difference between valley and mountain of the first four factors of the PCA was calculated for every cultivar. The resulting value (dF) was used to subgroup the cultivars.

2.2 Results and discussion

2.2.1 Biometric, physical and chemical evaluations on fruits

List of cultivars with the site location, full flowering and harvesting date are resumed in Table 3.

Table 3: Full flowering and harvest date of the apple cultivars on the 2 sites of Laimburg and Tarsch in increasing order of harvest date at Laimburg

	Cultivar	Full flowering date 2007		Harvest Date 2007			
		Laimburg	Tarsch	Laimburg	Tarsch	difference flowering date (days)	difference of harvest date (days)
1	Sansa	14.04.2007	21.04.2007	20.07.2007	06.08.2007	7	17
2	Silken	11.04.2007	17.04.2007	20.07.2007	17.08.2007	6	28
3	Coop 39 (Crimson Crisp®)	15.04.2007	n.a.	03.08.2007	22.08.2007		19
4	Early Red Gold	14.04.2007	21.04.2007	03.08.2007	22.08.2007	7	19
5	Nico Early	11.04.2007	18.04.2007	03.08.2007	22.08.2007	7	19
6	Gala Schnitzer (Schniga®)	13.04.2007	21.04.2007	06.08.2007	22.08.2007	8	16
7	Elanared	14.04.2007	20.04.2007	14.08.2007	22.08.2007	6	8
8	Civni (Rubens®)	13.04.2007	20.04.2007	14.08.2007	29.08.2007	7	15
9	Gradigold	15.04.2007	23.04.2007	14.08.2007	29.08.2007	8	15
10	Dalinbel (Antares®)	13.04.2007	18.04.2007	17.08.2007	29.08.2007	5	12
11	Delcoros (Autento®)	15.04.2007	22.04.2007	23.08.2007	05.09.2007	7	13
12	Milwa (Junami®)	12.04.2007	16.04.2007	27.08.2007	05.09.2007	4	9
13	Red Jonaprince (Red Prince®)	13.04.2007	19.04.2007	27.08.2007	05.09.2007	6	9
14	Ambrosia	12.04.2007	19.04.2007	27.08.2007	13.09.2007	7	17
15	Ariane (Les Naturianes®)	14.04.2007	n.a.	27.08.2007	13.09.2007		17
16	Ariwa	12.04.2007	18.04.2007	27.08.2007	13.09.2007	6	17
17	Dalinco	13.04.2007	20.04.2007	27.08.2007	13.09.2007	7	17
18	CIVG198 (Modì®)	10.04.2007	17.04.2007	27.08.2007	13.09.2007	7	17
19	Rucla	12.04.2007	18.04.2007	31.08.2007	05.09.2007	6	5
20	Azufu	13.04.2007	18.04.2007	31.08.2007	13.09.2007	5	13
21	La Flamboyante (Mairac®)	12.04.2007	19.04.2007	31.08.2007	13.09.2007	7	13
22	Nicoter (Kanzi®)	10.04.2007	16.04.2007	31.08.2007	13.09.2007	6	13
23	Pinova	13.04.2007	17.04.2007	31.08.2007	13.09.2007	4	13
24	Doriane	12.04.2007	n.a.	31.08.2007	19.09.2007		19
25	Nicola	14.04.2007	19.04.2007	31.08.2007	19.09.2007	5	19

26	Nicogreen (Greenstar®)	14.04.2007	20.04.2007	03.09.2007	13.09.2007	6	10
27	Delblush (Tentation®)	12.04.2007	17.04.2007	03.09.2007	19.09.2007	5	16
28	Scifresh (Jazz®)	12.04.2007	17.04.2007	03.09.2007	19.09.2007	5	16
29	Hapke Del.	n.a.	19.04.2007	04.09.2007	19.09.2007		15
30	Golden Del. Klon B	13.04.2007	19.04.2007	06.09.2007	19.09.2007	6	13
31	Topaz	11.04.2007	17.04.2007	07.09.2007	19.09.2007	6	12
32	Golden Orange	13.04.2007	19.04.2007	12.09.2007	19.09.2007	6	7
33	Huaguan	14.04.2007	20.04.2007	12.09.2007	27.09.2007	6	15
34	Caudle (Cameo®)	14.04.2007	19.04.2007	20.09.2007	03.10.2007	5	13
35	Gold Pink (Gold Chief®)	13.04.2007	21.04.2007	20.09.2007	03.10.2007	8	13
36	Delfloki	11.04.2007	18.04.2007	20.09.2007	10.10.2007	7	20
37	Joburn (Aurora Red®)	11.04.2007	16.04.2007	25.09.2007	03.10.2007	5	8
38	Catarina	12.04.2007	19.04.2007	25.09.2007	17.10.2007	7	22
39	Brak (Kiku® 8)	12.04.2007	19.04.2007	03.10.2007	10.10.2007	7	7
40	Coop 38 (GoldRush®)	11.04.2007	17.04.2007	11.10.2007	17.10.2007	6	6
41	Geneva	10.04.2007		31.07.2007			
42	Summerfree	14.04.2007		03.08.2007			
43	Reka	12.04.2007		09.08.2007			
44	Initial		19.04.2007		13.08.2007		
45	Rubinstep (Pirouette®)	13.04.2007		17.08.2007			
46	Delrouval (Cybele®)		17.04.2007		22.08.07		
47	Gamhong		20.04.2007		22.08.07		
48	Golden Mira	14.04.2007		23.08.2007			
49	Saturn		20.04.2007		29.08.2007		
50	Nevson (Sonya®)		18.04.2007		03.09.2007		
51	Rafzubex (Rubinette Rosso®)		19.04.2007		13.09.2007		
52	Red Idared		18.04.2007		19.09.2007		
53	Granny Smith	13.04.2007		20.09.2007			
54	Dalinette (Choupette®)	13.04.2007		25.09.2007			
55	Morgenduft Dallago	16.04.2007		25.09.2007			
56	Hongro		19.04.2007		27.09.2007		
57	Meran		19.04.2007		27.09.2007		
58	Sciros (Pacific Rose®)		20.04.2007		10.10.2007		
59	Cripps Pink (Pink Lady®)	11.04.2007		18.10.2007			

The difference in full flowering period ranges from a minimum of four days up to a maximum of eight days, depending on the cultivar. In average, in 2007, full flowering occurred six days later in Tarsch.

The difference in picking date between the two sites ranges from a minimum of five days for Rucla up to a maximum of twenty-eight days for Silken. In average between the two sites there was a difference of 14 days in terms of picking date, Tarsch being the later ripening site since it is located at a higher altitude than Laimburg.

Furthermore, while the production per tree was at a comparable level for the 2 sites (data not shown), fruit size was tendentially bigger in Laimburg than in Tarsch while the percentage of overcolour on bicoloured cultivars was clearly higher in Tarsch. Those results could be expected due to the climatic differences between the two sites mentioned in the introduction. As an example the colour and size grading results of 2 cultivars at the 2 sites are shown (Figure 3 a and 3b).

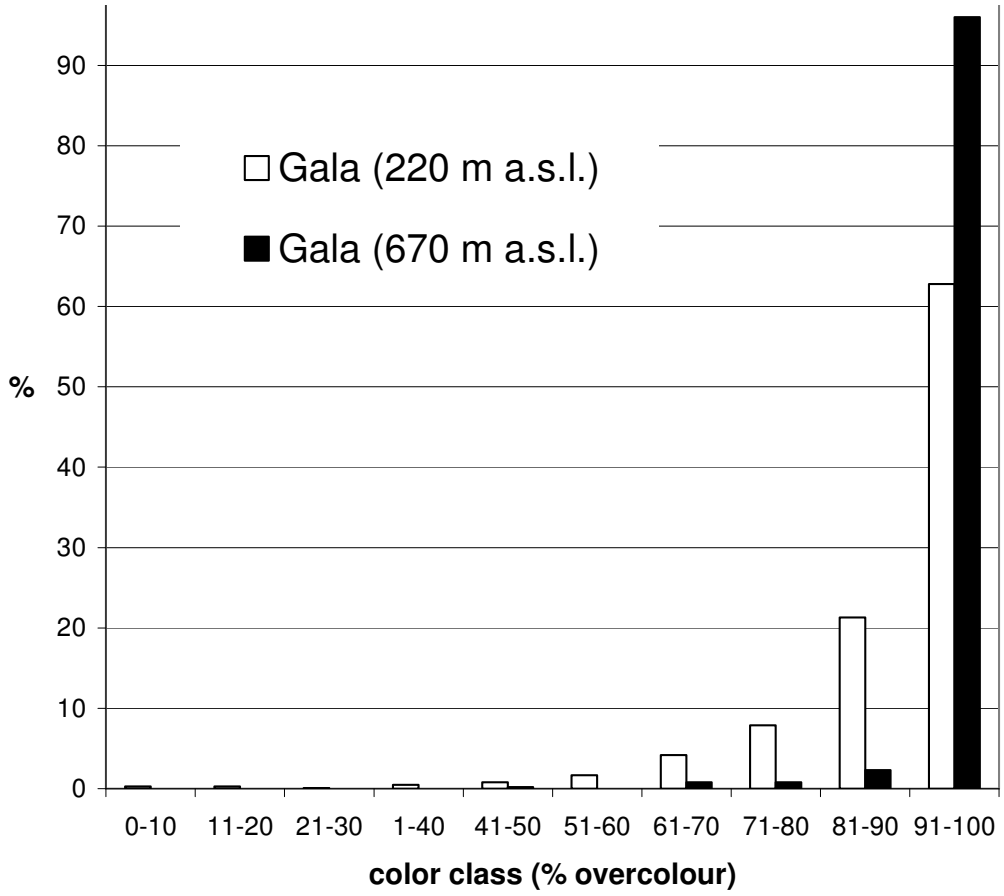


Figure 3a: Colour grading results of cv. Gala in the 2 sites Laimburg and Tarsch

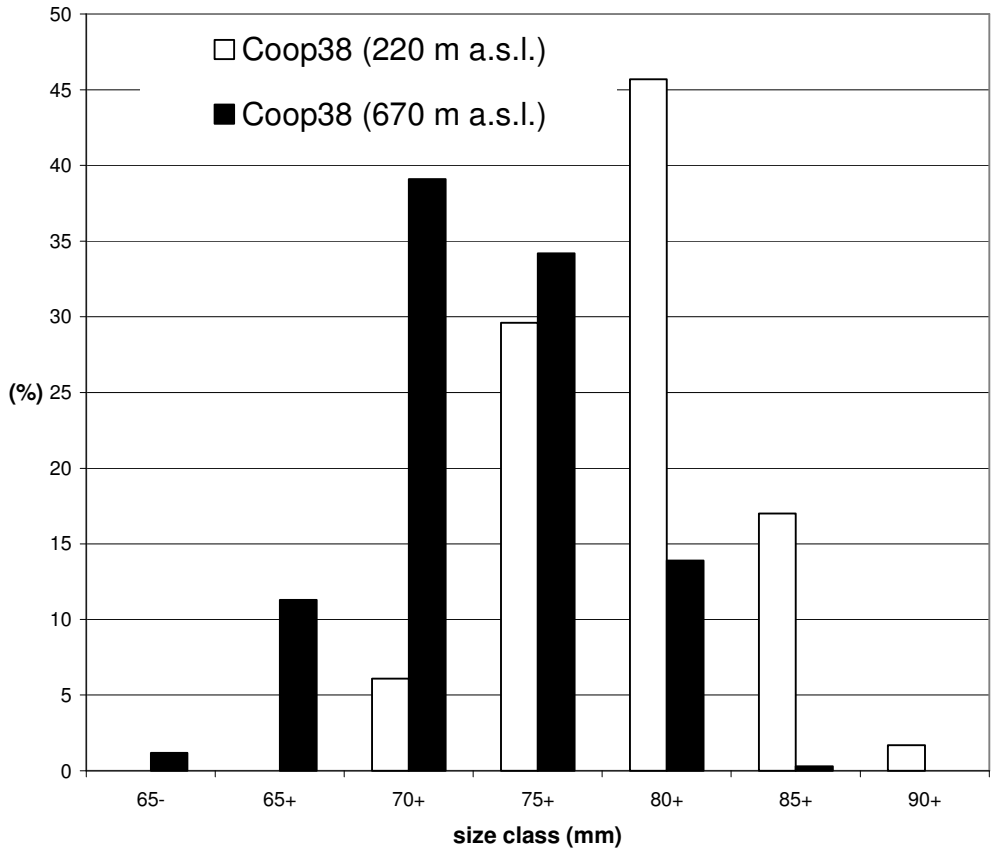


Figure 3b: Size grading results of cv. Coop38 on the 2 sites Laimburg and Tarsch

2.2.1.1Starch content

As fruit ripens on the tree, starch is broken down and converted into sugar through hydrolysis. Starch content is a maturity index used also on a commercial level: for the layman it is still the easiest and most meaningful maturity test. The less starch, the riper the fruit. However, it is not a fully reliable index, meaning that it is basically impossible to give absolute values with an overall validity. First of all, starch degradation patterns vary amongst climates and amongst cultivars. There are cultivars like Fuji (in the present work the 2 mutants Kiku 8 and Azufu are included) where the starch content can even increase for a certain period during the ripening process. It is therefore not a suitable maturity index for this specific cultivar.

Anyway in the present work starch index was the most important parameter to quantify the maturity status of the collected samples.

Table 4: Starch index (scale 1 to 5) of the 40 cultivars in alphabetical order determined on the 2 sites at harvest

cultivar	Tarsch	Laimburg
Ambrosia	3,1	2,6
Ariane	3,3	3,9
Ariwa	3,9	3,0
Azufu	3,5	4,3
Catarina	1,8	2,4
Caudle	3,1	3,0
Civni	2,5	2,3
Coop 38	3,0	n.a.
Coop39	3,5	2,6
Dalinbel	2,7	3
Dalinco	2,1	3,4
Delblush	3,2	2,5
Delcoros	2,0	2,7
Delfloki	3,0	3,0
Doriane	3,5	4,7
Early Red Gold	3,1	2,9
Elanared	2,5	2,6
G 198	3,8	3,3
Gala Schnitzer	3,0	2,8
Gold Pink	2,4	3,5

cultivar	Tarsch	Laimburg
Golden Del. Klon B	3,1	3,0
Golden Orange	n.a.	2,4
Gradigold	2,8	3
Hapke Del.	2,1	2,9
Huaguan	3,8	4,1
Joburn	2,4	3,2
Kiku 8	2,9	4,5
La Flamboyante	2,3	2,8
Milwa	1,5	2,5
Nico Early	n.a.	2,8
Nicogreen	1,8	2,4
Nicola	3,2	3,5
Nicoter	2,5	3,1
Pinova	3,6	3,0
Red Jonaprince	3,6	4,0
Rucla	2,6	3,8
Sansa	2,3	2,5
Scifresh	2,8	3,7
Silken	2,7	2,5
Topaz	2,7	3,2

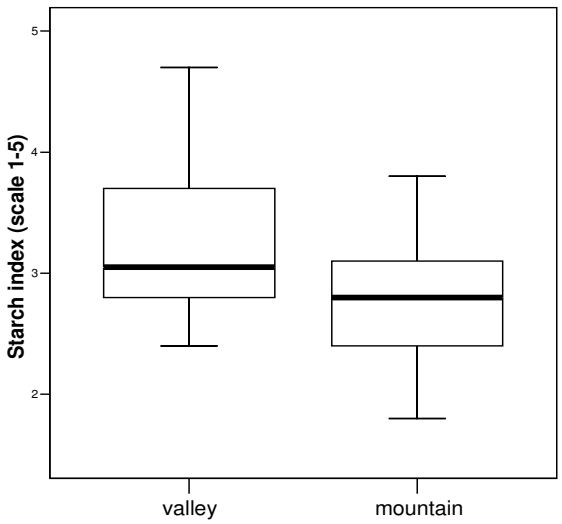


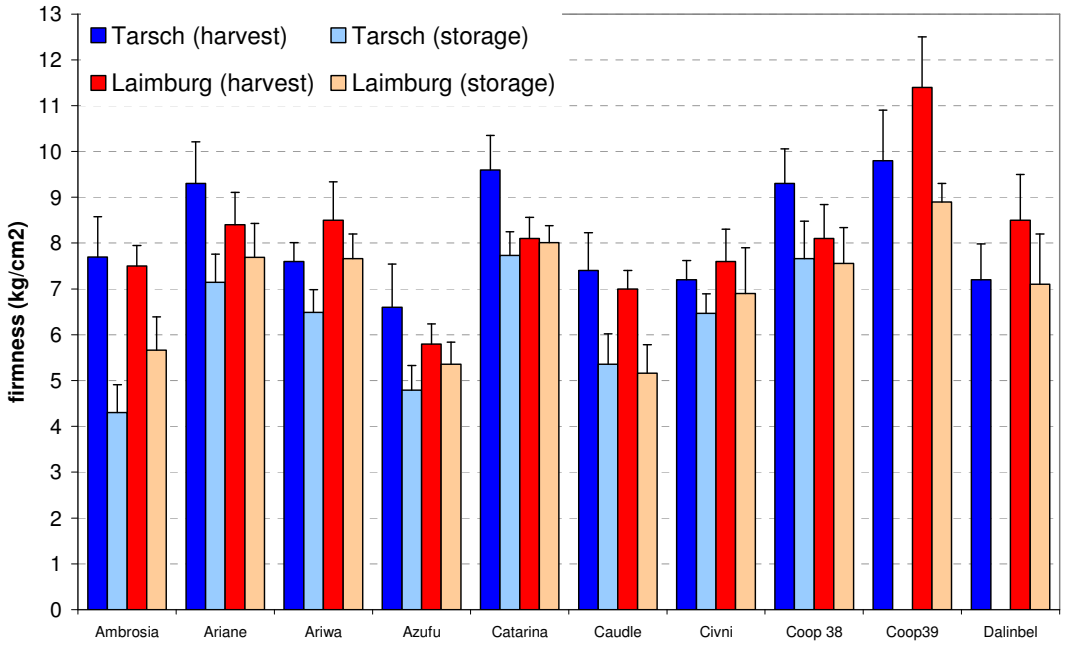
Figure 4: Boxplot representing the starch index of the 40 cultivars on the 2 sites Laimburg (valley) and Tarsch (mountain)

The starch content analysis at harvest showed on average a lower starch content (-0,3 points on the 1-5 scale) of the 40 cultivars collected on the mountain site compared to the respective cultivars in the valley site (table 4, Figure 4). This is in line with past observations and recommendations for commercial picking.

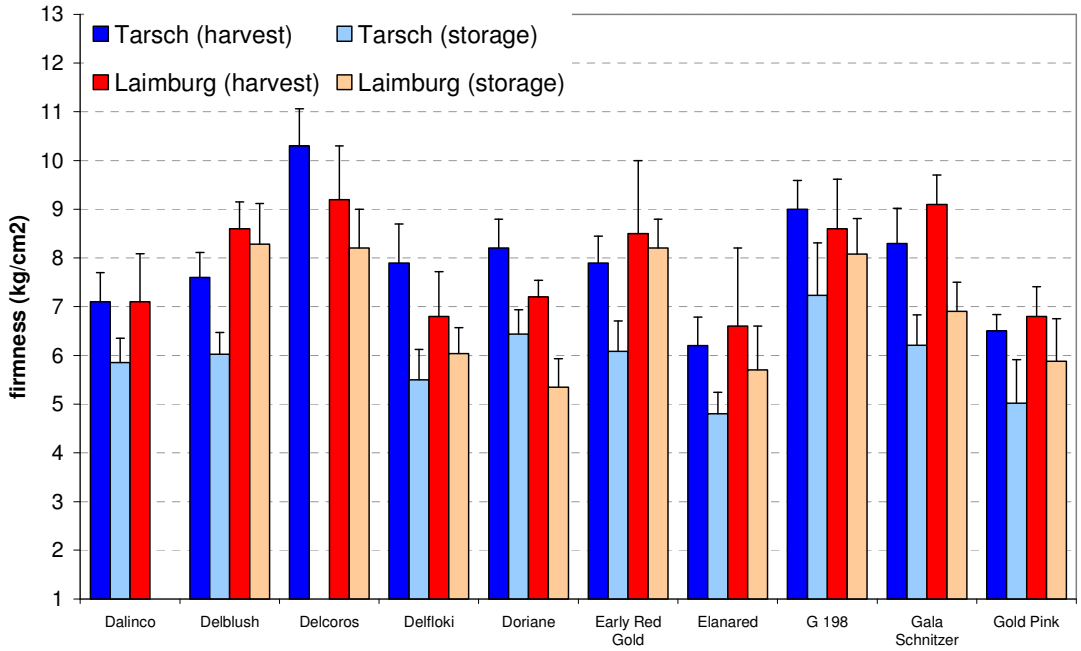
2.2.1.2Firmness

Fruit firmness is an important quality index, which varies among different cultivars and sites. The firmness of the fruit flesh normally decreases with ripeness, the fruit becomes softer. The ideal value at harvest is a narrow span. Especially the end of the ideal harvest date can be measured by the penetrometer. The firmness of the fruit flesh is of prime importance for the perishability in storage and after shipment. More and more, buyers require a minimum penetrometer value at acceptance of the shipment.

The results of the penetrometric measurements on the 40 cultivars in the 2 sites are shown in Figures 5a/b/c/d.

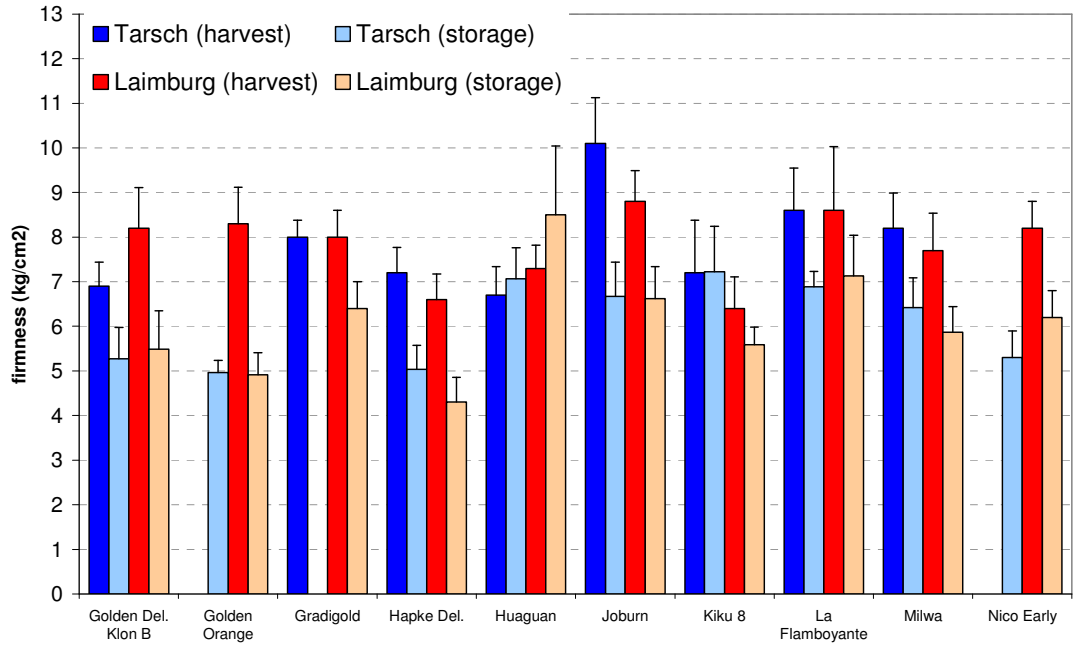


5a)

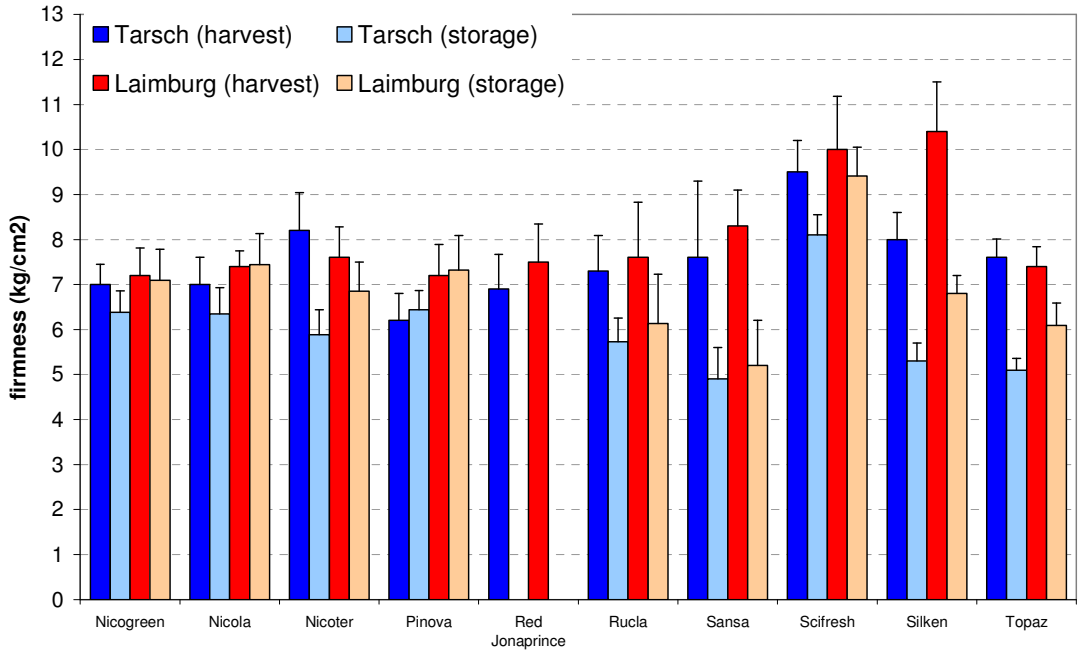


5b)

Figure 5a/b/c/d: Firmness measured by Pimprenelle instrument on the 40 cultivars in Tarsch and Laimburg at harvest and after storage. Cultivars are in alphabetical order and standard deviation is shown.



5c)



5d)

Figure 5a/b/c/d: Firmness measured by Pimprenelle instrument on the 40 cultivars in Tarsch and Laimburg at harvest and after storage. Cultivars are in alphabetical order and standard deviation is shown.

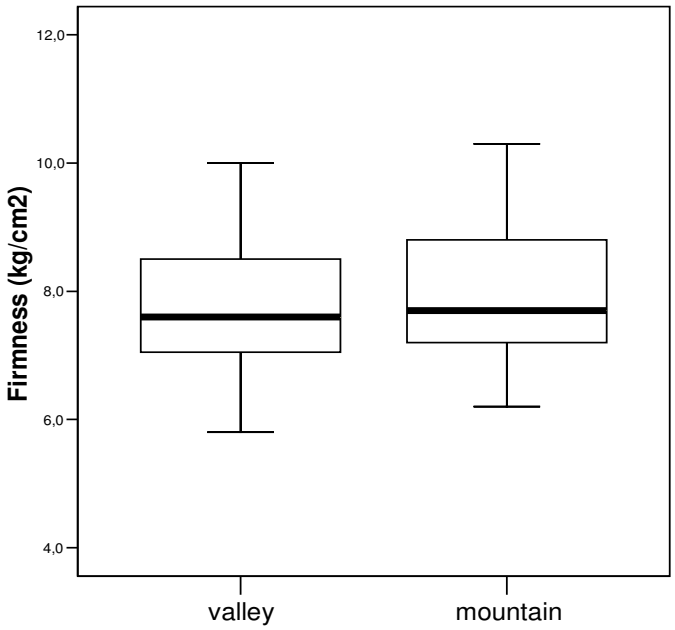


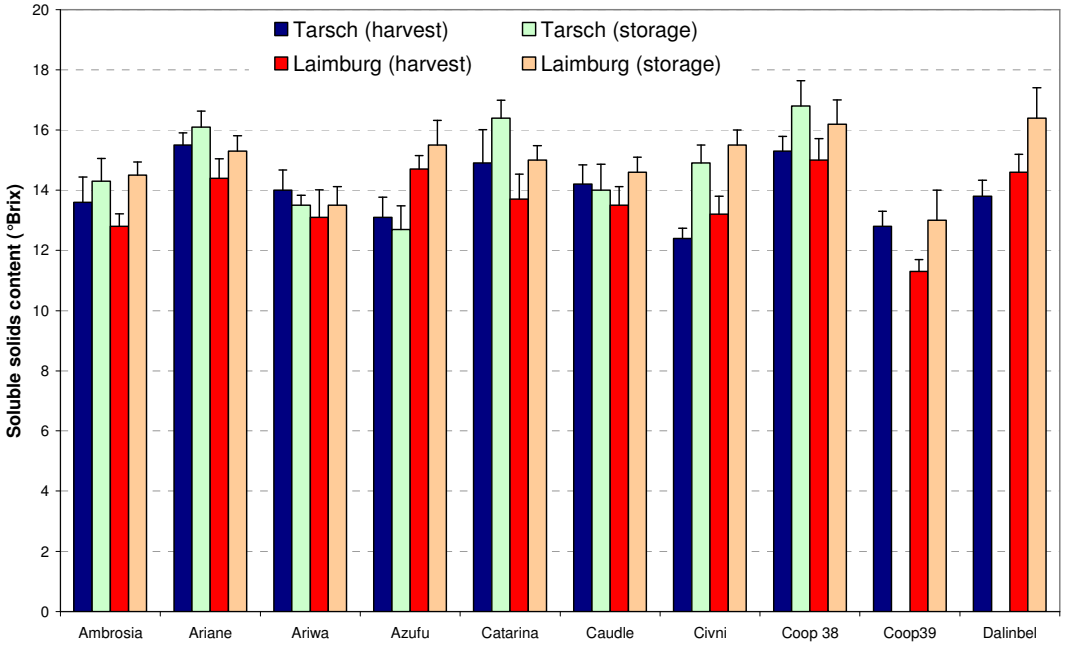
Figure 6: Boxplot representing the firmness of the 40 cultivars on the 2 sites Laimburg (valley) and Tarsch (mountain) at harvest

Fruits of the mountain area tended to have higher fimness values (Figure 6).

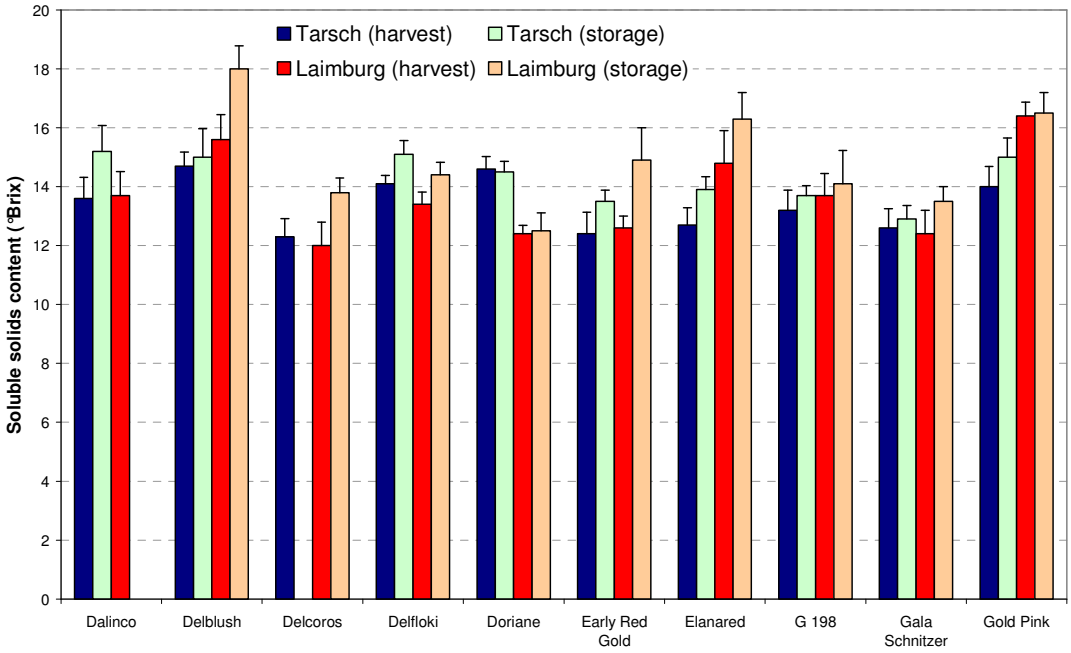
2.2.1.3Soluble solids content

The soluble solids content (SSC) is an indicator for the sugar content of the fruits and it is one of the most important parameters for internal quality. The minimum sugar content is of primary interest in determining ripeness. SSC increases during storage since the remaining starch is degraded mainly to the complex sugar sucrose and the reducing sugars fructose and glucose. The sugar content of the fruit ripening on the tree is influenced by many factors and can even fluctuate during the ripening process. Therefore for the layman this measurement isn't decisive in determining the harvest date until the final phase of ripening.

The results of the refractometric measurements of SSC on the 40 cultivars in the 2 sites are shown in Figures 7a/b/c/d.

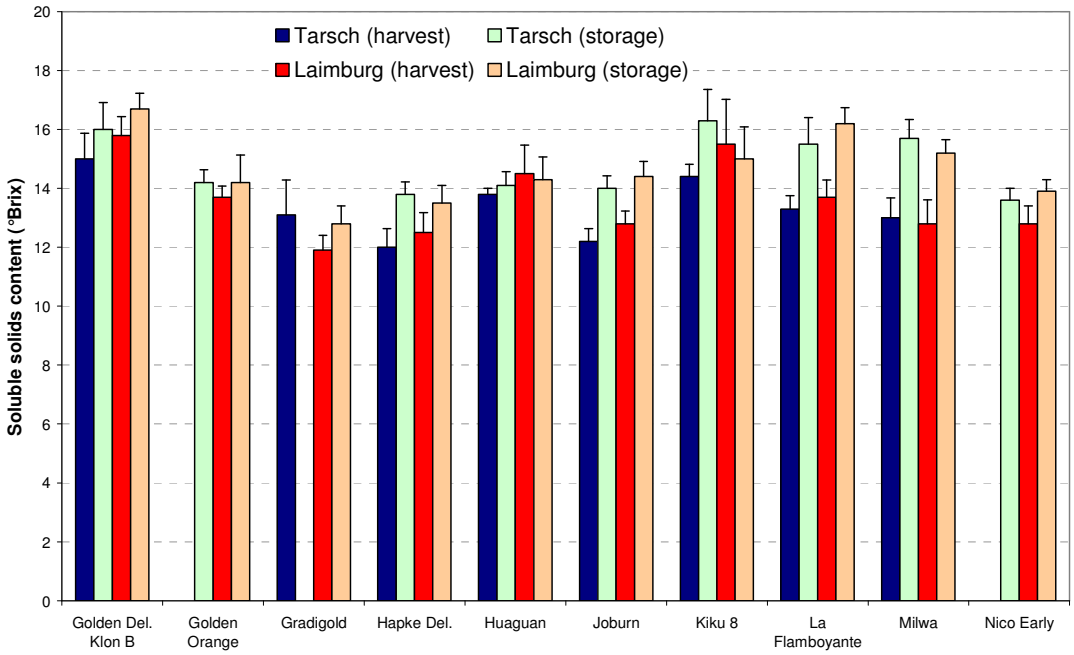


7a)

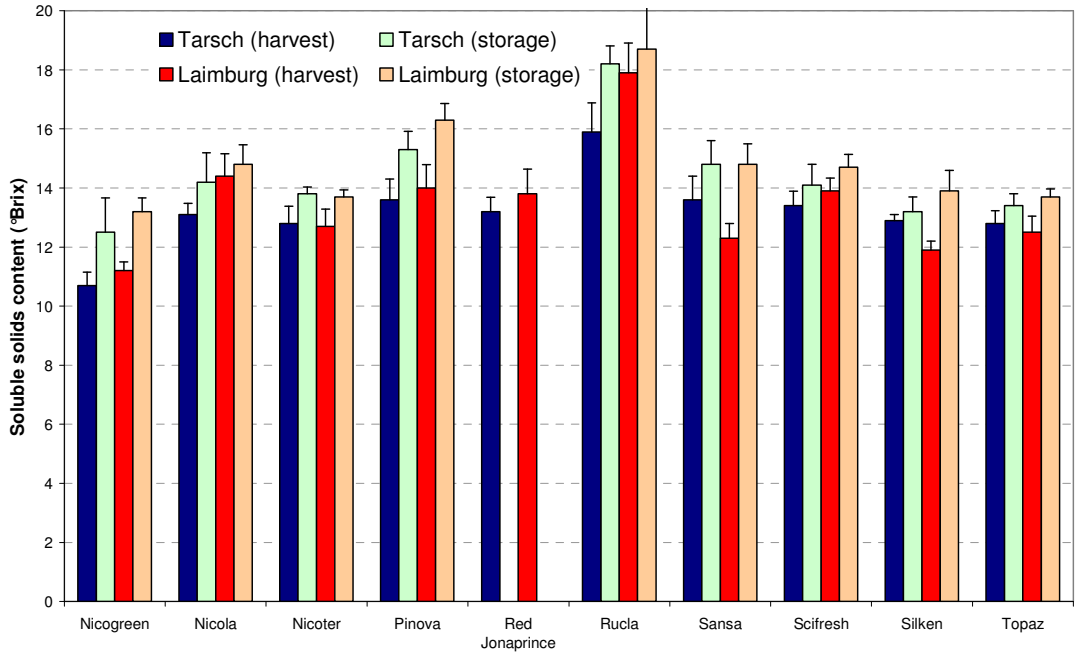


7b)

Figure 7a/b/c/d: SSC measured by Pimprenelle on the 40 cultivars in Tarsch and Laimburg at harvest and after storage. Cultivars are in alphabetical order and standard deviation is shown.



7c)



7d)

Figure 7a/b/c/d: SSC measured by Pimprenelle on the 40 cultivars in Tarsch and Laimburg at harvest and after storage. Cultivars are in alphabetical order and standard deviation is shown.

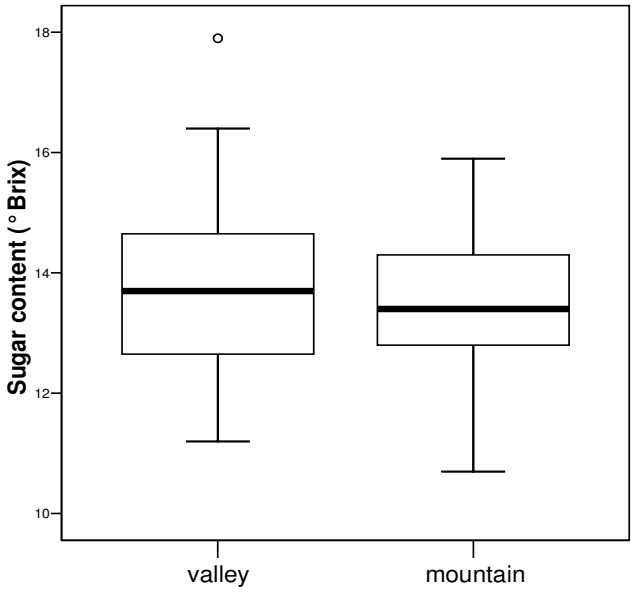


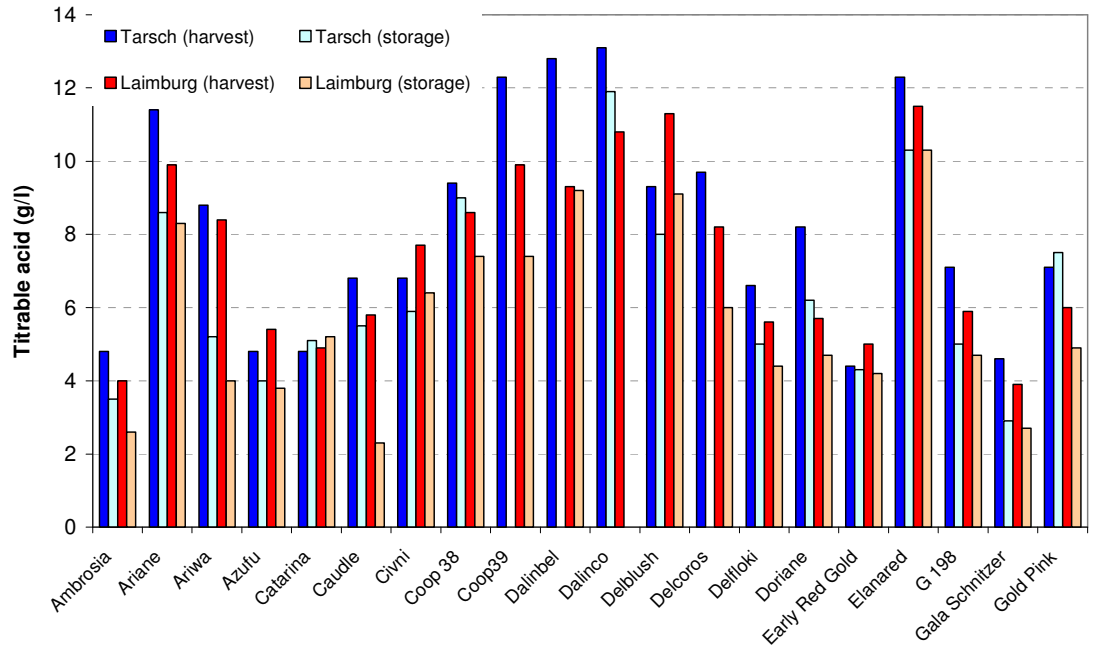
Figure 8: Boxplot representing the soluble solids content (SSC) of the 40 cultivars on the 2 sites Laimburg (valley) and Tarsch (mountain) at harvest

SSC measured with the refractometer was tendentially lower in the mountain area at harvest (Figure 8).

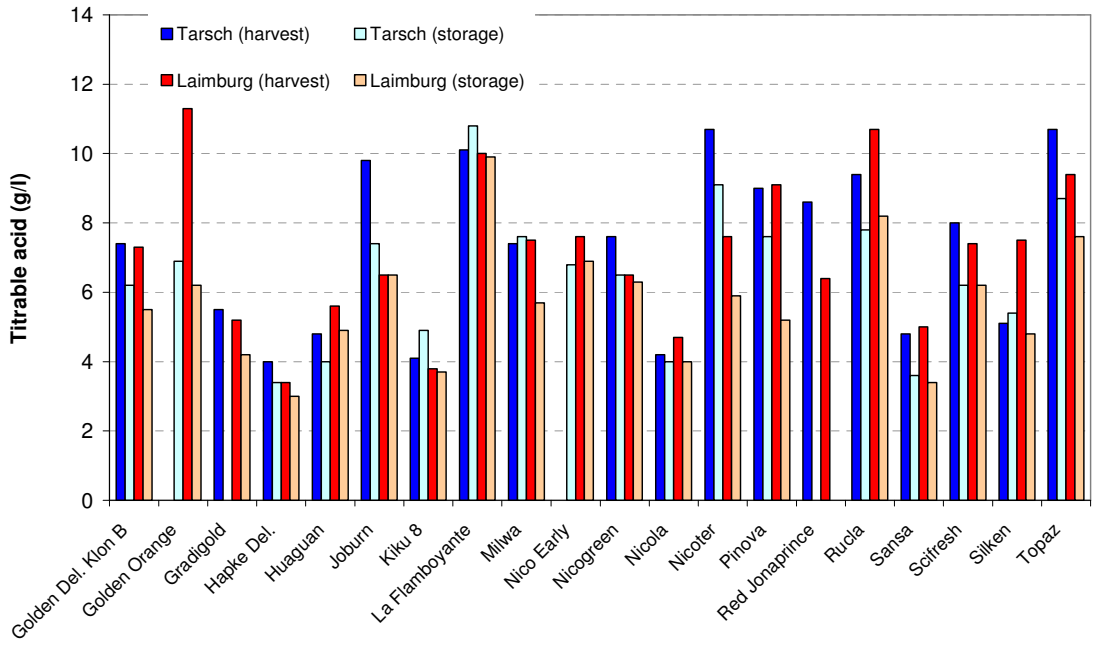
2.2.1.4Acidity

Acidity is, along with sugar and flavouring, of decisive importance for the quality, the flavour development and the perishability of the fruit. It is, however, a measurement of quality which scarcely gives clues as to determining the harvest date. The acidity of the fruit can vary greatly from year to year, influenced mainly by weather conditions. Even in the final stages of ripening, considerable variances are possible, but in general, acidity decreases with increasing ripeness. The process continues during storage. The three most important acids are malic acid, citric acid and tartaric acid. The titratable acids are expressed in g/l.

The results of the titration of the 40 cultivars in the 2 sites Laimburg and Tarsch are shown in Figures 9a/b/c/d.



9a)



9b)

Figure 9a/b: Titrable acidity measured by Pimprenelle on the 40 cultivars in Tarsch and Laimburg at harvest and after storage. Cultivars are in alphabetical order.

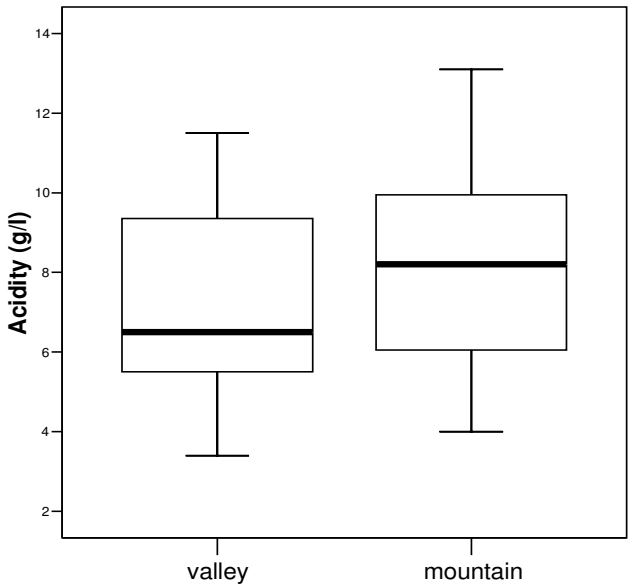


Figure 10: Boxplot representing the titrable acidity of the 40 cultivars on the 2 sites at harvest

Titrable acidity was tendentially higher in the mountain area at harvest (Figure 10).

2.2.2 Sensorial analysis

The results of the sensorial evaluation are shown in Table 5.

Table 5: Results of the sensorial evaluation of 59 cultivars. The numbers represent the intensity of the respective parameter on a 1 to 9 scale (1= very low intensity, 9= very high intensity).

cultivar	site	odour intensity	odour type	firmness	juiciness	mealiness	taste sweet	taste sour	flavour intensity	flavour type	fruitiness
Ambrosia	valley	1		4	4	4	6	3	4		5
Ambrosia	mountain	1		4	4	4	6	2	3		5
Ariane	valley	1		6	5	1	6	7	7	nuts	3
Ariane	mountain	3		6	5	2	6	7	5	exotic fruits	4
Ariwa	valley	1		7	6	1	6	4	6	grape	5
Ariwa	mountain	4		4	4	3	6	4	6	almond	5
Azufu	valley	1		4	7	3	7	3	3		5
Azufu	mountain	4		4	6	1	4	2	3		3

Catarina	valley	2		7	6	1	5	2	4		5
Catarina	mountain	3		4	4	1	5	3	5	berries	5
Caudle	valley	2		4	5	3	6	3	4		6
Caudle	mountain	2		3	4	2	6	3	5		5
Civni	valley	1		5	5	1	6	5	7		7
Civni	mountain	1		6	6	1	7	5	5	exotic	3
Coop 38	valley	1		6	4	1	6	6	6	citrus	5
Coop 38	mountain	1		5	2	1	6	6	6	floreal	6
Coop 39	valley	1		6	5	1	5	6	6	candy	4
Coop 39	mountain	4		6	5	1	6	7	7		2
Cripps Pink	valley	1		6	5	1	6	4	6		5
Dalinbel	valley	not available									
Dalinbel	mountain	5		4	4	3	6	6	4		6
Dalinco	valley	1		6	5	2	5	8	7	berries	3
Dalinco	mountain	1		4	5	1	4	6	5	citrus	5
Dalinette	valley	1		3	3	6	5	4	3		6
Delblush	valley	2		8	4	1	8	7	4	honey	6
Delblush	mountain	2		6	6	1	6	7	4	citrus	5
Delcoros	valley	1		7	6	1	6	6	3		6
Delcoros	mountain	4		7	3	1	6	6	5		4
Delfloki	valley	1		4	5	2	6	4	3		6
Delfloki	mountain	2		4	3	2	6	2	6	grape	3
Delrouval	mountain	1		4	5	4	5	6	3		2
Doriane	valley	2		3	3	4	5	3	6	grape	2
Doriane	mountain	4	quince	4	4	3	6	4	5	apricot	5
Early Red Gold	valley	3		7	5	1	7	3	5		8
Early Red Gold	mountain	2		4	5	3	6	2	3		2
Elanared	valley	1		2	3	3	5	7	5		6
Elanared	mountain	2		3	6	5	4	8	6	apricot	3
G198	valley	4		7	6	1	6	3	5	quince	2
G198	mountain	4		6	5	2	5	4	5	berries, grapes	4
Gala Schnitzer	valley	1		6	6	1	6	2	3		4
Gala Schnitzer	mountain	1		5	6	2	6	2	6	pear	3
Gamhong	mountain	5		8	5	1	6	2	7	pear	2
Geneva	valley	8		1	1	9	2	6	3		2
Gold Pink	valley	2		4	5	3	6	4	3		4
Gold Pink	mountain	2		4	3	2	6	3	4	banana	5
Golden Del. Klon B	valley	2		4	4	3	7	3	3		5
Golden Del. Klon B	mountain	2		4	5	2	6	4	4		5
Golden Mira	valley	1		4	4	4	5	4	8	moldy	2
Golden Orange	valley	1		2	4	5	6	4	4		6
Golden Orange	mountain	1		4	5	3	6	4	6	banana	5
Gradigold	valley	4	citrus	6	5	1	5	4	8	berries	4
Gradigold	mountain	6		5	4	3	6	4	8		3
Granny Smith	valley	2		4	7	1	4	6	4	green apple	4
Hapke Del.	valley	6		3	3	7	5	2	7	berries	2
Hapke Del.	mountain	4		2	3	6	6	2	6	berries	4
Hongro	mountain	2		5	4	2	5	4	6	exotic fruits	5
Huaguan	valley	2		6	6	1	6	3	3		5

Huaguan	mountain	3		6	6	1	7	2	5		2
Initial	mountain	6		4	3	3	4	4	7		5
Joburn	valley	1		5	3	4	6	3	4		5
Joburn	mountain	1		3	4	6	6	2	4		3
Kiku 8	valley	1		6	7	1	7	2	6	exotic fruits	6
Kiku 8	mountain	2		6	7	1	7	2	6	exotic fruits	6
La Flamboyante	valley	1		6	6	1	7	7	6	kaki, moldy	4
La Flamboyante	mountain	1		5	7	1	7	8	4		6
Meran	mountain	1		4	5	3	5	6	3		3
Milwa	valley	1		4	4	2	6	4	4		4
Milwa	mountain	2		5	6	1	6	5	5		4
Morgenduft Dallago	valley	1		3	1	7	5	3	4	moldy	1
Nevson	mountain	1		6	7	1	7	2	2		4
Nico Early	valley	4		3	4	2	6	4	4		5
Nico Early	mountain	5		4	5	2	7	5	5		3
Nicogreen	valley	5		6	7	1	5	5	5		3
Nicogreen	mountain	4		4	5	2	3	5	2		4
Nicola	valley	1		6	6	1	6	3	3		6
Nicola	mountain	1		5	6	1	4	4	4		4
Nicoter	valley	1		4	6	2	6	5	2		6
Nicoter	mountain	1		6	6	2	6	6	2		6
Pinova	valley	2		5	4	2	7	4	4		5
Pinova	mountain	1		6	6	1	6	6	4	citrus	6
Rafzubex	mountain	1		4	5	2	6	5	5	sulfur	6
Red Idared	mountain	2		3	4	3	4	4	3		3
Red Jonaprince	valley	2		5	5	3	6	4	6	exotic	3
Red Jonaprince	mountain	2		3	6	5	6	4	2		5
Reka	valley	4		3	3	5	4	7	8	citrus	5
Rubinstep	valley	3		5	5	3	6	7	6		3
Rucla	valley	1		5	5	1	8	4	3		6
Rucla	mountain	1		5	6	1	8	3	2		4
Sansa	valley	1		4	5	2	7	3	7	candy	4
Sansa	mountain	2		3	5	2	7	2	8	berries	5
Saturn	mountain	6		3	2	5	6	3	2		5
Scifresh	valley	1		8	6	1	7	5	3		7
Scifresh	mountain	1		7	6	1	7	7	4		6
Sciros	mountain	1		5	5	1	6	2	4		4
Silken	valley	2		5	4	3	6	4	7	exotic	4
Silken	mountain	3		3	3	6	5	4	6		3
Summerfree	valley	4	grassy	2	3	4	4	5	8	moldy	2
Topaz	valley	6		4	4	3	6	5	7		5
Topaz	mountain	7		4	5	1	6	5	7	spicy	5

The panelists could detect by smelling the fruits differences in odour intensity between the samples coming from the valley (Laimburg) and the mountains (Tarsch). In general, after storage and 6 days of shelf life mountain fruits were more intense in odour. In terms of aroma intensity the

apples coming from the valley were less intensively aromatic when they were tasted (Figure 11).

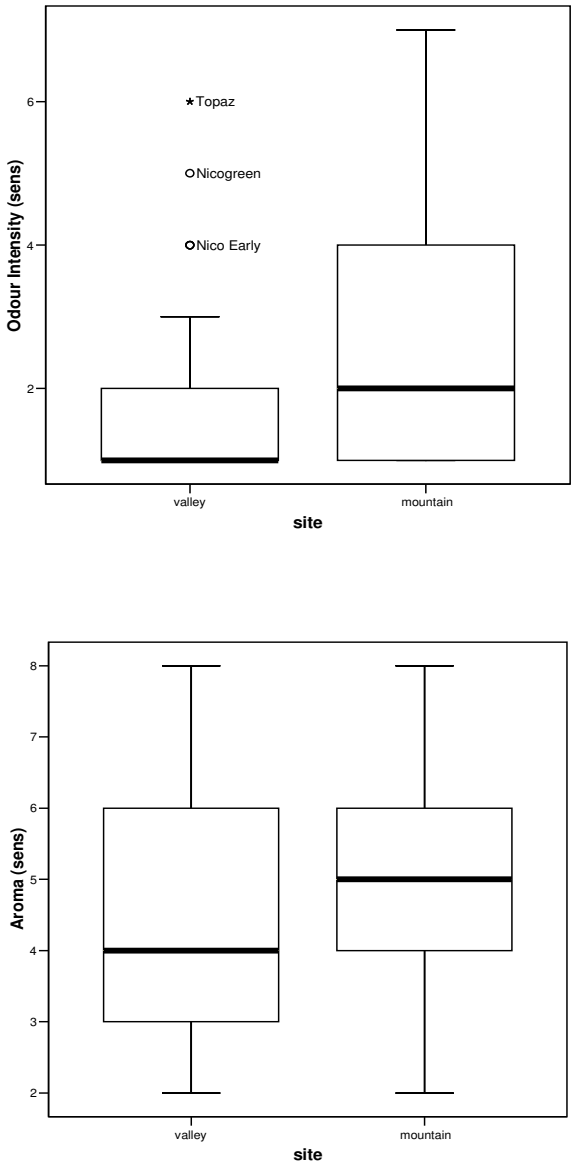


Figure 11: Results of the sensorial evaluations in terms of odour and aroma intensity.

Principal components analysis was performed to determine the correlation structure of the sensorial data (Table 6). PC1, PC2 and PC3 explain more than 60% of the total variance.

Table 6: PCA of the results of the sensorial evaluation

Component	% variance	% cumulated
1	32,130	32,130
2	18,583	50,712
3	12,656	63,368

	component		
	1	2	3
odour intensity	-0,489	0,502	-0,138
firmness	0,766	0,423	-0,111
juiciness	0,667	0,106	-0,476
mealiness	-0,788	-0,378	0,075
taste sweet	0,451	-0,282	-0,032
taste sour	0,318	0,431	0,694
fruitiness	0,507	-0,459	0,487
aroma intensity	-0,344	0,652	0,169

PC1, which explains more than 30% of the variance, mostly represents texture parameters like firmness, juiciness and mealiness. On the other hand, PC2 shows important positive loadings of odour and flavour parameters like odour intensity, fruitiness (typical apple aroma) and aroma intensity in general.

2.2.3 Correlation between instrumental and sensorial analysis

It was possible to find correlations between the sensorial perception of the panellists and the results of the Pimprenelle analysis. The most important correlations and their level of significance are resumed (Table 7).

Table 7: Correlations between instrumental and sensorial analysis (Pearson coefficient)

	Firmness (instrumental)	
Firmness (sens)	0,78	**
Mealiness (sens)	-0,55	**
Sourness (sens)	0,29	**
Juiciness (sens)	0,27	*
Fruitiness (sens)	0,25	*
Odour Intensity (sens)	-0,18	
Sweetness (sens)	0,15	
Aroma (sens)	-0,09	

	Acidity (instrumental)
Sourness (sens)	0,79
Mealiness (sens)	-0,18
Fruitiness (sens)	0,17
Odour Intensity (sens)	-0,09
Firmness (sens)	0,04
Juiciness (sens)	-0,03
Sweetness (sens)	-0,02
Aroma (sens)	-0,01

	Sugar content (instrumental)	
Sweetness (sens)	0,55	**
Fruitiness (sens)	0,37	**
Odour Intensity (sens)	-0,37	**
Mealiness (sens)	-0,28	**
Sourness (sens)	0,20	*
Aroma (sens)	-0,17	
Firmness (sens)	0,16	
Juiciness (sens)	-0,03	

	Juiciness (instrumental)
Juiciness (sens)	0,38
Mealiness (sens)	-0,29
Sweetness (sens)	-0,21
Aroma (sens)	-0,19
Odour Intensity (sens)	0,17
Firmness (sens)	0,15
Fruitiness (sens)	-0,08
Sourness (sens)	-0,04

Significance level: **=0,01 *=0,05

2.2.4 PTR-MS analysis of volatiles

The PTR-MS analysis resulted in a complex matrix of 180 measured mass units for 297 fruit samples. Only the results on cultivars which were present on the 2 sites were elaborated.

In order to better consider all the correlations between the mass peaks it was decided to perform a principal component analysis (PCA) on the data sets; having almost 200 masses, all the masses not showing relevant activities in the apple spectra were removed to simplify the system. For this reason, the difference between the spectra of apples and the background of the water saturated nitrogen have been calculated; thus, even if a mass

is not different from the background (in that case the difference being near to zero or even negative), it was removed and not used for the next analysis. The PCA analysis was performed on these simplified data sets, including KMO and Bartlett's test of sphericity. Only PCs with eigenvalues ≥ 1 were extracted. From this matrix eleven PCs were extracted.

The rotated component matrix is shown (loading > |0.4|) with the explanation of the total variance (Table 8 a/b).

Table 8a: Rotated component matrix (loading > |0.4|) with the explanation of the total variance

m/z	Component										
	1	2	3	4	5	6	7	8	9	10	11
m26						0,87					
m28						0,87					
m33								0,95			
m41				0,74							
m42		0,54									
m43				0,62							
m44				0,63							
m45		0,87									
m46		0,86									
m47		0,93									
m48		0,92									
m51								0,94			
m53							0,8				
m56				0,78							
m57				0,81							
m58				0,82							
m59										0,74	
m60		0,51								0,64	
m61				0,57							
m62				0,58							
m63		0,81									
m65		0,92									
m67	0,78										
m69	0,96										
m70			0,58								
m71		0,5	0,58								
m72			0,56								
m73		0,86									
m74		0,81									
m75			0,81								
m76			0,8								
m79									0,86		

m80									0,81		
m81	0,98										
m82	0,97										
m83	0,8										
m84											
m85				0,85							
m86				0,85							
m87		0,58		0,5							
m88		0,79									
m89		0,87									
m90		0,87									
m91											
m93	0,86										
m95	0,98										
m96	0,87										
m97	0,97										
m98	0,78										
m99					0,71						
m100					0,68						
m101			0,59								
m102		0,84									
m103			0,86								
m104			0,86								
m105							0,67				
m107	0,8										
m109	0,97										
m110	0,91										
m111	0,94										
m115					0,71						
m117		0,79									
m118		0,79									
m119	0,5	0,64									
m121	0,94										
m122	0,8										
m123	0,97										
m125	0,86										
m127					0,72						
m131		0,92									
m132		0,92									
m133		0,79									
m135	0,95										
m136	0,86										
m137	0,98										
m138	0,85										
m145		0,83									
m146		0,83									
m149	0,52										
m150	0,51										
m159			0,78								
m173					0,68						
m187			0,81								

Table 8b: Total explained variance

Component	total	% variance	% cumulated
1	35,7	43,1	43,1
2	15,0	18,1	61,2
3	6,8	8,2	69,4
4	4,1	4,9	74,3
5	2,8	3,4	77,7
6	2,0	2,5	80,2
7	1,9	2,3	82,4
8	1,6	1,9	84,4
9	1,4	1,7	86,0
10	1,2	1,4	87,4
11	1,1	1,3	88,7

The positive loadings of the variables m/z 67, 69, 81, 82, 83, 93, 95, 96, 97, 98, 107, 109, 110, 111, 119, 121, 123, 125, 135, 136, 137, 138, 149 and 150 yielded the most important contributions to the first PC which explains 43,1% of the total variance. The second PC was mainly characterized by positive contributions of the masses m/z 42, 45, 46, 47, 48, 60, 63, 65, 71, 73, 74, 87, 88, 89, 90, 102, 117, 118, 119, 131, 132, 133, 145 and 146. In the third dimension we find masses m/z 70, 71, 72, 75, 76, 101, 103, 104, 159, 187. Signals mainly contributing positively to the fourth PC were m/z 41, 43, 44, 56, 57, 58, 61, 62, 85, 86, 87. Masses m/z 99, 100, 115, 127 and 173 belong to the fifth PC. Only masses m/z 26 and 28 belong to the sixth PC. Masses m/z 53 and 105 positively contribute to the seventh PC. The eighth PC comprises positive contributions of masses m/z 33 and 51. Also the ninth PC has contributions of only 2 masses m/z 79 and 80. Finally, the tenth PC has shows positive contributions of masses m/z 59 and 60.

In such a complex matrix it the interpretation of the PCA and the masses contributing to the different dimensions is rather difficult and not straightforward. However, it is possible to try an interpretation of the first 4

dimensions which are the ones with the highest loadings (Table 9). From the fifth to the eleventh PC at the moment it is basically impossible to give a biochemical interpretation of the results.

Table 9: Most important masses m/z revealed by PTR-MS with their probable biochemical interpreation

m/z	formula	comment
57	C4H9+	fragment of C6 ester
69	C5H9+	isoprene
71	C5H11+	fragment of C7 ester
75	C3O2H7+	C3 esters
81	C6H9+	monoterpene fragment
85	C6H13+	fragment of C8 ester
89	C4O2H9+	C4 esters
93	C7H9+	monoterpene fragment
95	C7H11+	monoterpene fragment
103	C5O2H11+	C5 esters
107	C8H11+	monoterpene fragment
109	C8H13+	monoterpene fragment
117	C6O2H13+	C6 esters
119	C9H11+	monoterpene fragment
121	C9H13+	monoterpene fragment
131	C7O2H15+	C7 esters
137	C10H17+	monoterpene
145	C8O2H17+	C8 esters
149	C11H17+	fragment of sesquiterpene
159	C9O2H19+	C9 esters
173	C10O2H21+	C10 esters
205	C15H25+	sesquiterpene

The first PC is represented by the families of mono- and sesquiterpenes. The peak of mass m/z 137 is characteristic for terpenes, together with the masses m/z 81, 93, 109, 107 and 121. PTR-MS fragmentation patterns for terpenes were proposed for m/z 81 ($C_6H_9^+$), 93 ($C_7H_9^+$), 95 ($C_7H_{11}^+$), 107 ($C_8H_{11}^+$), 109 ($C_8H_{13}^+$), 119 ($C_9H_{11}^+$), 121 ($C_9H_{13}^+$) and 137 (loss of water for oxygen-containing terpenes; $C_{10}H_{17}^+$) in recent works (Mal07). The peak at mass m/z 205 would be typical for sesquiterpenes. In this experiment mass scans were performed from 18 to only 200 amu, therefore mass m/z 205

was not registered. On the other hand, mass m/z 149 and 150 most probably represents fragments of sesquiterpenes.
The PCs 2, 3 and 4 represent the important family of esters. Their splitting in more dimensions of the PCA proves once more that different cultivars biosynthesize many different kinds of esters (compare with Part 2 of the present work where on the cultivar Red Delicious the esters where concentrated on one dimension even though there were different treatments of the same cultivar); therefore different compounds behave in different ways depending on the variety. Traditional gas-chromatographic methods have showed that the qualitative and quantitative differences in volatile ester production are highly significant among apple cultivars (Fel00; Hol05; Lòp98). The presented results underline these findings.

The factors were furthermore scored with Anova. To get everything in one evaluation the multivariate analysis was chosen (Table 10 and 11).

Table 10: Multivariate test performed on the PCA factors

Effect		Value	F	Hypothesis df	Error df	Sig.
constant term	Pillai-Spur	,000	,000(a)	11,000	150,000	1,000
	Wilks-Lambda	1,000	,000(a)	11,000	150,000	1,000
	Hotelling-Spur	,000	,000(a)	11,000	150,000	1,000
	Roy's Largest Root	,000	,000(a)	11,000	150,000	1,000
cultivar	Pillai-Spur	8,423	13,412	429,000	1760,000	,000
	Wilks-Lambda	,000	21,084	429,000	1626,118	,000
	Hotelling-Spur	89,791	31,015	429,000	1630,000	,000
	Roy's Largest Root	30,335	124,452(b)	39,000	160,000	,000
sitevalley1mountain2	Pillai-Spur	,686	29,831(a)	11,000	150,000	,000
	Wilks-Lambda	,314	29,831(a)	11,000	150,000	,000
	Hotelling-Spur	2,188	29,831(a)	11,000	150,000	,000
	Roy's Largest Root	2,188	29,831(a)	11,000	150,000	,000
cultivar * sitevalley1mountain2	Pillai-Spur	6,293	5,486	429,000	1760,000	,000
	Wilks-Lambda	,000	7,802	429,000	1626,118	,000
	Hotelling-Spur	33,747	11,657	429,000	1630,000	,000
	Roy's Largest Root	14,347	58,860(b)	39,000	160,000	,000

Except for Factor 10 all others have interactions, therefore it is not possible to take general conclusions on cultivar and site effect.

Table 11: Test of Between-Subjects Effects

Source	Dependent Variable	Type III sum of squares	df	Mean Square	F	Sig.
cultivar * sitevalley1mountain2	A-R factor score 1 for analysis 1	66,336	39	1,701	8,994	,000
	A-R factor score 2 for analysis 1	79,730	39	2,044	27,581	,000
	A-R factor score 3 for analysis 1	27,429	39	,703	7,971	,000
	A-R factor score 4 for analysis 1	43,935	39	1,127	4,713	,000
	A-R factor score 5 for analysis 1	34,851	39	,894	5,684	,000
	A-R factor score 6 for analysis 1	23,790	39	,610	4,868	,000
	A-R factor score 7 for analysis 1	138,448	39	3,550	30,083	,000
	A-R factor score 8 for analysis 1	47,413	39	1,216	3,094	,000
	A-R factor score 9 for analysis 1	36,859	39	,945	1,174	,243
	A-R factor score 10 for analysis 1	88,047	39	2,258	7,688	,000
	A-R factor score 11 for analysis 1	49,824	39	1,278	4,736	,000

Source	Dependent Variable	Type III sum of squares	df	Mean Square	F	Sig.
cultivar	A-R factor score 1 for analysis 1	142,295	39	3,649	19,292	,000
	A-R factor score 2 for analysis 1	146,787	39	3,764	50,778	,000
	A-R factor score 3 for analysis 1	197,440	39	5,063	57,375	,000
	A-R factor score 4 for analysis 1	114,170	39	2,927	12,246	,000
	A-R factor score 5 for analysis 1	170,781	39	4,379	27,854	,000
	A-R factor score 6 for analysis 1	194,265	39	4,981	39,749	,000
	A-R factor score 7 for analysis 1	81,666	39	2,094	17,745	,000
	A-R factor score 8 for analysis 1	128,385	39	3,292	8,377	,000
	A-R factor score 9 for analysis 1	73,262	39	1,879	2,334	,000
	A-R factor score 10 for analysis 1	103,967	39	2,666	9,078	,000
	A-R factor score 11 for analysis 1	132,742	39	3,404	12,617	,000

Source	Dependent Variable	Type III sum of squares	df	Mean Square	F	Sig.
sitevalley1mountain2	A-R factor score 1 for analysis 1	,109	1	,109	,579	,448
	A-R factor score 2 for analysis 1	,624	1	,624	8,415	,004
	A-R factor score 3 for analysis 1	,013	1	,013	,148	,701
	A-R factor score 4 for analysis 1	42,649	1	42,649	178,415	,000
	A-R factor score 5 for analysis 1	8,214	1	8,214	52,245	,000
	A-R factor score 6 for analysis 1	,895	1	,895	7,139	,008
	A-R factor score 7 for analysis 1	,005	1	,005	,041	,841
	A-R factor score 8 for analysis 1	,325	1	,325	,828	,364
	A-R factor score 9 for analysis 1	,125	1	,125	,155	,694
	A-R factor score 10 for analysis 1	,000	1	,000	,001	,970
	A-R factor score 11 for analysis 1	13,271	1	13,271	49,193	,000

Component 1 and component 2 together explain more than 60% of the total variance (Table 8b). Scoring of the two most important factors of the PCA analysis of the PTR-MS results gives indication on the influence of cultivar and site on the production of terpenes and esters (Figure 12).

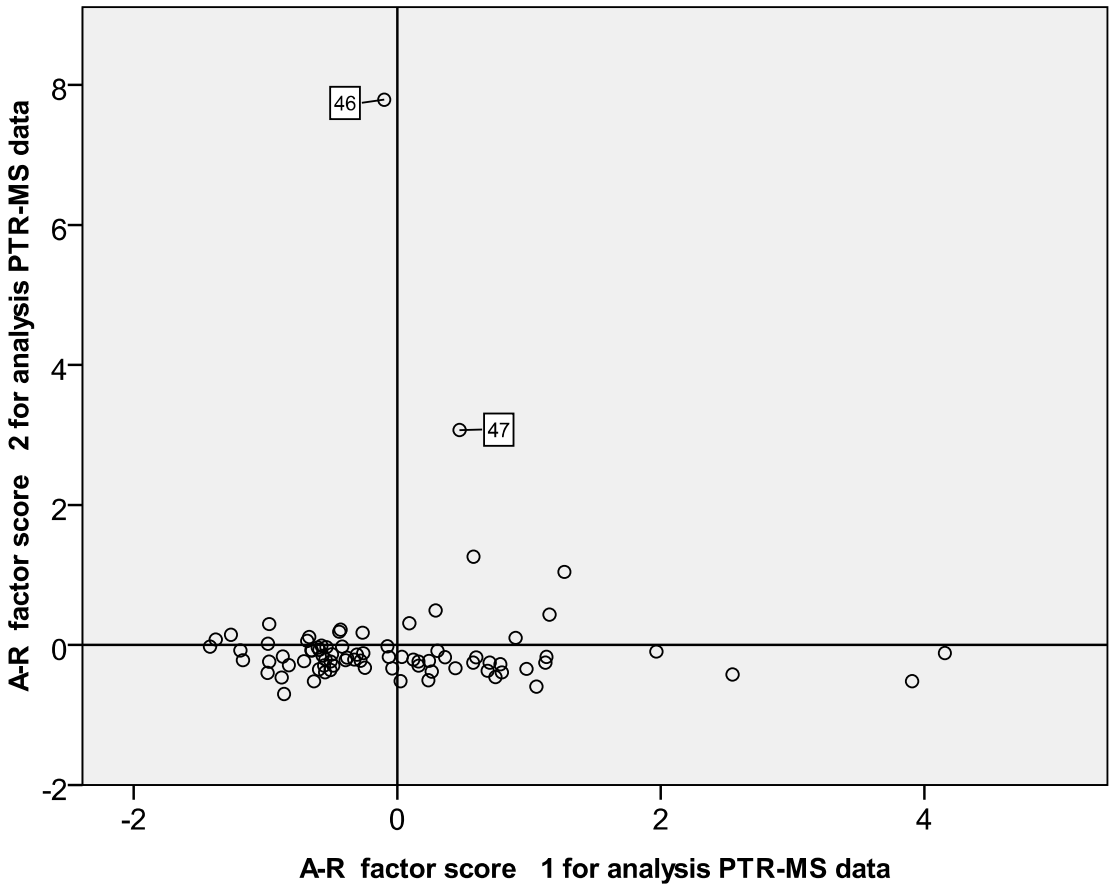


Figure 12: Scatter plot of Factor 1 and Factor 2 of the PCA of the PTR-MS results

Factor 2 has been biochemically interpreted as the main component of esters. The scoring of this factor shows a relatively low variability with a small gradient. There are only 2 exceptionally high scoring samples, labelled as 46 and 47 in the scatter plot. 46 represents the sample of the cultivar Gradigold coming from the mountain site while 47 is the sample of the cultivar Hapke Delicious picked in the valley site. Neither the biometric, physical and chemical nor the sensorial data allow an interpretation of those two exceptions inbetween the eighty considered samples. Besides

those two cases, in conclusion the gradient of esters related to Factor 2 is rather low both among cultivars and sites.

As explained before, the first PC is most probably represented by the families of mono- and sesquiterpenes. The scatter plot shows a clear gradient of the terpenes with a high variability among sites and cultivars. It is not possible to cluster the data based on the valley or mountain site.

By arbitrarily choosing a limit value of +1 and -1 for the 2 most important factors, factor 1 representing the terpenes and factor 2 representing esters, the cultivars can be divided in subgroups with low, medium and high amount of respectively terpenes related to factor 1 and esters related to factor 2 (Figure 13).

Figure 13: Subgrouping of the 40 cultivars on the 2 sites based on the value of factor 1 related to the family of terpenes and factor 2 related to a subfamily of esters. Subgroups with high amount are coloured in red, subgroups with medium amount are orange and subgroups with low amount are yellow.

	valley		mountain	
cultivar	Factor 1 (terpenes)	Factor 2 (esters)	Factor 1 (terpenes)	Factor 2 (esters)
Ambrosia	-0,6	-0,3	-0,9	-0,5
Ariane	-0,4	-0,2	0,1	-0,2
Ariwa	-0,3	-0,1	1,0	-0,3
Azufu	0,9	0,1	0,6	1,3
Catarina	0,8	-0,3	0,6	-0,2
Caudle	0,2	-0,2	-0,4	0,2
Civni	-1,4	0,1	-0,6	0,0
Coop 38	-1,0	-0,2	-1,0	-0,4
Coop 39	-0,4	-0,2	-0,5	-0,1
Dalinbel	-0,4	0,0	2,0	-0,1
Dalinco	-0,1	0,0	0,0	-0,2
Delblush	-0,1	-0,2	0,2	-0,3
Delcoros	-0,5	-0,3	1,1	-0,6
Delfloki	-0,3	-0,1	-0,7	0,1
Doriane	0,7	-0,4	0,8	-0,4
Early Red Gold	-0,5	-0,2	-0,9	-0,7

Elanared	-0,6	-0,1	0,2	-0,2
G198	-1,0	0,3	0,3	0,5
Gala Schnitzer	-0,3	-0,2	0,3	-0,4
Gold Pink	0,7	-0,3	0,0	-0,3
Golden Del. Klon B	-0,6	0,0	-0,6	-0,3
Golden Orange	0,7	-0,5	0,0	-0,5
Gradigold	1,3	1,0	-0,1	7,8
Hapke Del.	0,5	3,1	0,1	0,3
Huaguan	4,2	-0,1	1,2	0,4
Joburn	-1,2	-0,2	-0,7	-0,2
Kiku 8	-0,4	0,2	-0,3	0,2
La Flamboyante	1,1	-0,3	0,6	-0,3
Milwa	-0,7	-0,1	0,4	-0,3
Nico Early	-0,5	-0,4	-0,6	-0,5
Nicogreen	3,9	-0,5	0,4	-0,2
Nicola	-0,9	-0,2	-0,6	-0,2
Nicoter	-0,8	-0,3	-0,6	-0,1
Pinova	-0,3	-0,2	-0,5	-0,2
Red Jonaprince	-0,2	-0,3	2,5	-0,4
Rucla	-1,0	0,0	-0,5	0,0
Sansa	-1,4	0,0	-1,2	-0,1
Scifresh	-0,5	-0,4	0,2	-0,5
Silken	-0,7	0,1	-1,3	0,1
Topaz	1,1	-0,2	0,3	-0,1

There is evidence of an interaction between cultivar and site. An example of interaction is shown in Figure 14. Ambrosia has higher values of factor 1 in valley than in mountains, for Ariane the opposite situation occurs.

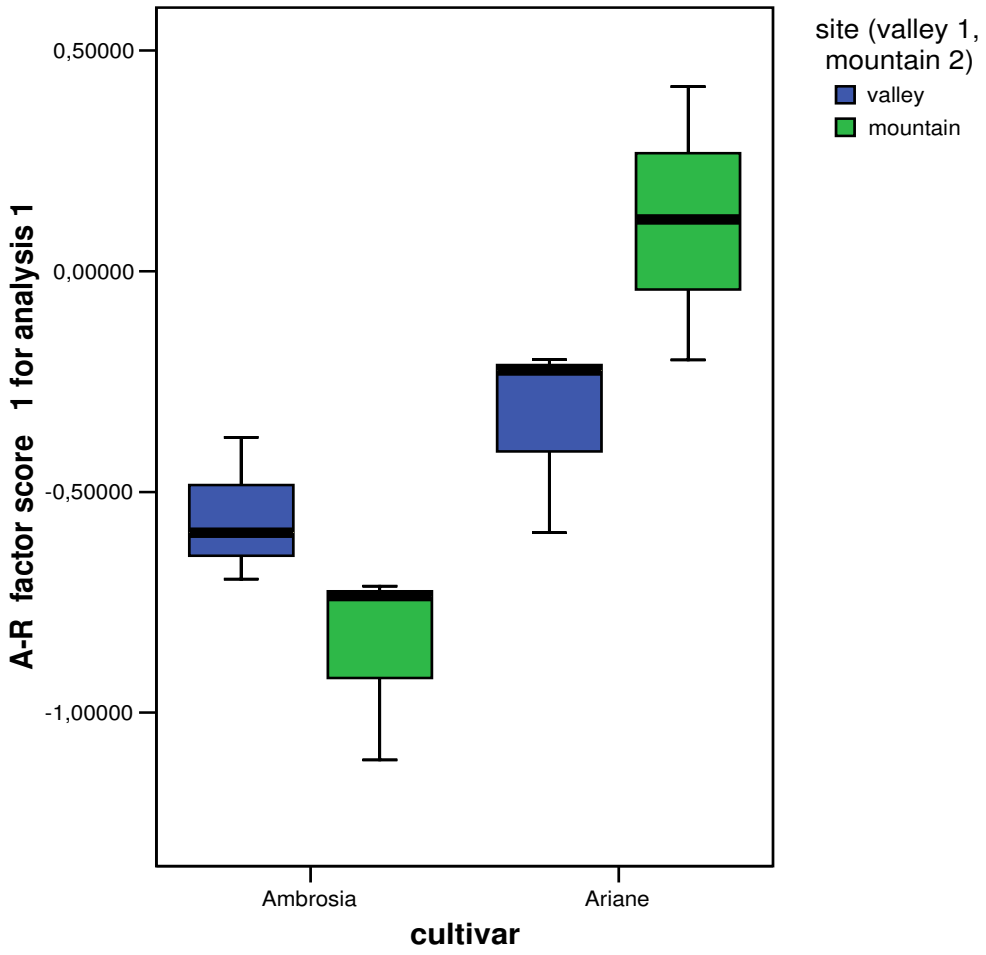


Figure 14: Boxplot of Factor 1 for 2 cultivars on the 2 sites considering the three measured fruits per site and cultivar

In order to perform a more detailed analysis of the interactions between cultivar and site the differences between valley and mountain of the means of the first four principal components were considered (Figure 15, 16, 17 and 18). This should give an estimation of the specific influence of the site on the cultivar related to the two analysed families of VOCs. The first PC is represented by the families of mono- and sesquiterpenes. The PCs 2, 3 and 4 represent the family of esters.

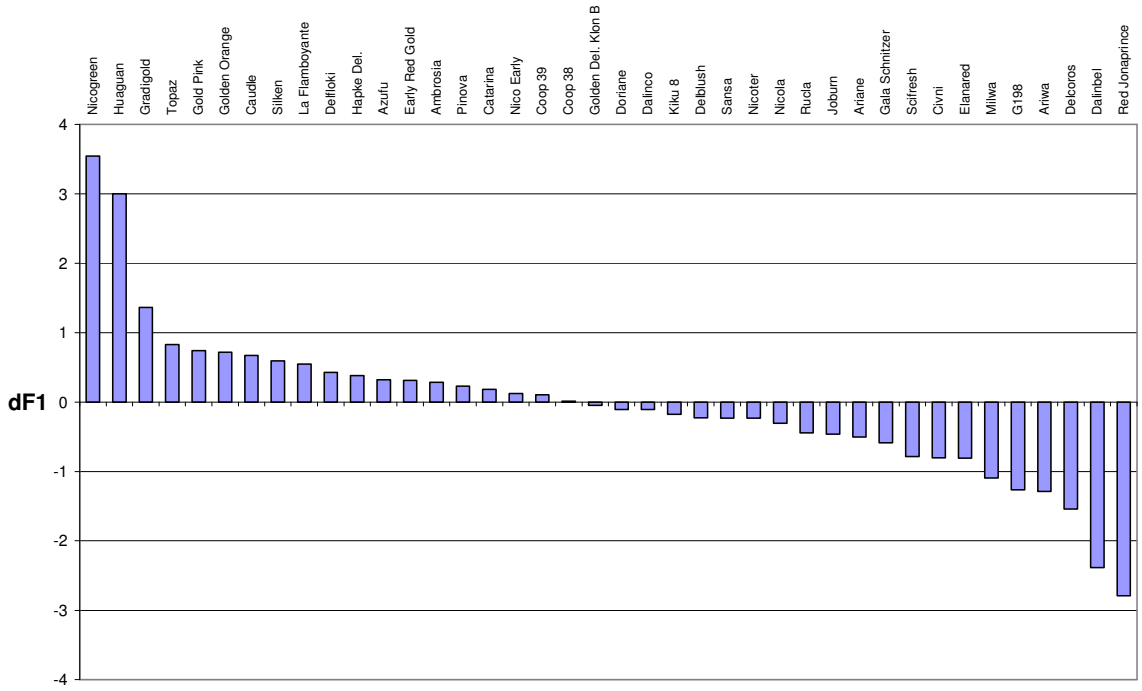


Figure 15: Difference between valley and mountain site of Factor 1 (dF1) of the PCA analysis of PTR-MS results. The forty cultivars are in decreasing order of dF1 value. Positive values mean higher amount in valley site.

The cultivars can be divided into 3 groups, if an arbitrary threshold of dF1 is chosen. Those cultivars with dF1 bigger than 1 show a strong increase of terpenes in the valley compared to the mountain site (Group A). Those cultivars with dF1 inbetween 1 and -1 show a moderate influence of the site onto the development of terpenes (Group B). Those cultivars with dF1 smaller than -1 show a strong increase of terpenes in the mountain compared to the valley site (Group C).

In this experiment the cultivars Nicogreen, Huaguan and Gradigold had a dF1 which was bigger than 1 (Group A). The cultivars Red Jonaprince, Rucla, Sansa, Scifresh, Silken and Topaz had a dF1 which was smaller than -1 (Group C). In comparison to the cultivars of those two groups, all the other cultivars showed a relatively moderate influence of the site onto the development of terpenes.

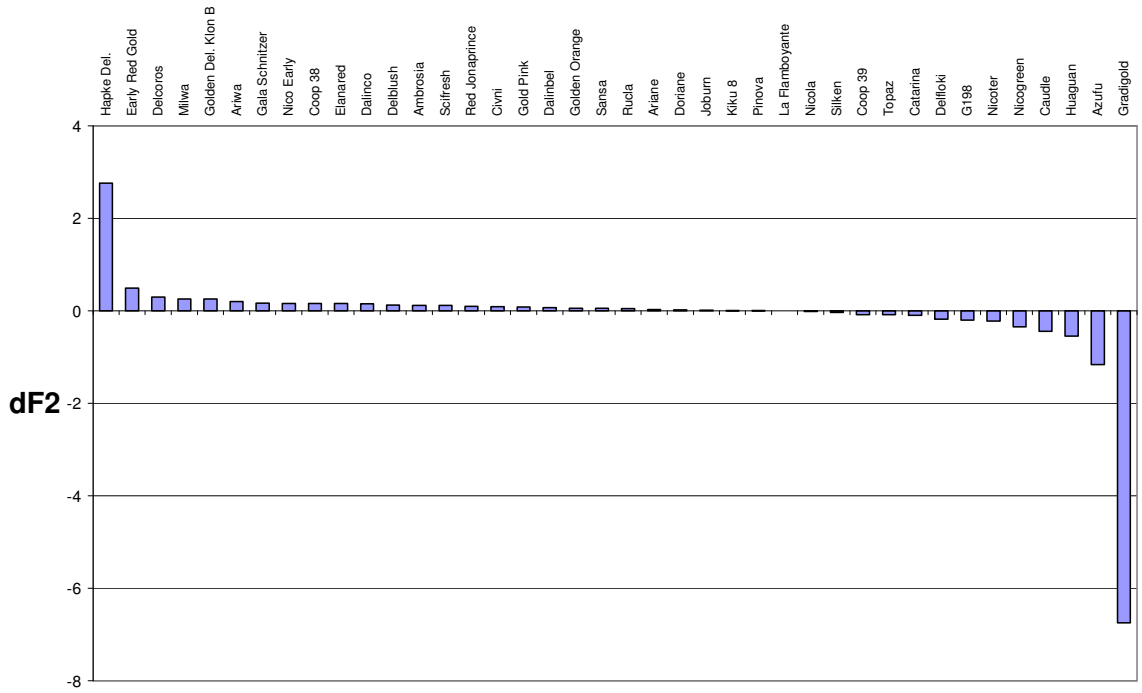


Figure 16: Difference between valley and mountain site of Factor 2 (dF2) of the PCA analysis of PTR-MS results. The forty cultivars are in decreasing order of dF2 value. Positive values mean higher amount in valley site.

Figure 16 shows the dF2 values in decreasing order. Factor 2 is representing together with Factor 3 and 4 the family of esters. With the exception of Hapke Delicious and Gradigold all other cultivars did not show significant influence of site onto the development of esters associated with Factor 1.

As mentioned before the family of esters could not be associated to one single PC as occurred with the family of terpenes. Figure 17 shows the dF3 and Figure 18 the dF4 values in decreasing order.

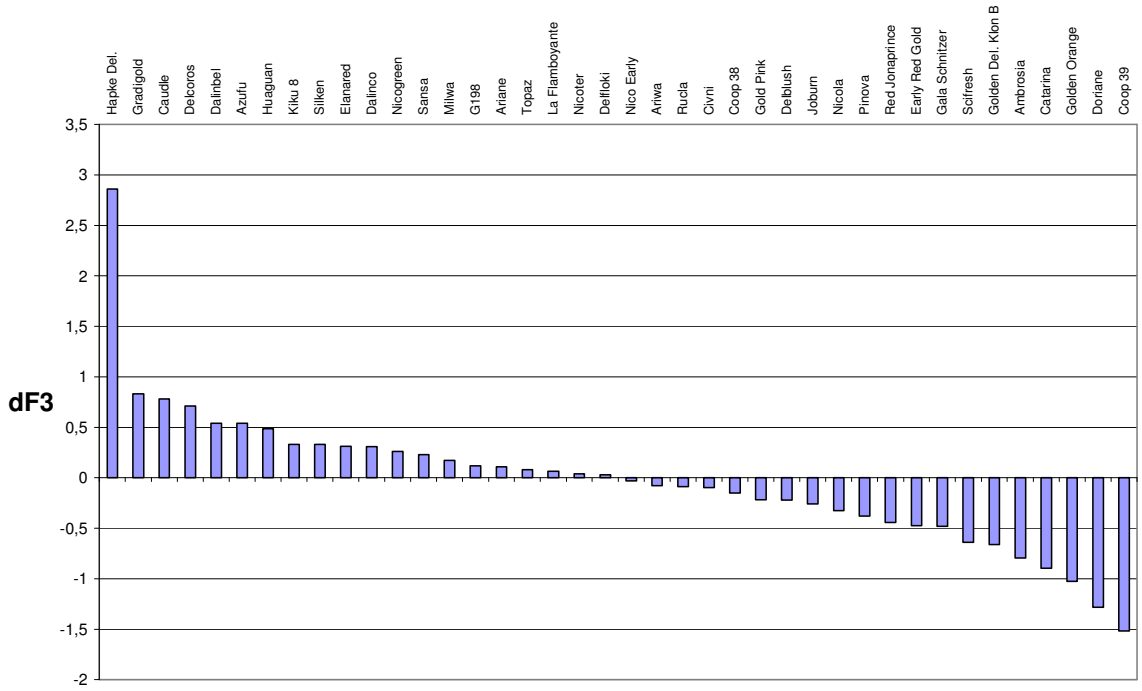


Figure 17: Difference between valley and mountain site of Factor 3 (dF3) of the PCA analysis of PTR-MS results. The forty cultivars are in decreasing order of dF3 value. Positive values mean higher amount in valley site.

Hapke Delicious showed a significant increase of esters associated to PC3 in the valley site. Coop 39, Doriane and Golden Orange showed a significantly positive influence of the mountain site on the development of esters associated to PC3. All the other cultivars were not significantly affected by site in terms of development of those kind of esters.

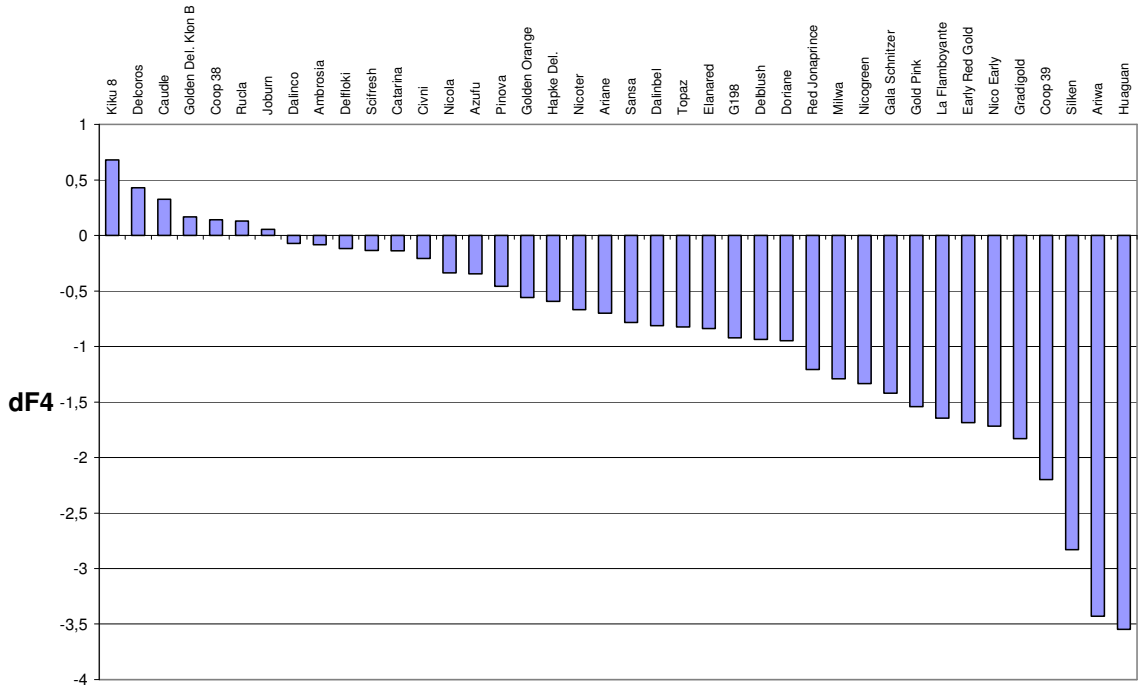


Figure 18: Difference between valley and mountain site of Factor 4 (dF4) of the PCA analysis of PTR-MS results. The forty cultivars are in decreasing order of dF4 value. Positive values mean higher amount in valley site.

A subgroup of cultivars with positive influence of the mountain site on the development of the esters associated to PC4 can be identified (Figure 18). This group comprises Huaguan, Ariwa, Silken, Coop 39, Gradigold, Nico Early, Early Red Gold, La Flamboyante, Gold Pink, Gala Schnitzer, Nicogreen, Milwa, Red Jonaprince. All other cultivars were not significantly affected by site in terms of development of those kind of esters associated to PC4. Also in this case the arbitrary value of dF4<-1 was chosen to divide cultivars in groups.

An interpretation of the overall results on the principal components associated with the family of esters is very difficult. Especially the achieved grouping based on the site influence is hardly interpretable with the data actually available. However, it can be stated that those results confirm the

high variability of the big family of esters among different cultivars. They do also behave in different ways depending on the variety (Figure 19).

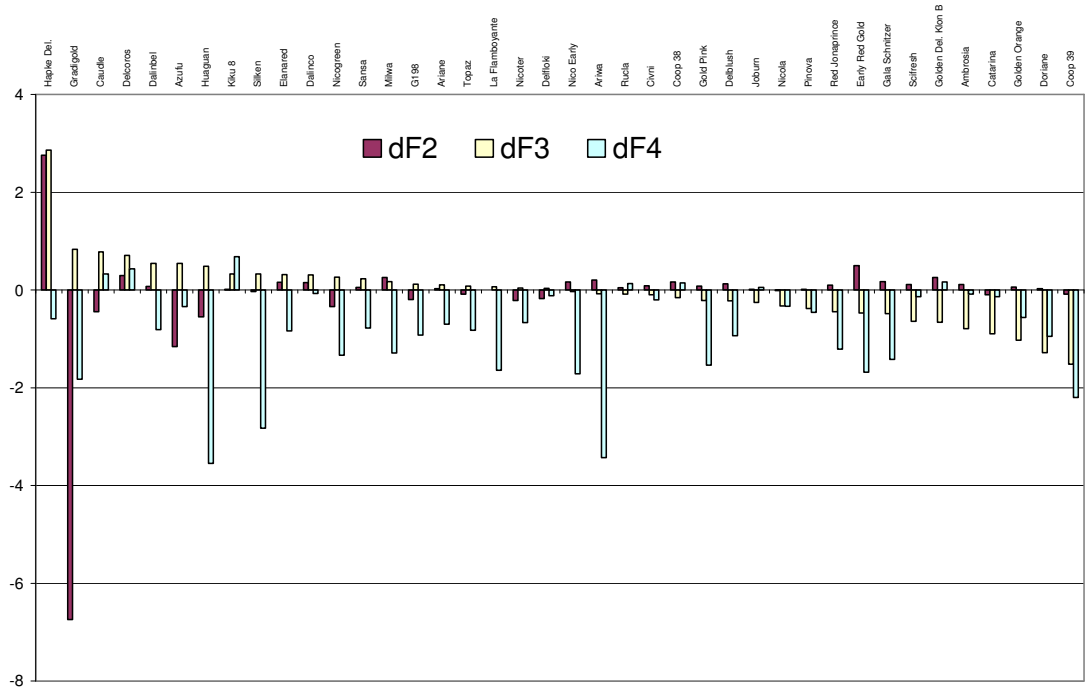


Figure 19: Difference between valley and mountain site of Factor 2,3 and 4 of the PCA analysis of PTR-MS results (dF2, dF3, dF4). The forty cultivars are in decreasing order of dF3 value. Positive values mean higher amount in valley site.

2.2.5 Correlation between sensorial and PTR-MS analysis

On one hand the PCA of PTR-MS results gave 11 factors; on the other hand the PCA of the sensorial analysis resulted in 3 factors, with the first 2 being the most important ones. To investigate a possible link between those two kinds of evaluation on the same range of cultivars a correlation analysis was undertaken. The result is resumed in Table 12.

Table 12: Correlation between PTR-MS results and sensorial analysis (Pearson coefficient)

		PC of PTR-MS analysis									
		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9	Factor 10
PC of sensorial analysis	Factor 1	-0,04	-0,29**	-0,26*	-0,21	-0,17	0,05	0,26*	-0,13	-0,38**	0,02
	Factor 2	0,19	0,27	-0,27*	-0,01	0,15	-0,04	-0,08	0,17	-0,08	-0,06
	Factor 3	-0,17	-0,15	-0,04	-0,08	0,03	-0,04	0,09	0,04	0,11	-0,27*

*significance level 0,01

** significance level 0,05

Table 12 shows some weak correlations with significance between the principal components of the sensorial data and the principal components of the PTR-MS data. In conclusion in this experiment it was not possible to correlate PTR-MS data with results of the sensory analysis. More comprehensive PTR-MS data which could be available in future might allow reaching the objective to find a link between human perception of aroma and flavour and those innovative instrumental measurements. Also a more detailed sensory analysis might help to reach the objective. In this work the panellists have evaluated some parameters as odour intensity and type, which directly depend on the VOC emission of whole apples. The other parameters which were evaluated during the sensory evaluation were registered after tasting by biting into the apple. The whole sensory impression of apple, like other food, is mainly determined by the combination and interaction of the chemical senses of taste and smell. Texture and many other properties contribute indirectly to flavour perception.

3 Part 2

3.1 Materials and Methods

3.1.1 Analysed apples, storage techniques and storage conditions

Apple fruit (Malus domestica Borkh.) cv 'Red Delicious', picked in the optimal harvest window for long-term CA storage (year 2007) in the Venosta Valley (~600 m above sea-level) of South-Tyrol (Italy), were stored for 7 months with four different long-term storage techniques. For each experimental condition, four replicates of approximately 60 fruits each were stored in gas-tight stainless steel containers (~235 kg/m^3) after seven days of pre-refrigeration at 2.5°C in normal air, in: i) optimal ultra low oxygen controlled atmosphere (ULO-CA) with 1.0 kPa O_2 plus 1.0 kPa CO_2; ii) dynamically controlled atmosphere (DCA) at extremely low O_2 (0.4 kPa) using the HarvestWatch® monitoring system (Satlantic Inc., Halifax, N.S., Canada). The monitoring system is based on the measurement of chlorophyll fluorescence by means of FIRM (fluorescence interactive response monitor, Satlantic Inc.) sensors during the whole storage period on samples of six apples. Other fruits were stored in iii) ILOS+ (repeated initial low oxygen stress): controlled atmosphere with 0.7-0.8 kPa O_2 plus 0.9 kPa CO_2, monitoring destructively the ethanol produced by the fruits due to two O_2 stress periods of one week each at 0.3 kPa O_2, maintaining the fruit ethanol content during the whole storage period in the range of 0.2-0.3 g/l ethanol; iv) treatment with 1-methylcyclopropene (0.625 µl/l 1-MCP; AgroFresh Inc./Rohm & Haas Company, Philadelphia, PA, USA), formulated as SmartFresh®, at 2.5°C for 24 h in the above mentioned gas-tight container with ventilation and monitoring of O_2 and CO_2, after six days of pre-refrigeration at 2.5°C in normal air. The 1-MCP-treated fruits were subsequently stored in ULO-CA. Temperature was kept at 1.3°C and relative humidity at ~98% for all storage methods.

3.1.2 PTR-MS analysis

The PTR-MS measurements were performed with a commercial high sensitivity proton transfer reaction mass spectrometer (hs-PTR-MS, Ionicon Analytik, Innsbruck, Austria).

For the analysis of VOCs emitted by apples, 1000-ml PFA jars (AHF Analysentechnik AG, Tübingen, Germany) equipped with two-way PFA covers (AHF Analysentechnik AG Tübingen, Germany) were used. An experimental set-up for simultaneous headspace VOC analysis of two parallel apple samples was designed. The jars were connected to the carrier gas supply (N2 Alphagaz 1, 99.999% – Air Liquide Italia S.R.L., Milan, Italy). In order to optimise experimental performance, a measuring time of ten minutes was chosen for one sample with parallel conditioning (for apple headspace equilibrium) of another one.

A total carrier gas flow of 500 ml/min of water saturated N_2 was used with the flow being equally divided between the two sampling jars, i.e. the headspace of each bottle was flushed at 250 ml/min. Constant temperature in the jars during analysis was ensured with a 20°C controlled water bath.

VOCs were ionised by way of proton transfer reactions from H_3O^+ ions and were mass-spectrometrically detected one atomic mass unit (amu) higher (M+1) than the molecular weight (M) of the neutral compounds. Mass scans were performed from 18 to 210 amu using a dwell time of 500 ms per amu. PTR-MS operating conditions were as follows: drift tube voltage, 600 V; drift tube pressure, 2.00 ± 0.05 mbar; drift tube temperature, 70°C; O_2+/ H_3O+ ratio ≤ 0.1 %; inlet temperature, 80°C. Memory effects (due to the sequential analysis of different samples) were reduced by the application of elevated inlet and drift tube temperatures and the use of inert Silcosteel® material for the inlet tubing.

Every sample was measured over ten scan cycles. The first three cycles are measured with pure N_2 only and are necessary for reaching stable operation conditions and for cleaning the instrument from residues of the

previous measurement. The last seven cycles, with the apple directly connected to the instrument, were used for data analysis.

3.1.3 Data analysis

Intensities of the signals were normalised to the primary ion intensities ($[H_3O^+]$ + $[H_3O^+ \cdot H_2O]$). The mean values of the signal intensities were calculated for scan cycles five to nine for each mass in the range from 18 m/z to 210 m/z. Data normalisation and calculations of means were conducted using Microsoft Excel version 2002. Principal component analyses (PCA), multivariance analysis and post hoc Tukey-b tests were performed using SPSS 12.0 for Windows. The significance level (α) was set at 0.05.

3.2 Results and discussion

3.2.1 PTR-MS peak analysis

It is reported that nearly 300 volatile organic compounds have been isolated from apple (Dim83). Of these, the alkylic esters (from C3 to C18), which are produced especially in apples' peel, are considered major contributors to the characteristic apple-like aroma and flavour in most cultivars and especially in "Delicious" apples (Gua71; Bra93). Moreover three acetic esters (butyl acetate m/z 116, 2-methylbutyl acetate m/z 130 and hexyl acetate m/z 144) are considered the most important and characteristic molecules of apples' flavour (Fel00). Because of the peculiarity of PTR-MS technique and of the great amount of isobaric and isomeric compound present in apple aroma it is not possible to assign a single peak of PTR-MS spectra to a single molecule, but is however possible to value the trend of the masses in the spectra. The masses of the three acetic esters are clearly present in the PTR-MS mass spectra and can be discriminated from the background. In fact, even if chemical ionisation, which takes place in the drift tube, is a soft ionisation, numerous

peaks related to the characteristic fragmentations of acetic esters (Apr07) were noticed in all apple mass spectra. These esters, having a hydrogen atom in the γ position to the carbonyl group, undergo the known McLafferty rearrangement giving characteristic fragment ion peaks of mass m/z = 61 and m/z = 43 (see Figure 20; R= CH3).

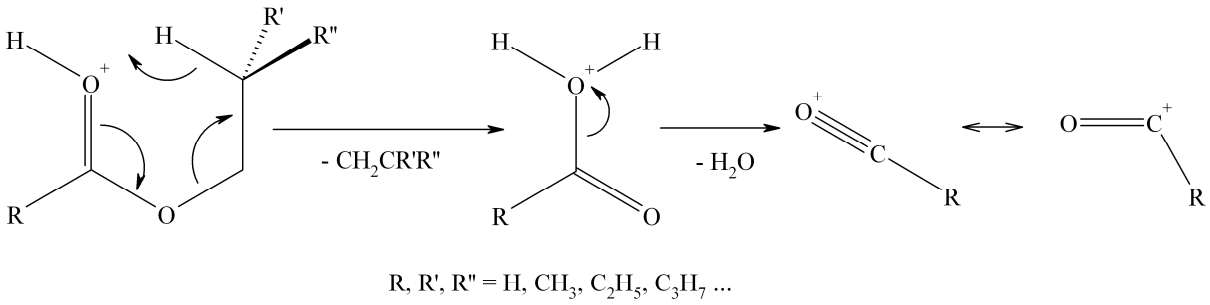


Figure 20. McLafferty rearrangement for esters in PTR-MS.

As an indication of this mechanism a linear correlation between mass 43 and 61 can be observed; this linear correlation ($r > 0.99$) has been found for all days and all apple spectra recorded. Coming from the contribution of all three esters, the signal intensities of these fragments exceed the intensity of the molecular ion peaks in every measurement. They are, in fact, the most intense peaks in the spectra together with the H_3O^+ ion (m/z = 19). Moreover, the stability of the cation m/z 43, due to the resonance form (see Figure 20), makes the fragmentation process easier. Considering the importance of acetic esters in apple aroma the development of mass m/z 43 and 61 can be chosen as an analytical index for the quantification of the total amount of aromatic molecules. Thanks to the potentialities of PTR-MS instrument it is possible to evaluate the aromatic degree of one apple in few minutes without using a destructive technique.

In order to better consider all the correlations between the mass peaks it was decided to perform a principal component analysis (PCA) on the data sets; having almost 200 masses, all the masses not showing relevant activities in the apple spectra were removed to simplify the system. For this reason, the difference between the spectra of apples and the background of the water saturated nitrogen have been calculated; thus, even if a mass is not different from the background (in that case the difference being near to zero or even negative), it was removed and not used for the next analysis. The PCA analysis was performed on these simplified data sets. Only PCs with eigenvalues ≥ 1 were extracted. From this matrix six PCs were extracted. In Table 13a the rotated component matrix is shown (loading > |0.4|) with the explanation of the total variance (Table 13b).

Table 13 a. Rotated component matrix (loading > |0.4|) with the explanation of the total variance

m/z	PC1	PC2	PC3	PC4	PC5	PC6
39.0	0.874					
40.0	0.847				0.434	
41.0	0.942					
42.0	0.960					
43.0	0.985					
44.0	0.982					
45.0	0.971					
46.0	0.960					
47.0	0.949					
53.0	0.476	0.616			0.481	
55.0	0.484	0.571	0.488			
56.0	-0.434			0.777		
57.0	0.990					
58.0	0.988					
59.0	0.933					
60.0	0.901					
61.0	0.987					
62.0	0.987					
63.0	0.978					
67.0		0.905				
69.0		0.841				
70.0		0.733				
71.0	0.884					
72.0	0.856		0.414			
73.0	0.700					
75.0	0.904					
76.0	0.901					
79.0	0.837	0.404				
81.0		0.989				
82.0		0.982				
95.0		0.984				
96.0		0.923				
97.0		0.943				
99.0	0.869					
101.0	0.698	0.497				
103.0	0.981					
104.0	0.979					
107.0		0.926				
109.0		0.973				
110.0		0.901				
111.0		0.902				
115.0	0.907					
117.0	0.989					
118.0	0.984					
119.0	0.833					
121.0		0.922				
122.0		0.872				
123.0		0.986				
124.0		0.936				
125.0		0.628				
127.0						
131.0	0.951					
135.0		0.983				
136.0		0.892				
137.0		0.982				
138.0		0.909				
139.0						0.984
145.0	0.980					
146.0	0.974					
149.0		0.976				
150.0		0.910				

83.0		0.912			159.0	0.948		
85.0	0.977				163.0		0.882	
87.0	0.934				173.0	0.850		
89.0	0.980				187.0		0.519	0.634
90.0	0.979				205.0		0.979	
91.0	0.935				206.0		0.936	
92.0			0.843					
93.0		0.966						

Table 13 b. Total explained variance

Components	Total	% of variance	% cumulative
1	35.780	47.706	47.706
2	25.771	34.361	82.067
3	2.172	2.897	84.964
4	1.858	2.478	87.442
5	1.373	1.831	89.273
6	1.242	1.656	90.930

The first PC was mainly characterized by positive contributions of the masses m/z 39, 40, 41, 42, 43, 44, 45, 46, 47, 53, 55, 57, 58, 59, 60, 61, 62, 63, 71, 72, 73, 75, 76, 79, 85, 87, 89, 90, 91, 99, 101, 103, 104, 115, 117, 118, 119, 131, 145, 146, 159 and 173. Only mass m/z 56 is characterized by negative contribution. The positive loadings of the variables m/z 53, 55, 67, 69, 70, 79, 81, 82, 83, 93, 95, 96, 97, 101, 107, 109, 110, 111, 121, 122, 123, 124, 125, 135, 136, 137, 138, 149, 150, 163, 187, 205, 206 yielded the most important contributions to the second PC. In the third dimension we find masses m/z 55, 72 and 187. Signals mainly contributing positively to the fourth PC were m/z 56 and 92. Masses m/z 40 and 53 belong to the fifth PC, and only mass m/z 139 belongs to the sixth PC.

The masses of the three acetic esters belong to the first dimension. Together with the molecular ions and the McLafferty fragment ions it is possible to recognise the fragment of the respective alcohols, from which the esters are biosynthesized (Fel00), due to the loss of a water molecule ($C_4H_9^+$ m/z = 57, $C_5H_{11}^+$ m/z = 71, $C_6H_{13}^+$ m/z = 85; Table 9).

What characterized this dimension is a typical trend for isomeric compounds. All signals of C3 (m/z = 75) to C10 (m/z = 173) esters are present together with their characteristic fragments. Every group of signals was separate from another by 14 amu (CH2 fragment).

The second PC is mainly characterized by another family of molecules which plays an important role in apple aroma: the terpenes (Val05). This mixture of molecules is biosynthesized from isoprene (C5H9 = m/z 69 M+H+) through known head-tail reactions. Only monoterpenes (C10H17 = m/z 137 M+H+) and sesquiterpenes (C15H25 = m/z 205 M+H+) made up by two and three isoprene units are revealed by PTR-MS. Higher molecular weight terpenes are not volatile enough to be detected.

All the other masses in this dimension can be predominantly assigned to fragmentation in proximity of double C-C bonds (for example in (E,E)-α-farnesene or ocimene) present in the carbon backbone of the terpenes (see Figure 21).

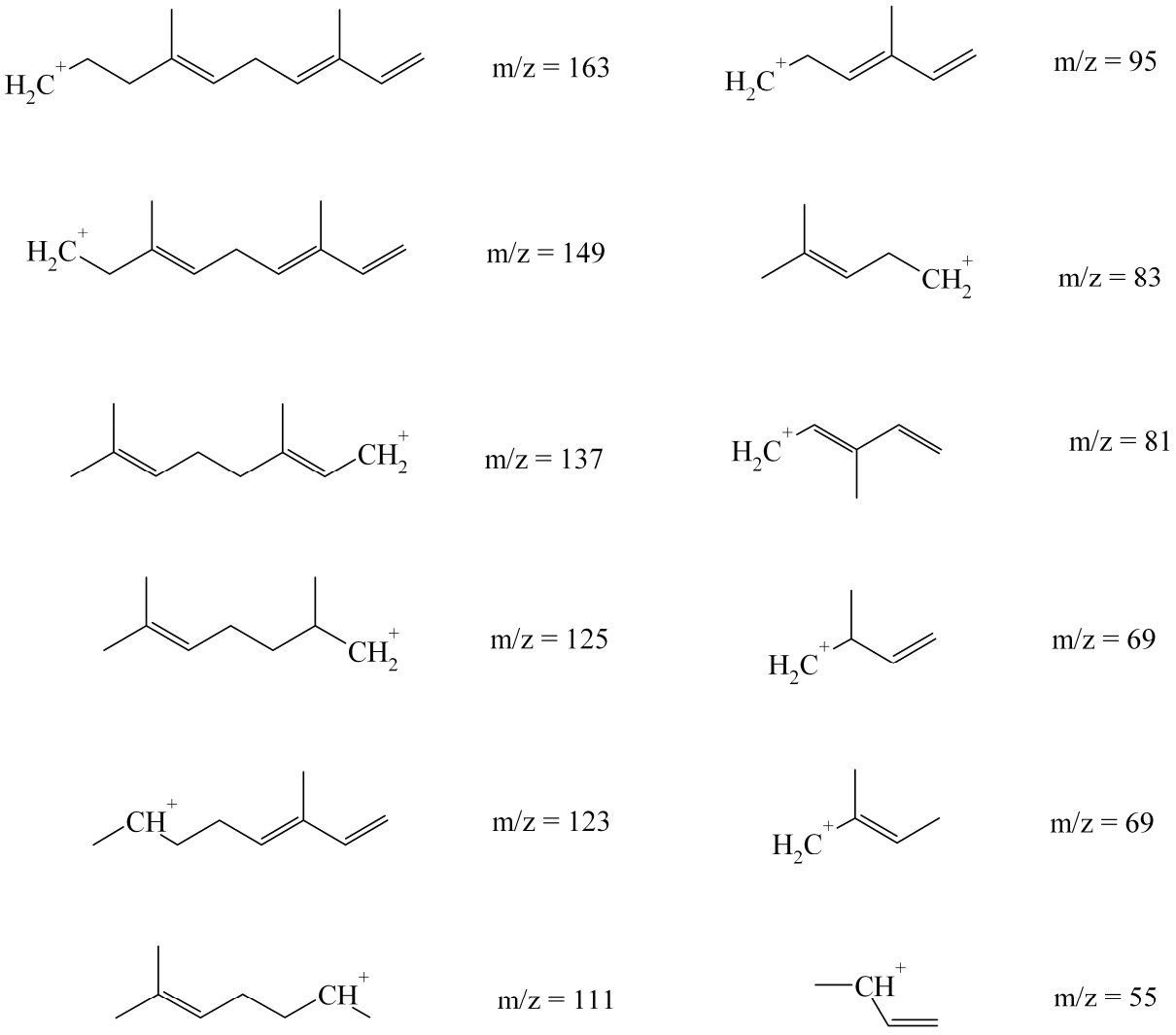


Figure 21. Main fragments of (E,E)-α-farnesene molecule.

In fact it is known that (E,E)-α-farnesene is one of the most abundant component of Red Delicious head space (Pal97). Similar fragmentations also occur in other mass spectrometry techniques (for detailed mass spectra of organic compounds see: http://webbook.nist.gov/chemistry) but the peak of the molecular ion is not as intense as in PTR-MS spectra.

From the third to the sixth dimension there are few loadings > |0.4| and, moreover, they are relative to masses already present in dimension one and two. The only exceptions are mass m/z 92 for PC4 and mass m/z 139

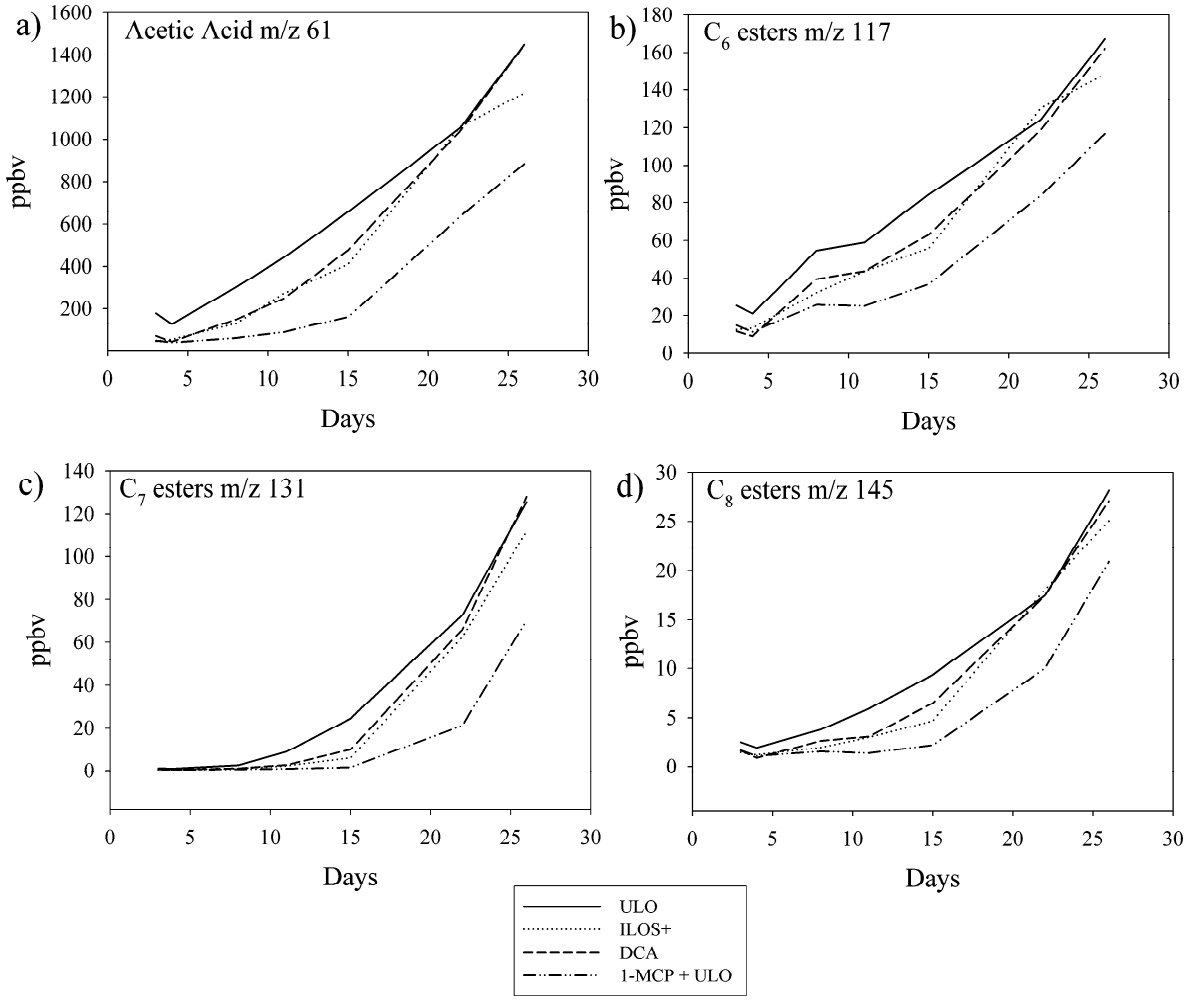
for PC6. But with only this limited information a clear assignment cannot be made.

3.2.2 Shelf-life of apples

Apples stored for 26 days at constant temperature (20°C) and relative humidity of 60-70% were analysed at seven-day intervals in order to evaluate the development of aromas in this period and to detect differences between the four storage methods.

As shown in Figure 22 a/b/c/d, all the masses of the first PC have a similar behaviour during shelf-life: they are almost constant for the first eight days and then drastically increase until day 26.

Figure 22 a/b/c/d. Shelf-life of molecular ions of the main substance for PC1: a) m/z 61 acetic acid b) m/z 117 C6-ester c) m/z 145 C8-ester d) m/z 131 C7-ester.



There are, however, slight differences between for the esters; C6 and C8 esters (except for C8 esters in apples treated with 1-MCP as it is shown in Figure 22d) increase in a similar way during the course of shelf-life (see Figure 22b and 22d), on the other hand, the concentration of C7 esters remains quite constant for eight days and then increases exponentially until day 26 when reaching the highest concentration (see Figure 22b).

However, apples treated with 1-MCP and then stored under ULO conditions produce the smallest quantity of esters. This is in agreement with the fact that inhibition of ethylene receptors by 1-MCP significantly blocks the maturation process of apples.

As far as the second PC dimension is concerned, the trend is completely different compared to the esters (see Figure 23).

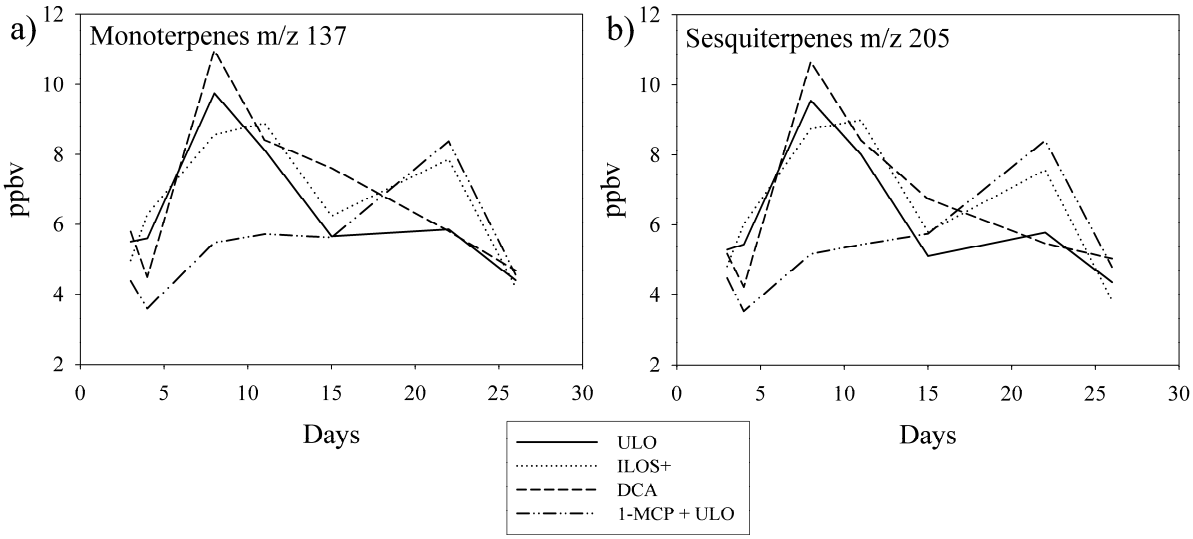


Figure 23. Shelf-life of molecular ions of the main substance for PC2: a) m/z 137 monoterpenes b) m/z 205 sesquiterpenes.

These molecules (for apples stored under ULO, ILOS+ and DCA conditions) increased significantly in the first eight days and then decreased in a similar way (there is only a little difference in apples stored under ILOS+ condition) until day 26.

Apples stored with DCA reach the highest concentration of these molecules, followed by ULO and ILOS+. As seen in the first PC dimension, apples treated with 1-MCP had a completely different behaviour: in the first 15 days the formation of terpenes in apples is small, rising around the 22nd day thereby reaching a maximum level (which, however, is still lower than

that of other storage conditions) and than falls to the common level. The reason for this behaviour is that α-farnesene production is linked to ethylene production and it is well documented that inhibition of ethylene production and binding to its receptor by 1-MCP treatment inhibits α-farnesene accumulation in the fruit skin (Tsa07).

In order to improve the estimation of differences of VCO development in relation to the different storage methods, post hoc Tukey-b tests were performed.

Table 14. Post hoc Tukey-b test for PC1. The means of groups of homogeneous subsets are shown. The method is based on the sum of squares. Error is the mean of squares.

PC1 Day 3

Storage Method	Subsets 1
ILOS+	-0.8447778
ULO+MCP	-0.8173519
ULO	-0.6016296
DCA	-0.5863250

Error = 0.024

PC1 Day 15

Storage Method	Subsets 1	2
ULO+MCP	-0.7013808	
ILOS+	-0.3301372	
DCA	-0.0394633	
ULO		0.7201458

Error = 0.154

PC1 Day 4

Storage Method	Subsets 1	2
ULO+MCP	-0.8879244	
ILOS+	-0.8051991	-0.8051991
DCA	-0.7635390	-0.7635390
ULO		-0.6351885

Error = 0.008

PC1 Day 22

Storage Method	Subsets 1
ULO+MCP	0.2295168
ILOS+	1.0663145
DCA	1.1956678
ULO	1.5281396

Error = 0.637

PC1 Day 8

Storage Method	Subsets 1	2	3
ULO+MCP	-0.8004843		
ILOS+	-0.5987099	-0.5987099	

PC1 Day 26

Storage Method	Subsets 1
ULO+MCP	0.5863758
ILOS+	1.3427553
DCA	1.8577009

DCA	-0.4683163	
ULO		-0.1167546

Error = 0.017

ULO	2.5879266

Error = 0.996

PC1 Day 11

Storage Method	Subsets 1	2
ULO+MCP	-0.7587475	
ILOS+	-0.4642214	
DCA	-0.4636230	
ULO		0.3140486

Error = 0.063

Regarding the first PC dimension (see Table 14) the main differences are present between days four and eight where apples treated with 1-MCP are clearly different from ULO apples. DCA and ILOS+ apples categorisation to subset 1 or 2 is not defined for all days. From day 11 to day 15 there is a clear difference between ULO apples and the others, which are categorised all together in a different subset. In the last days (from day 22 to day 26) no difference is noticed.

Table 15. Post hoc Tukey-b test for PC2. The means of groups of homogeneous subsets are shown. The method is based on the sum of squares. Error is the mean of squares.

PC2 Day 3

Storage Method	Subsets 1
ULO+MCP	-0.8458367
ILOS+	-0.6655590
ULO	-0.3717987
DCA	-0.3473012

Error = 0.437.

PC2 Day 15

Storage Method	Subsets 1
ULO	-0.5479517
ULO+MCP	-0.3376077
ILOS+	-0.2025564
DCA	0.3018089

Error = 0.994.

PC2 Day 4

PC2 Day 22

Storage Method	Subsets	
	1	2
ULO+MCP	-1.1499837	
DCA	-0.7123888	-0.7123888
ULO	-0.1428966	-0.1428966
ILOS+		0.0431889

Error = 0.258.

Storage Method	Subsets	
	1	2
ULO	-0.5882829	
DCA	-0.1582625	-0.1582625
ULO+MCP		0.5094076
ILOS+		0.7636843

Error = 0.301

PC2 Day 8

Storage Method	Subsets
	1
ULO+MCP	-0.3253993
ILOS+	1.0432715
ULO	1.2761945
DCA	1.4989275

Error = 0.941.

PC2 Day 26

Storage Method	Subsets
	1
ULO+MCP	-0.9015744
ILOS+	-0.7140897
ULO	-0.6413076
DCA	-0.5314939

Error = 0.259.

PC2 Day 11

Storage Method	Subsets	
	1	2
ULO+MCP	-0.4184187	
DCA	0.4667996	0.4667996
ULO	0.7265535	0.7265535
ILOS+		1.1063342

Error = 0.362

In the second PC dimension (see Table 15) the main differences are found (from day 4 to 11) between 1-MCP and ILOS+. For this dimension DCA and ULO apples cannot be assigned to a specific subset. In the last days (from day 22 to day 26), as seen for the first dimension, no difference is revealed.

In the other four PC dimensions there are still differences mostly in the fourth and fifth PC where 1-MCP apples are completely different from ULO apples. On the other hand, DCA and ILOS+ apples seem to sometimes behave like ULO apples and sometimes like 1-MCP-treated apples (see Table 16, 17, 18 and 19).

Table 16. Post hoc Tukey-b test for PC3. The means of groups of homogeneous subsets are shown. The method is based on the sum of squares. Error is the mean of squares.

PC3 Day 3

Storage Method	Subsets
	1
ILOS+	-0.7874002
DCA	-0.7631665
ULO+MCP	-0.1042103
ULO	0.2703242

Error = 0.263.

PC3 Day 15

Storage Method	Subsets	
	1	2
ULO+MCP	-0.4962747	
DCA	0.4613130	0.4613130
ILOS+	0.6968427	0.6968427
ULO		1.3259460

Error = 0.409.

PC3 Day 4

Storage Method	Subsets	
	1	2
ILOS+	-0.8675139	
DCA	-0.5826632	
ULO+MCP	-0.3081319	-0.3081319
ULO		0.4917872

Error = 0.259.

PC3 Day 22

Storage Method	Subsets	
	1	2
ULO	-0.9253663	
DCA	-0.7582876	-0.7582876
ULO+MCP	-0.5913192	-0.5913192
ILOS+		0.2300268

Error = 0.272.

PC3 Day 8

Storage Method	Subsets
	1
DCA	-0.8639170
ILOS+	-0.7782652
ULO+MCP	-0.6528390
ULO	0.4101860

Error = 0.586.

PC3 Day 26

Storage Method	Subsets
	1
ULO	-0.6026196
DCA	-0.5098526
ULO+MCP	0.2349499
ILOS+	0.3549941

Error = 0.351.

PC3 Day 11

Storage Method	Subsets	
	1	2
ULO+MCP	0.6347871	
ILOS+	1.1481015	
DCA	1.5133050	
ULO		3.1361531

Error = 0.669.

Table 17. Post hoc Tukey-b test for PC4. The means of groups of homogeneous subsets are shown. The method is based on the sum of squares. Error is the mean of squares.

PC4 Day 3

Storage Method	Subsets
	1
ULO	1.6525772
ULO+MCP	1.7776393
ILOS+	1.8985127
DCA	2.7972274

Error = 0.488.

PC4 Day 15

Storage Method	Subsets	
	1	2
ULO+MCP	-1.4015038	
ILOS+	-1.0109516	
DCA		0.0227310
ULO		0.1375973

Error = 0.248.

PC4 Day 4

Storage Method	Subsets	
	1	2
ILOS+	0.1319618	
ULO+MCP	0.3091908	
DCA	0.9824055	0.9824055
ULO		1.3521431

Error = 0.190.

PC4 Day 22

Storage Method	Subsets
	1
ULO+MCP	-1.2233422
ILOS+	-0.5786225
DCA	-0.4125499
ULO	-0.3295333

Error = 0.490.

PC4 Day 8

Storage Method	Subsets	
	1	2
ULO+MCP	-0.6471952	
ILOS+	-0.3378987	
DCA		0.5201312
ULO		0.6755405

Error = 0.125.

PC4 Day 26

Storage Method	Subsets	
	1	2
ULO+MCP	-1.2021100	
ILOS+	-0.3717673	-0.3717673
DCA	0.3391253	0.3391253
ULO		0.9922570

Error = 0.892.

PC4 Day 11

Storage Method	Subsets	
	1	2
ULO+MCP	-0.4698031	
ILOS+	-0.3661838	
DCA	-0.0751737	-0.0751737
ULO		0.7426003

Error = 0.240.

Table 18. Post hoc Tukey-b test for PC5. The means of groups of homogeneous subsets are shown. The method is based on the sum of squares. Error is the mean of squares.

PC5 Day 3

Storage Method	Subsets
	1
DCA	0.0655083
ULO+MCP	0.2919261
ILOS+	0.5773268
ULO	0.6360183

Error = 0.164.

PC5 Day 15

Storage Method	Subsets		
	1	2	3
ULO+MCP	0.0452291		
ILOS+	0.6328283	0.6328283	
DCA		1.0291590	
ULO			1.8051882

Error = 0.124.

PC5 Day 4

Storage Method	Subsets	
	1	2
ILOS+	-0.7691806	
ULO+MCP	-0.3136226	-0.3136226
DCA	-0.2749391	-0.2749391
ULO		0.2324876

Error = 0.122.

PC5 Day 22

Storage Method	Subsets	
	1	2
ULO	0.6547933	
ULO+MCP	0.6782122	
DCA	1.0308152	1.0308152
ILOS+		1.5533400

Error = 0.094.

PC5 Day 8

Storage Method	Subsets	
	1	2
ILOS+	-0.4124951	
ULO+MCP	0.0012929	
DCA	0.3310904	
ULO		1.6334108

Error = 0.240.

PC5 Day 26

Storage Method	Subsets
	1
ULO	-1.7615509
DCA	-1.1042502
ULO+MCP	-0.7802841
ILOS+	-0.3299615

Error = 0.481.

PC5 Day 11

Storage Method	Subsets	
	1	2
ILOS+	-1.5326498	
ULO+MCP	-1.3016575	-1.3016575
DCA	-1.0715202	-1.0715202
ULO		-0.3559791

Error = 0.293.

Table 19. Post hoc Tukey-b test for PC6. The means of groups of homogeneous subsets are shown. The method is based on the sum of squares. Error is the mean of squares.

PC6 Day 3

Storage Method	Subsets
	1
ULO	-0.1815410
ULO+MCP	-0.0704099
DCA	0.3056503
ILOS+	1.3682865

Error = 0.959.

PC6 Day 15

Storage Method	Subsets
	1
ULO+MCP	-0.4499703
ILOS+	-0.0463248
ULO	0.0114068
DCA	0.3173596

Error = 0.143.

PC6 Day 4

Storage Method	Subsets
	1
ULO+MCP	-0.3590597
ULO	-0.0694000
ILOS+	-0.0642057
DCA	-0.0509862

Error = 0.083.

PC6 Day 22

Storage Method	Subsets
	1
ULO	-0.5147386
DCA	-0.1153091
ILOS+	0.1065908
ULO+MCP	0.3206115

Error = 0.173.

PC6 Day 8

Storage Method	Subsets
	1
ULO+MCP	-0.4651514
ULO	-0.4031285
DCA	-0.3751502
ILOS+	0.0803261

Error = 0.072.

PC6 Day 26

Storage Method	Subsets
	1
ULO	-0.1970794
ILOS+	0.0787651
ULO+MCP	0.1191658
DCA	0.1991096

Error = 0.184.

PC6 Day 11

Storage Method	Subsets
	1
ULO	-0.4021435
DCA	-0.3351611
ULO+MCP	-0.1044635
ILOS+	0.0223758

Error = 0.074.

4 Conclusions

In Part 1 of the work 40 apple cultivars coming from two different macroclimatic areas of South Tyrol were characterized in three different ways. Chemical-physical analyses were carried out with the semiautomatic Pimprenelle machine, an expert panel performed a sensorial analysis and the volatile organic compounds were measured with a Proton Transfer Reaction Mass Spectrometry (PTR-MS) instrument (Lin93).

The Pimprenelle measurements revealed that fruits of the mountain area tend to have a higher firmness and acidity and a lower soluble solid content than fruits of the same cultivar harvested in the valley, both at harvest and after 3 months of normal cool storage.

Also the sensorial panel detected significant differences between cultivars and between sites. After cold storage and 6 days of shelf life fruits coming from the mountain were more intense in odour. In terms of aroma intensity the tasting showed that apples coming from the valley were less intensively aromatic.

Highly significant correlations between the instrumental Pimprenelle analysis and the sensorial analysis were found. The penetrometric value showed a highly significant positive correlation with the sensorial perception of firmness and a negative correlation with mealiness, as it could be expected. The measured titrable acidity correlated with the intensity of sourness which has been perceived by the panellists. The refractometric index was positively correlated with the perception of the intensity of sweetness and fruitiness (apple like aroma), while there was a negative correlation with odour intensity.

PTR-MS can provide, without pre-treatments like derivatization, a rapid fingerprint of the volatile profile. Because there is evidence of the correlation of PTR-MS fingerprinting also with genetic features of fruits this method should provide a bridge between sensory assessment of quality

and genetic control of apple. This was not possible in the present work (Part 1), since the correlation between the results of the sensorial analysis and the PTR-MS data is not significant enough. This might be explained by the assumption that certain odours and aromas are not perceived in a linear way by the human nose while the statistical elaboration of the data presumes a linear model. Most probably this is due to the fact that human perception of odour and flavour depends not only on the quantity of VOCs but for sure on their quality and their combination.

However, the result of the principal component analysis (PCA) of the PTR-MS allows to identify the most important families of molecules which are the major contributors to the characteristic apple-like aroma. The analysis of the loadings of the PCA performed on the PTR-MS data, combined with biochemical considerations, allowed to associate the four most important PCs (out of eleven which had been found) to the families of terpenes (PC1) and the big family of esters (PC2, PC3, PC4). On one hand the splitting of the family of esters in more dimensions of the PCA confirms that different cultivars biosynthesize many different kinds of esters. On the other hand, with very few exceptions, the variability of the single factors PC2, PC3 and PC4, related to esters, was rather low (Figure 12). In fact this result shows the actual limits of the investigated VOC measurement method. It is not yet well enough established and calibrated for apple to allow not only a quantification but also a more detailed and comprehensive qualification of VOCs. The relatively high number of analysed cultivars (even coming from two macroclimatically very different sites) probably result in a too complex system. The available biochemical information were, alone, not enough to simplify the data and filter out clear conclusions.

The terpenes associated to PC1 showed a clear gradient between cultivars and sites, with evidence of interaction among environment and cultivar. In this experiment the cultivars Nicogreen, Huaguan and Gradigold showed a

lower production of terpenes in the mountain site, while the cultivars Red Jonaprince, Rucla, Sansa, Scifresh, Silken and Topaz showed a higher amount of terpenes in the valley. All the other cultivars were affected less significantly by the site regarding this family of VOCs.

In Part 2 of the work the dynamics of aroma development has been investigated by means of PTR-MS in apples of a single cultivar (Red Delicious) during ripening under shelf-life conditions, after storage for seven months with four different techniques. The most important molecules of apple aroma have been identified and characterised through ionisation induced with H_3O^+. The first PC dimension included the principal esters (C6 esters m/z 116, C7 esters m/z 130 and C8 esters m/z 144) which showed a similar behaviour during shelf-life. On the other hand, monoterpenes and sesquiterpenes, which belonged to the second PC dimension, had a completely different behaviour compared to the esters.

In order to evaluate differences between the storage methods, post hoc Tukey-b tests were performed. Main differences have been found between apples treated with 1-MCP and untreated ones, stored in different controlled atmospheres. This behaviour could be due to the inhibition of the ripening process by 1-MCP also during shelf-life conditions, where room temperature and regular air composition accelerate ripening and senescence processes of apples well preserved in cool storage at very low O_2 and high CO_2 concentrations. Apples treated with 1-MCP developed the smallest quantity of VOCs during shelf-life.

C6 and C8 esters increased in a similar way during the course of shelf-life, the concentration of C7 esters remained quite constant for eight days and then increased exponentially until day 26 when reaching the highest concentration. Apples treated with 1-MCP and then stored under ULO conditions produced the smallest amount of esters. This is in agreement with the fact that inhibition of ethylene receptors by 1-MCP significantly

blocks the maturation process of apples. For the terpenes the trend was completely different. They increased significantly in the first eight days and then decreased in a similar way until day 26. Apples stored with DCA reached the highest concentration of these molecules, followed by ULO and ILOS+. As seen in for esters, apples treated with 1-MCP had a completely different behaviour: in the first 15 days the formation of terpenes in apples was small, rising around the 22nd day thereby reaching a maximum level and than falling to the common level.

Due to online real-time measurements and easy data acquisition by means of the PTR-MS technique it has been possible to analyse the “dynamics of aroma” development in apple samples in an easy and quick way (focusing on the most important masses, e.g. m/z 43 and 61, the analysis of one apple became quicker, i.e. ~ 3 minutes). These results, in fact, could represent a further step to make PTR-MS a standard technique for apple aroma analysis combined with the traditional sensory tasting panels.

Comparing the results of the 2 parts of the work, it is interesting to see that in Part 1 the main factor discriminating the 40 cultivars in the 2 sites was related to terpenes while in Part 2 of the work the main factor was related to Esters. In fact, the single cultivar Red Delicious analysed in Part 2 had already been analysed in Part 1 (Hapke Delicious which is a sport of Red Delicious). Also in Part 1 Red Delicious considered on its own showed a bigger variability of esters than terpenes between the mountain and valley site.

Even if several compounds could be associated with some masses with reasonable accuracy, it is preferable, before commenting on this point, to extend the experimental database on fragmentation in PTR-MS. To better interpret the results of PTR-MS it will be necessary to evaluate proton-transfer reactions for a series of reference compounds and identify their fragmentation patterns. This investigation is crucial for recognizing known

and characterizing unknown VOCs during development and ripening of apple, which PTR-MS analysis should or could evidence. With the availability of fragmentation patterns of the most important apple volatile compounds (Table 1) a better analysis and interpretation of the results in Part 1 might be possible. Moreover it would be possible to validate the PTR-MS results with chromatographic spectra of the same compounds.

However, the fast and individual measurement, the total absence of pre-treatment (apples are virtually unaffected by the measurement process), and the promising discriminative power of the proposed approach are good bases for the development of an “on-line quality/product control method” which could implement the apple agro industrial processing plants.

In this work the measurement of each single fruit required only eight minutes and other seven to clean gas lines between measurements. At the end the whole procedure could be, at least theoretically, totally computer controlled from the sampling phase to the discriminant analysis and data representation on the computer monitor. This is one of the most qualifying aspects of this proposed method and could be the basis for a complete automatic system for practical application with a very short time from sample preparation to data displaying.

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