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Impact of Agricultural and Environmental Factors on Strawberry (*Fragaria x ananassa* Duch.) Aroma – A Review

Jens Rohloff

The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway

Corresponding author: * **jens.rohloff@bio.ntnu.no**

ABSTRACT

The cultivated strawberry (*Fragaria x ananassa* Duch.) is an important berry crop worldwide due to its flavourful taste, and high content of nutrients and health-beneficial phytochemicals. Derived from interspecific hybridization of the octoploids *F. virginiana* and *F. chiloensis*, a vast number of strawberry varieties have been developed adopted to varying growth environments, and in order to meet consumer demand and preferences by the food industry. Hitherto, more than 360 volatile aroma compounds have been described in varietal genotypes, thus underscoring the complexity of aroma patterns in strawberry comprising hydrocarbon acids, esters, alcohols, aldehydes, and ketones, terpenes, aromatic structures, and furanones. Different extraction and analysis techniques, among others gas chromatography (GC), and sensory evaluation, which all are applied in the quality assessment of strawberry fruit, are presented. The impact of varietal and genetical differences, agricultural and environmental factors, post-harvest conditions and processing on strawberry aroma content and composition is highlighted by numerous examples from own research studies utilizing solid-phase microextraction (SPME) coupled with GC. The significance of inheritance and aroma compound metabolism on allover strawberry quality is emphasized with specific focus on future breeding efforts in *Fragaria* sp.

Keywords: breeding, extraction, gas chromatography (GC), sensory, solid-phase microextraction (SPME), volatile

Abbreviations: **AAT**, alcohol acyltransferase; **AECA**, aroma extraction concentration analysis; **AEDA**, aroma extraction dilution analysis; **CA**, controlled atmosphere; **CAR**, carboxen; **CCD**, carotenoid cleavage dioxygenase; **DHF**, 2,5-dimethyl-4-hydroxy-3(2H)-furanone; **DHS**, dynamic headspace; **DMF**, 2,5-dimethyl-4-methoxy-3(2H)-furanone; **DOXP**, deoxyxylulosephosphate pathway; **DVB**, divinylbenzene; **EESI-QTOF-MS**, Extractive electrospray ionization coupled with quadrupole time-of-flight MS; **Fa**, *Fragaria x ananassa* Duch.; **FD**, dilution factor; **FT-IR**, Fourier transform infrared spectroscopy; **Fv**, *Fragaria vesca* L.; **GB**, glycine betaine; **GC**, gas chromatography; **GC/FID**, GC coupled with flame ionization detection; **GC/MS**, GC coupled with mass spectrometry; **GC/MS-O**, GC/MS linked to olfactometry; **GC-O**, GC linked to olfactometry; **GM**, genetically modified; **HS**, headspace; **IPP**, isopentenyl phosphate; **JA**, jasmonic acid; **LOX**, lipoxygenase pathway; **MA**, modified atmosphere; **MAB** marker-assisted breeding; **MAE-SPME**, microwave-assisted extraction coupled with SPME; **MeJA**, methyl jasmonate; **MEV**, mevalonate pathway; **OAV**, odour activity value; **PA**, precursor atmosphere; **PAR**, photosynthetically active radiation; **PCA**, principal component analysis; **PDMS**, polydimethyl siloxane; **PET**, polyethylene terephtalate; **PINS**, pinene synthase; **PTR-MS**, Proton transfer reaction linked to MS; **PVC**, polyvinyl chloride; **SAFE**, solvent assisted flavour evaporation; **SBSE**, stir bar sorptive extraction; **SDE**, simultaneous distillation extraction; **SHS**, static headspace; **SPME**, solid-phase microextraction; **VAB**, valeric acid betaine

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INTRODUCTION

The cultivated strawberry (*Fragaria x ananassa* Duch.) is an economically valuable berry crop worldwide compared to other staple crops. Strawberry world production has expanded by 68% in the past 30 years (FAOSTAT 2010) and accounted for almost 1.9 billion $ in the USA in 2009. The productivity calculated as yield (t/ha) on the other hand, has only increased by 21% because of the selection of high-yielding varieties and cultivation method improvements. Although strawberry cultivation in Northern Europe (Norway, Sweden and Finland) is limited due to harsh climatic

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conditions and short summer seasons, the total production in the Nordic countries has increased by 28% since 1987 (FAOSTAT 2010). In Norwegian horticulture, strawberry represents the most important berry species. On average, strawberry is grown on a total area of 1.700 ha with a first-hand value of more than € 38 million per year (Haslestad 2006).

Today's cultivated strawberry originates from the accidental interspecific hybridization of the octoploid (2n = 8x = 56) species *F. chiloensis* from Chile and *F. virginiana* from eastern North America in the 18th century (Hummer and Hancock 2009). This hybrid combines significant traits such as an appreciated deep-red colour, flavourful taste and increased berry size and high yield compared to other utilized but less productive species in the genus *Fragaria*, e.g. the diploid woodland strawberry (*F. vesca* L.) and the hexaploid musk strawberry (*F. moschata* Duch.). Important quality factors of *F. x ananassa* for both consumers and industry are sugar and acid content, but also the characteristic aroma and flavour composition of strawberries (Rohloff *et al.* 2004) is regarded as a valuable fruit trait. In addition to its nutritional value and aesthetic qualities, strawberry is mainly appreciated for its substantial content of health-beneficial phytochemicals, and a growing interest in strawberry production due to the berries medicinal active and health-beneficial components has been recognized in recent years (Meyers *et al.* 2003; Anttonen *et al*. 2006; Paredes-López *et al.* 2010). Strawberries are a significant source of so-called antioxidants exhibiting functional roles in the plant related to plant growth, metabolism, defence and stress response, and showing biological and health-beneficial activity in human nutrition (Tulipani *et al.* 2008). The biological activity of strawberries is mainly based on the abundance of phenolics (Aaby *et al.* 2007), comprising anthocyanins, hydroxycinnamic and hydroxybenzoic acids and ellagic acid structures, but also includes high amounts of ascorbic acid (vitamin C).

Recent studies in strawberries have been focusing on possibilities to improve their phytochemical content by focusing on the biosynthesis of core metabolites within the phenylpropanoid pathway (Lunkenbein *et al.* 2006a), genotype selection and development of varieties (Davik *et al.* 2006; Khanizadeh *et al.* 2008). On the other hand, breeding focus on traits such as firmness, shelf-life, and harvest time has led to a genetic erosion of sensory characteristics and loss of berry aroma (Ulrich *et al*. 2007) and thus, negatively affected consumer acceptance. In general, the aroma of strawberry fruit shows quite complex chemical patterns and comprise more than 360 hitherto detected volatile compounds (Zabetakis and Holden 1997; Schwab *et al.* 2009) belonging to different chemical classes: hydrocarbon acids, esters, alcohols, aldehydes, and ketones, terpenes, aromatic structures, and furanones. However, less than 20 volatiles have been shown to have aroma-impact properties based on determined dilution factors (Schieberle *et al*. 1997; Ulrich *et al*. 1997), odour threshold values (Latrasse 1991; Larsen *et al*. 1992) or calculated odour activity values (Jetti *et al*. 2007). Newer studies try to address these questions with focus on the genetic background and inheritance patterns (Olbricht *et al*. 2008; Zhang *et al*. 2009b), in order to promote the breeding of flavourful strawberry varieties.

The present review tries to outline recent trends in the development and application of aroma analysis tools for the characterization of volatile patterns of strawberry. Different extraction methods and analysis techniques will be briefly presented. Based on the author's and coworker's experience in the utilization of the solid-phase microextraction (SPME) technique for volatile profiling of strawberries in particular, the review tries to highlight the significance of various factors including genetics, cultivation methods, and post-harvest handling, which directly or indirectly affect the aroma composition and thus quality of strawberry fruit. Finally, a scientific outlook discussing flavour and aroma aspects and future implications in *Fragaria* breeding and strawberry production is presented.

STRAWBERRY AROMA ANALYSIS

Analytical methods and techniques

1. Aroma volatile extraction methods

When describing methods and techniques which are applied toward aroma characterization of food in general, one has to make a distinction between instrumental and sensory approaches. In most cases, isolation and enrichment of strawberry aroma volatiles has to be carried out prior to instrumental analysis. In contrast to *flavour*, which describes the perception of food sensory characteristics by the senses of taste *and* smell, the concept of *aroma* and *aroma volatile*/ *aroma compound* will be consistently utilized in order to describe compound mixtures or single compounds. The use of these terms implies the volatile character of chemical structures which can be detected by the olfactory system of the human nose, and simultaneously underscore the necessity of adequate isolation and detection by technical devices. However, it has to be mentioned that single aroma volatiles also add to the overall flavour impression of food sensed by the tongue, e.g. furaneol (sweet), eugenol (sweet, warm) and methyl anthranilate (sweet, fruity) (The Good Scents Co. 2010). Terms such as *odour*, *fragrance* and *scent* which are often used to describe the subjective impression of food aroma, will be omitted in order not to confuse the reader.

Strawberry aroma is composed of a complex matrix of volatile compounds which derive from different biosynthetic pathways. Aroma volatiles can be classified based on their *chemical structure* (aliphatic, aromatic, heterocyclic), and attached *functional groups* defining the chemical classes, such as hydrocarbyls in esters (alkyl-, alkenyl-, phenyl-, benzyl-), oxygen-containing groups in alcohols (hydroxy-), acids (carboxy-), aldehydes (aldo-), ketones (keto-), ethers (alkoxy-), and nitrogen-containing groups (amino). Less common and just recently described classes comprise sulphur-containing thiols and sulfides (Du *et al*. 2010a). Depending on molecular weight, chemical class, boiling point and vapour pressure, and the interaction between compounds and berry texture, suitable extraction methods and analysis techniques for strawberry aroma description have to be considered. In many cases the isolation and detection of 20-30 major aroma volatiles is sufficient enough to characterize e.g. effects of crossing (Olbricht *et al*. 2008), cultivation (Rohloff *et al.* 2004) or storage conditions (Pérez and Sanz 2001) on aroma compositional changes. In terms of method development and application of advantageous technology, one might prefer to present detailed aroma profiles (> 40 compounds) in order to emphasize the innovative character above other techniques (da Silva and das Neves 1997; Aubert *et al*. 2005; Kafkas *et al*. 2005). In the following paragraphs, commonly applied extraction and analysis techniques will be presented. Benefits and drawbacks regarding sensitivity, artefact formation, compound discrimination and thermostability will be briefly discussed.

Research on strawberry aroma started in the 1950s, but first the introduction of new separation technology based on gas chromatography (GC) in the 1960s led to increased scientific interest regarding detailed characterization of strawberry aroma profiles and the identification of single compounds. During the past 20 years, innovative developments have changed laboratory work and instrumentation from macro- to micro-scale and thus, altered extraction and analytical procedures. High-throughput systems, minimal sample size, high sensitivity and information technology are keywords which characterize the direction of miniaturization and automation in modern science.

Isolation and extraction techniques comprise solvent-based extraction, distillation and headspace methods. *Solvent-based extraction* requires the application of adequate solvents, which are capable of isolating the aroma volatiles from raw purées or centrifuged, clear juices. In order to keep extracts free of highly polar metabolites such as sugars,

flavonoids, tannins and di-/tricarboxylic acids, two requirements have to be fulfilled: solvents have to be unpolar but semi-polar enough to extract aroma volatiles of different polarity, and simultaneously show low solubility in water (< 10%). The most commonly applied solvents are dichloromethane (Polarity Index = 3.1), diethyl ether (2.8) often mixed with pentane (0.0), and tert-butyl methyl ether (2.5). Regarding strawberry aroma, the extraction type might also be called liquid-liquid extraction (LLE) when using strawberry purée, pressed juice or juice after centrifugation – unless one studies e.g. aroma volatiles from dried fruits (liquid-solid extraction LSE). Upon solvent removal and thus concentration of analytes, extracts can be directly subjected to GC separation. Solvent extraction excellently recovers aroma volatiles belonging to different chemical classes, but also involve the application of relative large amounts of hazardous halogenated solvents (dichloromethane) or explosive chemicals (diethyl ether). Depending on the polarity of the chosen solvent or solvent mixture, LLE might favour the extraction of more polar structures and furanones compared to headspace techniques such as SPME; this method seems to be generally better suited for highly volatile and non-polar compounds (Kafkas *et al*. 2005). The lower detectability of e.g. furanones, due to their relatively lower vapour pressure, might be circumvented by adding sodium chloride to the liquid. NaCl increases ionic strength of the sample, and simultaneously decreases analyte solubility, and thus improves the extraction of more polar volatiles. Moreover, newer methods try to reduce the utilization of large solvent volumes as in the case of LLME (liquid-liquid microextraction) (Aubert *et al*. 2005).

Distillation techniques are often used in food aroma extraction, of which simultaneous distillation-extraction (SDE) is the most commonly applied technique (Escriche *et al*. 2000; Talens *et al*. 2002; Jeleń *et al*. 2005). More advanced methods have been developed such as solvent assisted flavour evaporation (SAFE) (Engel *et al*. 1999). Artefact formation might occur in SDE due to elevated temperatures and Maillard or Strecker reactions; the aroma-impact compound *furaneol* is known to be discriminated by SDE, and highly volatile compounds might be lost (reviewed by Engels *et al.* 1999). SAFE might be advantagous above SDE, since much lower temperatures (e.g. 20 to 30°C) are applied, which in turn take care of critical and thermolabile aroma volatiles; however, SDE has been shown to be more applicable in aroma volatile research. This fact applies also for the supercritical fluid extraction which has been used as a preparative tool in strawberry aroma analysis (Polesello *et al*. 1993). Compound selectivity makes SFE a rather unsuitable method, and it might only be applied for the extraction of potential pesticides in strawberry fruits (Pearce *et al*. 1998).

Headspace extraction (HS) represents the most versatile technique today for the isolation and analysis of food aroma. One might distinguish between static headspace (SHS) often used in SPME (Rohloff *et al.* 2004; Olbricht *et al.* 2008) or gas sampling (Rizzolo *et al*. 2007), and dynamic headspace techniques (DHS) by sweeping the sample vial with a carrier gas and subsequent concentration on a sorptive material, also called *purge-and-trap* technique (da Silva and das Neves 1997, 1999; Hakala *et al*. 2002). Trapped volatiles might be directly subjected to GC analysis, or have to be solvent-eluted from the sorptive material prior to analysis. Techniques such as hyphenated HS extraction with GC is solvent-free and a fast sampling technique as further discussed in section 2.1.2 “Aroma volatile analysis – Detection and identification”. Automated HS linked to thermal desorption (TD) and coupled GC/MS or GC/FID is a feasible tool in the study of volatile organic compounds from food (MacNamara *et al*. 2010). Nevertheless, in many cases samples need to be freshly prepared prior to analysis, making TD-GC analysis in strawberry analysis a rather uncommon approach except of stir bar sorptive extraction (SBSE). SBSE was introduced in 2000 and is based on a similar type of extraction. However, the HS step is omitted, since the stir bar is placed in the sample vial, and analytes are directly bound to the sorptive material (polydimethylsiloxane, PDMS) covering it (Kreck *et al*. 2001; Du *et al*. 2010b). Upon manual or automated application, extracted analytes are desorbed in a gas chromatograph in a TD unit. SBSE is a fast and highly sensitive technique for volatile extraction comparable to SPME. The latter can be considered as a HS technique in terms of strawberry aroma analysis. SPME has been frequently applied by the author and co-workers, and its application, advantages and drawbacks will be described in detail in alter section.

2. Aroma volatile analysis – Detection and identification

The analytical instrumentation most commonly applied today for the separation of aroma mixtures and subsequent analyte detection is gas chromatography (GC), which already has been mentioned in connection with aroma extraction. The sample (solvent-based, absorbed on a sorbent, or gaseous) is introduced in the injection port of the GC and vaporized at elevated temperature. Analytes are transported by an inert carrier gas (He, N, H) through a capillary column where they are separated based on the chosen instrumental parameters (column length, diameter, packing material, gas pressure and velocity, temperature programming) and the analytes’ physicochemical properties (boiling point, molecular weight, polarity). Finally, analytes are detected by a detector system, of which mass spectrometry (MS) and flame ionization detection (FID) are mostly applied. In general, modern GC and detection instrumentation is capable of high-efficiency separations of complex sample matrices and highly sensitive down to pg-levels, and more than 50 volatile analytes might be easily recovered in one analytical run (da Silva and das Neves 1999). Detector signals can further be used for quantitative purposes based on signal intensity, peak area or height. GC coupled with MS (GC/MS) has the great advantage of mass sensitive detection, delivering mass spectra which are characteristic for each analyte (MS fingerprint), and allow for reliable compound identification through automated or manual MS database search. FID, on the other hand, is often used simultaneously for quantifications; however, GC/FID analysis alone requires the use of reference compounds for compound identification based on retention time. In order to gain a better resolution of peaks from complex volatile mixtures, 2D GC (GC x GC) might be applied using two capillary columns of different polarity. In other approaches, GC samples are split and run on two different columns being detected by two detectors simultaneously. Gas chromatography linked to olfactometry (GC-O) is a specific type of sensory aroma analysis where instrumental analysis is coupled with the sense of the human nose (Zellner *et al*. 2008), and will be presented in section “Sensory evaluation and taste panels”.

A simplified and fast approach toward total berry aroma using MS technology is provided by headspace fingerprinting mass spectrometry (HF-MS) (Berna *et al*. 2007). Without any chromatographic separation, the headspace sample is subjected to ionization in the MS detector. A mass spectrum of all aroma volatiles is generated and can be used as a fingerprint for the strawberry sample, based on the presence and intensity of the detected fragment ions. Data from several samples might further be analyzed by multivariate statistical analyses, e.g. Principal Component Analysis (PCA). Extractive electrospray ionization coupled with quadrupole time-of-flight MS (EESI-QTOF-MS) has been recently introduced by Chen and co-workers (2007), and establishes a highly sensitive method for the generation of volatile fingerprints based on total berry aroma. Being quite similar to HF-MS, also EESI-QTOF-MS needs to be followed up by unsupervised chemometric methods (multivariate statistics) for pattern recognition and sample classification.

Proton transfer reaction-mass spectrometry (PTR-MS) is yet another analysis tool for plant volatile research (Tholl

et al. 2006), which has been applied for berry aroma volatile analysis (Boschetti *et al*. 1999; Carbone *et al*. 2006; Aprea *et al*. 2009). In contrast to traditional GC/MS, PTR-MS is much faster by analyzing the whole sample within a few minutes. Based on 'weak' proton ionization at atmospheric pressure, no molecule fragmentation occurs and the protonated molecule mass of each single compound is depicted in a sample mass spectrum. Although the technique's sensitivity is comparable to GC detection, compound selectivity is rather low with regard to fast-reacting terpenes and isobaric compounds (alcohols and acids) (Tholl *et al.* 2006). Sensitive pulsed flame photometric detection coupled with gas chromatography (GC-PFPD), a detector type which has been developed 20 years ago, has recently been successfully applied for the identification of new strawberry sulphur volatiles (Du *et al*. 2010a), and might be conidered as a useful tool for specific applications. Also Fourier transform infrared spectroscopy (FT-IR) might be directly linked to GC separation in order to supplement GC analysis approaches (Tholl *et al*. 2006). Only few examples of FT-IR approaches in strawberry aroma research exist (Hakala *et al.* 2001) not least because of rather complicated data handling; this technique is better suited for the detection of nutritional compounds (Kim *et al*. 2009).

Sensory evaluation and taste panels

The sensory quality of strawberry fruit is based on a combination of sweetness and acidity, aroma, texture and appearance (Ulrich *et al*. 2007). These parameters can be simultaneously assessed by trained taste panels with regard to quality control and product development, and be used to distinguish between varieties (Jetti *et al*. 2007; Jouquand *et al.* 2008) and quality differences (Azodanlou *et al*. 2003; Han *et al*. 2005; Almenar *et al*. 2009a). In terms of aroma compounds and the perception of smell (olfaction) from food, one might consider a possible high variation between the results from different taste panels. This question has been addressed by Ferreira *et al*. (2006) by studying the relation between orthonasal and retronasal detection of aroma compounds with regard to delivery mechanisms, compound volatility and persistence. Aroma release during eating is significantly influenced by retronasal perception and intensity, and can be calculated as a function of transfer and volatility in the food matrix (Trelea *et al*. 2008). Moreover, consumer perception and quality grading of strawberry fruit flavour has been shown to be strongly positively correlated with both the amount of aroma volatiles and total sugar content, while higher fruit firmness seemed to have a negative effect (Azodanlou *et al.* 2003). The heterogeneity of samples and anatomophysiological differences of panellists might explain variation in sensory impression and thus results, and have led to the development of reliable instrumentation trying to technically mimic the properties of human senses such as olfaction (*electronic nose*) and taste (*electronic tongue*) as described later.

Gas chromatography coupled with olfactometry (GC-O) is a combined technique based on both instrumental analysis and olfaction (Ulrich *et al*. 1997; Zellner *et al*. 2008). After GC separation, the aroma volatiles are directed to a sniffing port(s) where one or several panellists perform an aroma description. For compound identification purposes, GC might be followed by MS detection and simultaneous olfactometry. Several types of approaches might be applied e.g. aroma extraction concentration analysis (AECA), or aroma extraction dilution analysis (AEDA). The latter can be used to calculate dilution factors (FD), which describe the aroma-impact and detection level of volatiles (Schieberle and Hofmann 1997). Aroma patterns derived from GC-O, or alternatively from the quantitative composition by GC/MS combined with odour activity values (OAV) might give similar results as shown by Nuzzi *et al.* (2008). However, GC-O has to be considered as a rather time-consuming and expensive method due to the training of panellists.

The development of *electronic noses* (E-nose) started in the 1980s and generated technological devices which today partly fulfil industrial needs regarding accuracy, precision and applicability for routine analyses (Zhang and Gongke 2010). In general, an E-nose is built up of a sample delivery unit coupled to a detector which is linked to a computer. Volatile compounds (headspace above liquid or solid sample) are transported to the detector system consisting of a sensor such as surface acoustic wave (SAW) quartz microbalance (zNose™), metal oxide semiconductor (MOS), conducting polymers (CP), or field effect transistors (MOSFET). Such instruments are based on sensor array technology and can detect signals from single volatiles or volatile mixtures. Detector signals are subsequently analyzed by multivariate statistic analyses for pattern recognition and sample identification purposes. Newer E-nose devices are also available as portable instruments; specific types might even be coupled to ultra-fast GC separation (zNose™) (D'Auria *et al*. 2007) or a quadrupole mass analyser (Smart Nose™) (Gabioud *et al*. 2009), thus bridging the gap between advanced laboratory equipment for chromatography and miniaturized E-noses for solely pattern recognition. Potential applications in agricultural and food chemistry comprise aspects related to the classification of varieties (McKellar *et al*. 2005; Laureati *et al*. 2010), maturation, quality and shelf-life (Li *et al*. 2009; Clifford *et al*. 2010), and food processing (Buratti *et al*. 2006; Dalmadi *et al*. 2007). The MS Nose™ is a highly specialized E-nose which might be applied for real time detection of aroma volatiles from the human nose during mastication of food (Harker and Johnston 2008; Yang *et al.* 2011), also known as breath-by-breath analysis. No chromatographic steps are required, and compound masses are detected after 'soft' ionization at atmospheric pressure. In terms of food aroma and berry research, the technique might be utilized to study the significance of fruit texture and firmness on aroma volatile release and subsequent retronasal detection, and thus represents a versatile tool for food product development based on the consumers' perception and preferences.

Electronic tongues (E-tongue) for sensory analysis and the supplement of human judgement were introduced in the 1990s (Riul *et al*. 2010). E-tongue measurement is based on a sensor array which detects both single compounds and complex chemical matrices in liquid media. Compared to the electronic nose, mainly polar compounds are recognized by the E-tongue. However, the instrument is also capable of sensing potential aroma volatiles such as hydrocarbon alcohols, acids, aldehydes and esters (Legin *et al*. 2005; Rudnitskaya *et al*. 2006; Hruškar *et al*. 2010a). Recent technological developments mainly rely on the application of electrochemical measurements (*potentiometric*) and impedance spectroscopy (*voltametric*). In contrast to other quantitative methods such as GC separation and detection, flavour and taste sensing by E-tongue technology is generally based on classification and requires the use of multivariate statistical methods and artificial neural networks for pattern recognition followed by sample identification (Hruškar *et al*. 2010b). In conclusion, both E-tongue and E-nose instrumentation are highly promising technologies which potentially might replace highly costly and time-consuming sensory panels in the future, and allow for the automated high-throughput of food samples and quality control in industrial processes.

Aroma analysis by solid-phase microextraction (SPME)

SPME can be considered to be one of the mostly utilized extraction methods to approach the aroma volatile composition in strawberry fruit or related berry crops. The following section will *solely* focus on the application of SPME for berry aroma volatile extraction from fresh fruit or otherwise processed samples. The reader is referred to relevant reviews and articles about environmental, agricultural and food research (Rohloff 2004; Ouyang and Pawliszyn 2006; Risticevic *et al*. 2009), in order to get a full overview of the

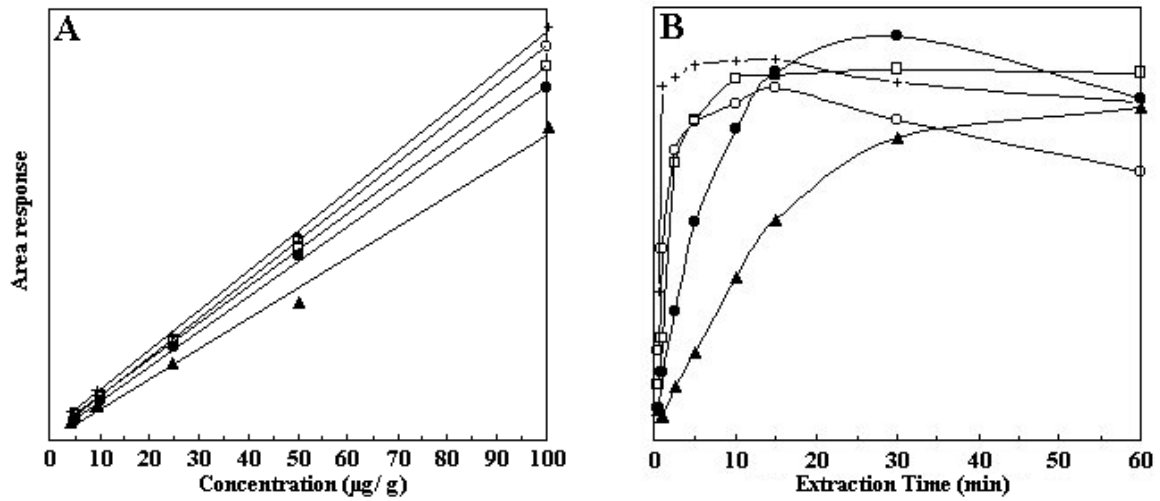

Fig. 1 Influence of concentration (**A**) reference compounds and extraction time (**B**) reference compounds added to strawberry juice) on SPME fibre absorption (dimensionless area response) of selected volatile compounds naturally occurring in strawberry aroma: ● linalool, ▲ nerolidol, ○ methyl hexanoate, + ethyl butanoate, □ ethyl acetate). *Source*: Rohloff 2001 (unpublished results).

method's applicability and flexibility with regard to extraction approaches and analytical platforms.

SPME in strawberry aroma analysis is almost exclusively used in SHS mode, and commonly followed up by GC/MS or GC/FID separation and detection. Extracted volatiles might be manually applied (Rohloff *et al*. 2004) or automatically injected into the GC (Olbricht *et al*. 2008), thus underscoring the feasibility and versatility of this method. SPME is solvent-free, potentially based on non-destructive *in situ* sample preparation, and requires only minimum sample processing prior to extraction. The principle of SPME is based on a sorptive fibre, which is capable of extracting volatiles from the headspace of a sample vial or the sample's environment. Depending on the polarity of the chosen sorbent material, analytes are readily absorbed or adsorbed on the fibre. Various fibre coatings, also with differing fibre thickness are commercially available for the suitable extraction of unpolar or/and semi-polar volatiles for both standard and trace compound analysis. The unpolar PDMS-coated fibre of 100 µm in diameter has earlier been used as the "standard" fibre type (Ulrich *et al.* 1995; Ibañez *et al*. 1998; Holt 2001; Rohloff *et al.* 2004). As newer fibre types became available throughout the years, it was shown that combined sorbents provided better suitability toward the complex strawberry aroma consisting of volatiles of different polarity and concentration level (Azodanlou *et al*. 1999 and 2003). Today, the following multi-component fibre types are most frequently applied: the 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre (de Boishebert *et al*. 2004) and the 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DBV/CAR/PDMS) (Urruty *et al.* 2002; Jetti *et al*. 2007; Aprea *et al.* 2010). A recently developed method established for essential oil profiling of herbs and medicinal plants – microwave-assisted extraction linked with microextraction (MAE-SPME) – might also be successfully applied for the characterization of strawberry aroma (Zhang *et al*. 2009a).

Due to a restricted surface and volume of the SPME fibre, extraction time normally does not exceed 20-30 min. However, SPME sample extraction conditions must be kept strictly controlled since small variations in fibre extraction time and exposure depth, temperature, and headspace volume might lead to variation, and reduced comparability of analysis results (Azodanlou *et al*. 1999; Holt 2001; Rohloff 2004). Volatiles partition between the sample matrix, headspace *and* the SPME fibre, but reach an equilibrium when the concentrations of analytes in the different phases are quite stable (**Fig. 1B**). However, not necessarily real sample concentrations and compositions are measured, since the aroma volatiles of the headspace gas, and not the sample

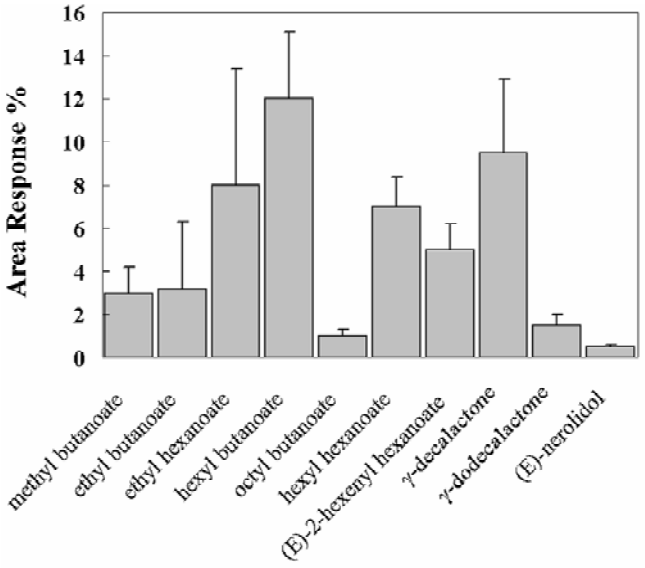

Fig. 2 Reproducibility (n=9) of main aroma volatiles from a homogenized strawberry sample detected by SPME-GC/MS. Note that the variation of single compounds does not exceed 5% deviation. *Source*: reproduced with permission of Lyngved (1999).

directly is assessed. Compound discrimination, i.e. the selective extraction of single volatiles might occur (Holt 2004). This is particularly true for highly volatile and trace level compounds, and analytes showing less affinity toward the utilized fibre type. Nevertheless, SPME shows strong extraction linearity toward differing concentration levels of single compounds or compound mixtures as depicted in **Fig. 1A** and described by Holt (2004). Moreover, the SPME method shows also a high degree of reproducibility in subsequent extractions from the same sample (n=9) with variations <6% (**Fig. 2**) (Lyngved 1999). Keeping minimum exposure times and equilibrium settlement, after determination of suitable experimental conditions, assures a proper SPME extraction. In many studies, the pre-processing of strawberry samples is preferred (purée or clear juice after centrifugation). By this, the headspace volume in the sample vial can be more easily controlled due to a fixed sample volume. One should be carefully in sample preparation not to crush the seeds since "green" notes (C_6-compounds or so-called *green leaf volatiles*) will be added to the fruit aroma. Moreover, the liquid sample might be agitated by a magnetic stirrer to improve flavour release from the watery

phase. Also the addition of 20-25% NaCl (Rohloff *et al*. 2004; Jetti *et al*. 2007; Olbricht *et al*. 2008) and the pH stabilization by buffers further facilitate volatile release and thus, SPME extraction efficiency and reliability.

In the next section, several examples from own investigations and the application of SPME will be used to emphasize the impact of agricultural and environmental factors on the aroma volatile composition of strawberries.

STRAWBERRY AROMA QUALITY AND PRODUCTION FACTORS

The study of volatile compounds produced by strawberry has been of significant interest in the past three decades due to the fruit's pleasant and flavourful aroma. These chemical structures are considered to belong to the so-called group of *secondary metabolites* being produced by plants from precursors of primary metabolism mainly via the lipoxygenase, phenylpropanoid/benzenoid and terpenoid pathways (Negre-Zakharov *et al*. 2009). The strong fruity, but also sweet, herbal and floral notes of strawberry aroma blends serve the purpose to increase the berries' attractiveness with regard to potential seed dispersal. Moreover, many of these volatiles show also distinct biological activity and might be involved in plant defence mechanisms in terms of repellent function, predator attraction and toxic action. The main pathways of aroma volatile biosynthesis and products will here be briefly described. For more details on regulation of gene expression and biochemistry, the reader is referred to scientific reviews e.g. Klee (2010) and Schwab *et al*. (2009).

Esters comprise the largest group of aroma-impact volatiles in strawberries. Their biosynthesis is strongly induced during fruit ripening due to morphological-dependent and developmental regulation of gene expression of corresponding catalyzing enzymes, and the presence of necessary substrates. Many C_6-volatiles, either alcohols or aldehydes (e.g. (Z)-3-hexenol, hexanal), are directly derived from the lipoxygenase pathway (LOX)-derived fatty acids, and further serve as precursors in the formation of esters. Other precursors include branched (e.g. 3-methylbutanol from leucine) and aromatic (e.g. 2-phenylethanol from phenylalanine) alcohols and aldehydes, which are directly generated from certain amino acids. The final step in ester production in fruits and berries is catalyzed by so-called alcohol acyl transferases (AAT). An AAT identified in strawberry (SAAT) has been shown to be responsible for the formation of many characteristic fruit esters during ripening (Aharoni *et al*. 2000a). This work was later supplemented by the identification of a VAAT in the woodland strawberry (*F. vesca*) (Beekwilder *et al*. 2004). Cinnamic acid-derived esters, methyl and ethyl cinnamate, are supposed to be synthesized due to the action of the multifunctional UDP-glucose:cinnamate glucosyltransferase FaGT2 (Lunkenbein *et al*. 2006c). These volatiles are typically found in *F. vesca* and strongly contribute to the characteristic aroma of the woodland strawberry, but reasonable amounts have also been detected in certain octoploid strawberry varieties (Jetti *et al*. 2007).

The chemical class of terpenes in strawberries has been of particular interest since these compounds have characteristic sensory properties and simultaneously, are known to exert biological activity against microorganisms (fungi, bacteria). Volatile terpenes derive from either the mevalonate pathway (MEV) in the cytosol, or plastidal metabolism via the deoxyxylulosephosphate pathway (DOXP). Both lead to the formation of the precursor isopentenyl diphosphate (IPP) for further biosynthesis of terpenic structures, of which linalool and nerolidol are the most prominent in cultivated strawberry. A functional nerolidol synthase in *F. x ananassa* (FaNES1) was identified by Aharoni *et al*. (2004), leading to the formation of the monoterpene linalool and the sesquiterpene nerolidol. FaNES1 is thought to be mainly responsible for terpene synthesis in cultivated strawberry, while pinene synthase activity found in *F. vesca* (FvPINS) is obviously absent in *F. x ananassa* fruit (Aharoni *et al*. 2004). However, the surprisingly high variability of terpenic structures identified in *F. x ananassa* other than linalool and nerolidol can not only be explained by the multifuctionality of (FaNES1) and suggests other terpene synthases and pathway-related enzymes to be involved.

The group of phenylpropanoid/benzenoid-derived volatile structures plays a minor role in their contribution to the overall strawberry aroma. Nevertheless, since these pathways also lead to health-beneficial compounds with potential antioxidant action such as anthocyanins, flavonols and other phenols, much attention has been paid to these secondary structures. Beside the already mentioned cinnamates, also the aroma-impact compound eugenol (Ulrich *et al*. 1997) derives from cinnamic acid, while phenylpropanoids (e.g. 2-phenylethanol) and benzenoids are biosynthesized from the precursor benzoyl-CoA. The formation of benzenoids leads to characteristic aroma volatiles with benzyl- and benzoic acid structure such as benzaldehyde, benzyl acetate, and ethyl benzoate.

Furanones represent a small group of aroma volatiles significantly contributing to the overall strawberry aroma. The furanones comprise aroma-impact compounds such as *furaneol* (2,5-dimethyl-4-hydroxy-3(2H)-furanone DHF) and *mesifurane* (2,5-dimethyl-4-methoxy-3(2H)-furanone DMF), both characterized by a caramel-like and sweet aroma and flavour impression. These compounds have a strong "strawberry-like" aroma with extremely low aroma threshold values; thus they are also termed strawberry furanone (DHF) and berry furanone (DMF). Due to the food and flavour industries' interest in furanones, DHF was already in 1965 technically synthesized. First recently, the last steps in biosynthesis of these structures were functionally characterized in strawberry fruit: the formation of DHF via an enone oxireductase (FaEO) (Klein *et al*. 2007), and the metabolic step from DHF to DMF via a catalyzing *O*-methyl transferase (FaOMT) (Lunkenbein *et al*. 2006b).

Another important group of aroma volatiles comprise the so-called ionones, which are produced through degradation of carotenoids in strawberry fruit during ripening. The derived structure β-ionone can be considered as an important aroma-impact compound due its low aroma threshold value, adding floral notes to strawberry aroma (Ulrich *et al.* 1997). Recently, a carotenoid cleavage dioxygenase FaCCD1 has been functionally characterized (García-Limones *et al*. 2008), which is supposed to be involved in carotenoid catabolism and volatile production in strawberry fruit *in vivo*. Methyl anthranilate is yet another aroma-impact compound in strawberry fruit being directly synthesized from the shikimic pathway via chorismate as precursor and a final methylation step. The compound is present both in aroma from *F. vesca* (Pyysalo *et al*. 1979) but also frequently detected in cultivated strawberry (Ulrich *et al.* 1995, 2007).

Varietal aroma profiles and inheritance

The aroma of strawberries has been exhaustively studied in the past 50 years. Despite the high complexity of aroma patterns in berries compared to other fruits, only a small range of up to 20 volatiles have been identified as aroma-impact compounds (Latrasse 1991; Larsen *et al*. 1992) mainly contributing to the overall strawberry aroma. Significant compound classes comprise esters, furanones, lactones, aldehydes, ketones, acids, aromatic structers and terpenes (Schieberle and Hofmann 1997; Ulrich *et al.* 1997). Due to the different biosynthetic origin of related compounds as described in the introductory part of the previous section, it becomes clear that aroma volatile patterns of strawberry varieties are strongly genetically (*varietally*) determined.

The specifity of AAT enzymes from 6 strawberry cultivars was shown to reflect varietal differences based on aroma profiles (Olías *et al*. 2002), and thus underscored the significance of the genetic background for strawberry classification into certain *aromatypes*. Furthermore, concentration levels and sensory properties of aroma compounds can

Table 1 SPME study of aroma volatile composition of 6 strawberry cultivars being tested at The Plant Biocentre, Dragvoll, in 1996 as part of the «Strawberry Project - 98». *Source*: compiled with permission of Holt (1999).

COMPOUND	Korona	Bounty	Senga S.	Jonsok	Nora	Glima	Aroma description
methyl butanoate	*	*	*				ethereal fruity
ethyl butanoate	*	*	*	*		*	fruity sweet, apple
2-hexenal		*	**			*	green fruity
methyl hexanoate	*	**	**	*		*	fruity pineapple
butyl butanoate						tr	fruity sweet
ethyl hexanoate	**	**	*	***	*	***	fruity pineapple
hexyl acetate	***	***	*	***	***	*	fruity green
(*E*)-2-hexenyl acetate	****	****	**	****	****	**	sweet green, apple
limonene						tr	sweet citrus, peely
linalool		*	*	*		tr	floral citrus
benzyl acetate	tr	tr		tr	tr	tr	sweet floral fruity
hexyl butanoate	***	*	**	**	***	***	green waxy fruity
(*E*)-2-hexenyl butanoate	****	**	****	***	***	****	green fruity apple
ethyl octanoate		tr	**	*	tr	*	fruity waxy
hexyl hexanoate	*	*		tr	*	tr	green herbal, fruity
octyl butanoate	*	*	*	*	*	*	fruity, green waxy
(*E*)-2-hexenyl hexanoate			*	*		*	green apple, herbal
γ-decalactone					**		fruity peach
(*Z*)-nerolidol		**	*	*	tr	*	mild floral

tr <1.0 %; * 1-5 %; ** 5-10 %; *** 10-20 %; **** >20 %

be linked to specific genotypes as described in several reports (Larsen *et al*. 1992; Ulrich *et al*. 1997; Hakala *et al*. 2002; Bursać *et al*. 2007; Jetti *et al*. 2007). Results from our lab studies in 1996 (**Table 1**) revealed clear aroma differences based on the abundance of significant volatiles in 6 varieties ('Korona', 'Bounty', 'Senga Sengana', 'Jonsok', 'Nora', and 'Glima') (Holt 1999). Relative high levels of terpenes in particular could be used to distinguish among genotypes ('Bounty', 'Senga Sengana', and 'Jonsok'), but also other compounds related to the class of esters and acids might be used for classification purposes as pointed out by Pelayo-Zaldívar *et al.* (2005).

The furanones, normally detected in minor amounts, comprise the aroma-impact compounds such as furaneol (DHF) and mesifurane (DMF), both adding caramellic-sweet notes to strawberry aroma. DMF levels increase during ripening while DHF simultaneously decreases (Jetti *et al*. 2007), but both show similarly low odour threshold values in water (Ulrich *et al*. 1997). The structurally-related lactones of which γ-decalactone represents the most abundant compound, might also be used for variety classification (Jetti *et al*. 2007; Nuzzi *et al*. 2008; Ulrich *et al*. 2008).

The aroma of the diploid "wild" woodland strawberry has long been of interest in strawberry volatile research (Drawert *et al*. 1973), also in comparison to the aroma of cultivated, octoploid varieties (Pyysalo *et al*. 1979; Ueda *et al*. 1997). Beside reasonable amounts of the monoterpene linalool and the sesquiterpene nerolidol (**Table 1**), these compounds are quite often accompanied by trace levels of other terpenic structures in strawberry varieties, such as pinenes, limonene, γ-terpinene, terpinolene, 4-terpineol, α-terpineol, nerol, and myrtenyl derivatives (Rohloff, unpublished results; see also review by Zabetakis and Holden 1997). The latter aroma compounds (myrtenal, myrtenol, myrtenyl acetate) have been proposed to be solely produced in diploid *F. vesca* in comparison with *F. x ananassa* (Aharoni *et al*. 2004) due to species-genetic differences and the absence of the necessary enzyme PINS. However, reasonable amounts are easily detectable by HS-SPME in varieties 'Calypso' and also 'Bounty' (Rohloff, unpublished results), which is also confirmed by studies of other cultivars (Ghizzoni *et al.* 1997; da Silva and das Neves 1999; Ulrich *et al.* 2007).

The aroma in strawberry fruit has first recently attracted attention as a potential breeding goal, starting with studies by a US American (Carrasco *et al*. 2005), Chinese (Zhang *et al*. 2009b) and by a German research group (Olbricht *et al*. 2008), in order to characterize inheritance patterns of distinct aroma compounds. The comprehensive study from

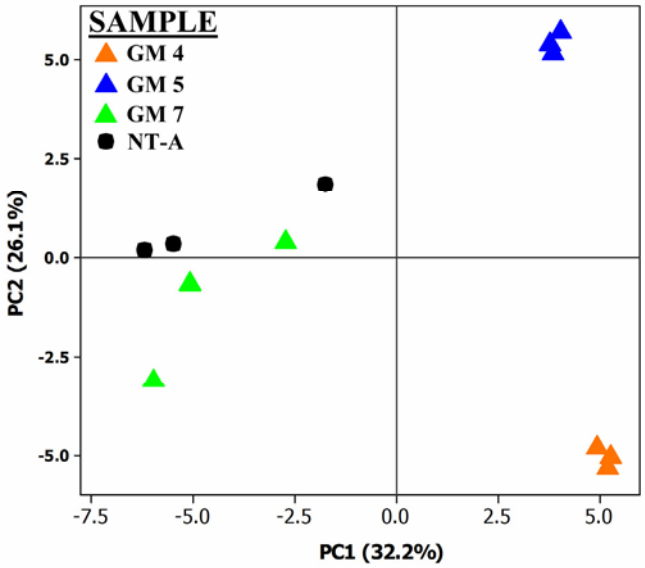

Fig. 3 Principal component analysis (PCA) of strawberry volatile profiles from non-transformed (**NT**) and GM plants (lines **GM4**, **GM5**, and **GM7**) from greenhouse cultivation. The principal components PC1 and PC2 were computed based on the total of 66 aroma volatiles. *Source*: Rohloff 2005 (unpublished results).

the German group revealed the occurrence of reasonable amounts but also high variability of the aroma-impact compound methyl anthranilate in genotypes of the F1-population, which was generated from two contrasting parents, the aroma-rich variety 'Mieze Schindler' and the modern cultivar 'Elsanta'. These results exemplify the significance for the selection of suitable genotypes for breeding purposes toward aroma traits. However, multi-generation studies need to be performed in order to verify the stability of observed aroma traits and the selection of breeding lines as emphasized in recent studies by Olbricht and co-workers (2011).

The commercial use of genetic modification (GM) for the introduction of new gene functions into plants and the alteration of crop traits has an almost 20 year old tradition. Today most of the commercialized GM traits are related to herbicide tolerance and insect and virus resistance in major staple crops, but also modification of nutritional quality of certain plant species has attained more interest in recent years, e.g. amino and fatty acids, and starch (GMO COM-

PASS 2010). The modification of food aroma or generally flavour has not been commercially approached so far. However, attempts toward the purposeful change of nutritional and flavour-related traits of strawberry have been made and several patents have already been granted (Aharoni *et al*. 2000b, 2002; Schwab *et al*. 2003). One major safety issue regarding the use of GM plants in human nutrition is the possibility that traits and biological functions other than those related to the modification, might have been affected. Potential effects include changes in protein metabolism and biosynthesis, which might be approached through proteomics and metabolomics in order to validate the substantial equivalence, i.e. the similarity of health and nutritional characteristics between the original and GM plant. As part of a European study of a GM strawberry with improved resistance against *Botrytis cinerea* (Project: TSP-EEES/QLK5-CT-1999-01479), HS-SPME of aroma profiles of strawberries from different GM lines was carried out (**Fig. 3**). Based on 66 detected compounds, PCA analyses revealed clear differences between at least two of the GM strawberry lines based on slightly changed aroma volatile patterns. In terms of quantitative considerations and substantial equivalence however, the observed changes were not significant.

Ontogenetic, seasonal and geographic variation

The composition of aroma volatile patterns is strongly dependent on the genetic background of the plant as already pointed out for the varietal aromatype of distinct strawberry genotypes. However, ontogenetical, seasonal and geographic factors significantly influence the metabolism and levels of aroma compounds. While ontogenetic factors have to be considered as internal factors based on the organ- and time-dependent gene expression and metabolic changes in maturing strawberry fruit, both seasonal and geographical parameters are determined by environmental variables differing periodically from year to year (light, temperature, water) and locally (microclimate, soil).

Ontogenetic Factors – Morphological and physiological changes occur throughout the ontogenetic development after blossoming from green to ripe strawberry fruit. Cell division continues for about one week after petal fall, whereas cell size increase and vacuolation is initiated immediately (Knee *et al*. 1976). Also the content of nutritionally important metabolites such as sugars and acids changes continuously. Levels of glucose, fructose, sucrose and malic acid increase, while concentrations of the main Krebs' cycle product citric acid decrease steadily and thus, the levels of titratable acidity (Ménager *et al*. 2004). About three weeks after blossoming, pigmentation of the berry starts; while levels of total phenols decrease, anthocyanin concentrations increase simultaneously (Montero *et al*. 1996; Ferreyra *et al*. 2007). In general, the harvesting of strawberries is based on the parameters soluble solids (°Brix value) and pigmentation, i.e. anthocyanin content. In the case of long-distance transport or fruit export, berries might also be picked at an earlier stage because of the fruits' softness and relatively rapid decay at full mature stage. However, aroma quality parameters are generally not considered as a decision criterion for the time point of harvest as explained in the next paragraph.

In terms of berry development, the aroma volatile composition and concentration levels dramatically change in ripening strawberry fruits. As depicted in **Fig. 4**, a higher number of detectable peaks representing aroma volatiles can be found in fully ripe strawberries. Moreover, the total level of identified compounds and volatile esters in particular was enhanced as shown in **Table 2**. Increased concentrations of aroma-impact volatiles (Ulrich *et al*. 1997) such as methyl and ethyl butanoate (compound no. 2 and 3) and methyl and ethyl hexanoate (compounds no. 7 and 9) are responsible for adding strong fruity notes to ripe strawberries. Additionally, also levels of the significant aroma compound mesifurane with its fruity-caramel-like character

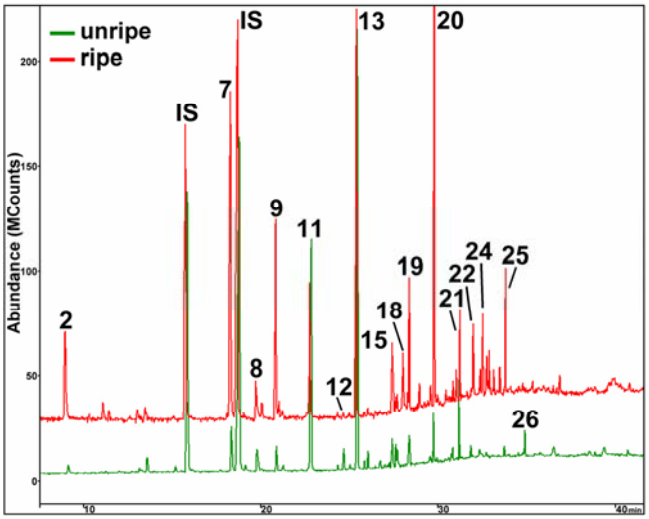

Fig. 4 Overlayed GC/MS chromatograms showing aroma volatile profiles of unripe and ripe strawberries (var. 'Bounty') extracted by HS-SPME. Peak numbers refer to compounds listed in **Table 2**. IS = internal standards added to the samples. *Source*: Holt 1996 (project report «Strawberry Project - 98»; unpublished results).

Table 2 SPME study of aroma volatile composition of unripe and fully ripe strawberries (var. 'Bounty'). *Source*: Holt 1996 (project report «Strawberry Project - 98»; unpublished results).

No.*	AROMA VOLATILE	UNRIPE	RIPE
1	methyl acetate	4.28E+06	2.11E+06
2	methyl butanoate	7.33E+06	1.25E+08
3	ethyl butanoate	9.85E+05	1.15E+07
4	propyl butanoate	5.04E+05	3.17E+06
5	hexanal	2.22E+06	7.04E+06
6	isoamyl acetate	4.00E+06	1.21E+05
7	methyl hexanoate	7.54E+07	4.38E+07
8	(*E*)-hexenal	4.49E+07	5.35E+07
9	ethyl hexanoate	2.49E+07	3.42E+07
10	styrene	7.09E+06	3.99E+06
11	hexyl acetate	2.32E+08	1.74E+08
12	(*Z*)-3-hexenyl acetate	4.99E+06	2.46E+06
13	(*E*)-2-hexen-1-yl acetate	3.84E+08	2.63E+08
14	hexanol	9.59E+04	1.73E+06
15	2-ethylhexyl acetate	3.01E+07	6.31E+07
16	(*Z*)-2-hexen-1-ol	2.31E+07	7.69E+06
17	(*E*)-2-hexen-1-yl propanoate	1.62E+07	1.13E+07
18	methylhexyl butanoate	6.22E+05	6.95E+06
19	hexyl butanoate	1.59E+07	3.68E+07
20	(*E*)-2-hexen-1-yl butanoate	4.55E+07	4.22E+08
21	linalool	4.64E+07	7.27E+07
22	mesifurane (DMF)	3.11E+05	2.31E+07
23	hexyl hexanoate	2.31E+06	6.06E+06
24	octyl butanoate	5.21E+04	8.85E+06
25	(*E*)-2-hexenyl hexanoate	8.15E+06	6.66E+07
26	α-muurolene	2.30E+07	0
	TOTALT	1.00E+09	1.45E+09
	ESTERS	8.58E+08	1.28E+09
	ALCOHOLS	2.32E+07	9.42E+06
	ALDEHYDES	4.71E+07	6.06E+07
	TERPENES	6.95E+07	7.27E+07

* Compound numbers refer to **Fig. 4**

were increased in red fruit. These results are in accordance with several other studies (e.g. Ménager *et al*. 2004). Since favourable aroma impression, consumer preference and aroma ester production coincides with the degree of ripeness/sweetness of strawberry fruit as shown in the detailed analytical and sensory studies by Azodanlou *et al*. (2003, 2004), it is not necessary to consider the aroma trait as a decision parameter for berry quality toward harvest time point.

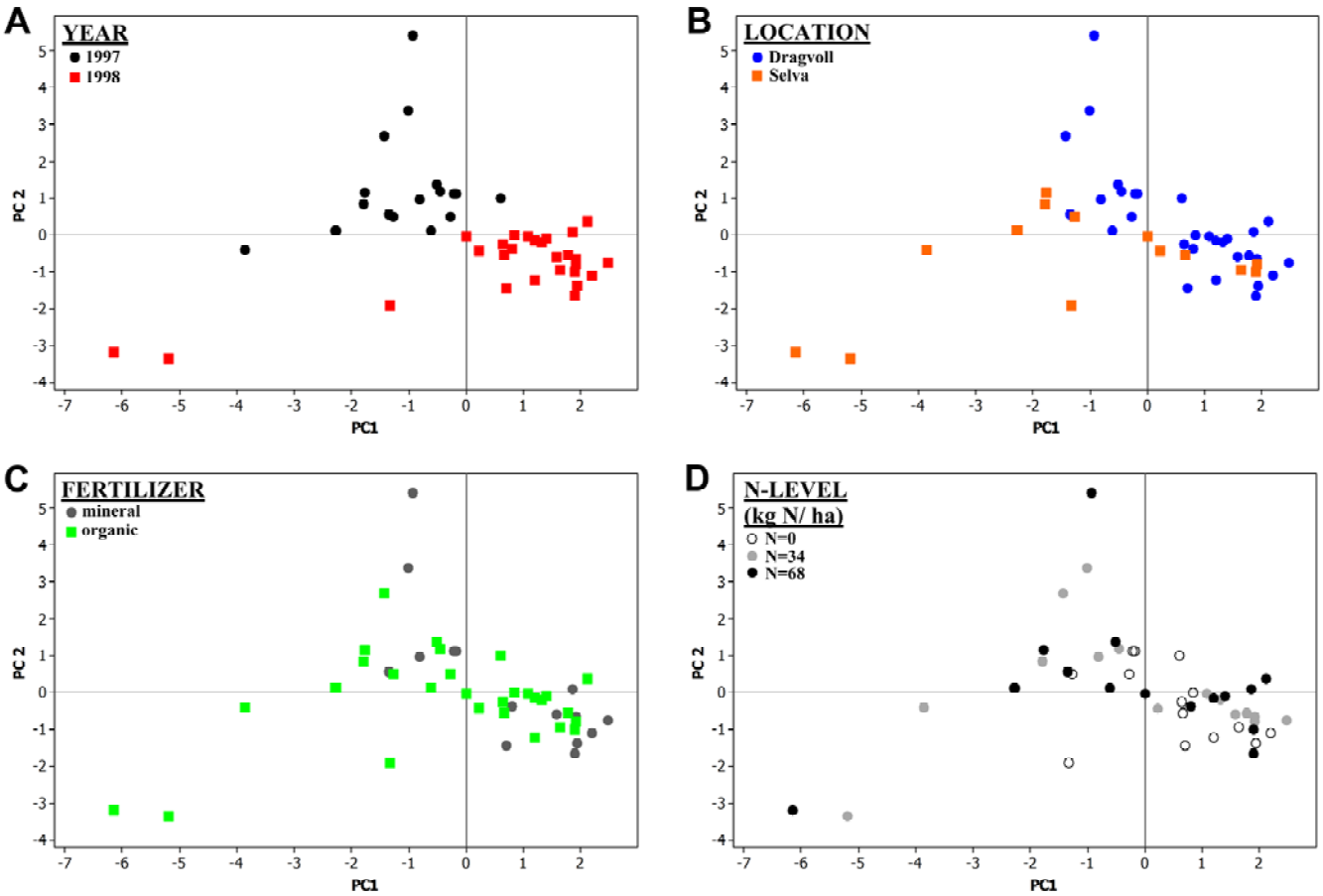

Fig. 5 Principal component analysis (PCA) of aroma profiles of strawberry variety 'Korona' with regard to the following factors: (**A**) seasonal variation (1997 and 1998), (**B**) location (Dragvoll or Selva), (**C**) type of fertilizer (mineral or organic), and (**D**) nitrogen level (N=0, 34, or 68 kg/ha). PC1 and PC2 explain 44.5% and 18.1% of variation, respectively. PCAs are based on the pre-selection of 10 aroma volatiles – hexyl butanoate, (*E*)-2-hexenyl butanoate, hexyl hexanoate, (*E*)-2-hexenyl hexanoate, octyl butanoate, γ-decalactone, 1-nonanol, (*Z*)-nerolidol, 1-dodecanol, and γ-dodecalactone –, which were detected in all 45 samples. *Source*: compiled with permission of Lyngved (1999).

Seasonal Factors – Since metabolism and thus biosynthetic reactions are highly dependent on the presence of necessary assimilates from photosynthesis, both primary but also secondary metabolism are strongly influenced by external factors such as temperature and light. Both nutritional compounds such as sugars (Rohloff *et al*. 2002) and aroma volatile composition might differ significantly from year to year (Hakala *et al*. 2002). In terms of light intensity, a decrease in photosynthetic active radiation (PAR) does not necessarily lead to obvious changes in aroma profiles as in the case of rain roof cultivation of strawberries (Rohloff *et al*. 2004). The parameter temperature generally shows a much higher impact on aroma variability when comparing berries harvested in different years from the same location. However, shading of plants resulted in significantly reduced levels of distinct aroma volatiles as pointed out by Watson *et al*. (2002). In an interesting study comparing the effect red and black plastic mulch on berry taste and aroma quality (Kasperbauer *et al*. 2002), the red mulch was observed to positively affect both sugar levels and aroma ester production. On the other hand, levels of terpenic compounds in berries were enhanced by plant cultivation on black mulch, which actually was also shown in the aromatic plant sweet basil (Loughrin and Kasperbauer 2001). Light quality effects might be explained as a change of FR/R levels influencing phytochrome-mediated metabolic pathways. In general, seasonal factors (sampling year 1997 and 1998) (Lyngved 1999) showed strongest effect on sample clustering compared to other parameters as depicted in **Fig. 5**, representing PCA studies of aroma profiles of strawberry variety 'Korona'. Within-seasonal variations (May and August) of aroma compounds from Californian strawberries have also been described (Pelayo-Zaldívar *et al*. 2005),

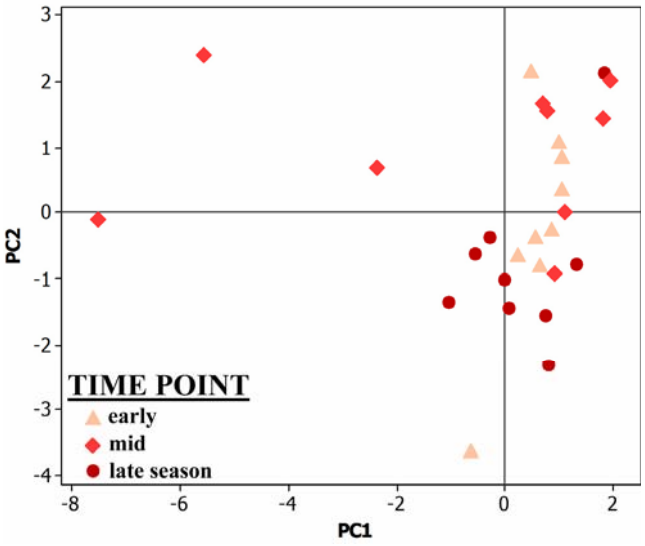

Fig. 6 Principal component analysis (PCA) of aroma profiles of strawberry variety 'Korona' with regard to harvest time point (early, mid, and late season) from locations Dragvoll and Selva in 1998. PC1 and PC2 explain 33.6% and 33.4% of variation, respectively. PCAs are based on the pre-selection of 10 aroma volatiles: (hexyl butanoate, (*E*)-2-hexenyl butanoate, hexyl hexanoate, (*E*)-2-hexenyl hexanoate, octyl butanoate, γ-decalactone, 1-nonanol, (*Z*)-nerolidol, 1-dodecanol, and γ-dodecalactone –, which were detected in all 27 samples. *Source*: compiled with permission of Lyngved (1999).

Table 3 Aroma analysis of strawberries cultivated with mineral (11-5-17) and organic fertilizer (cattle liquid manure). Average results from trial years 1997 and 1998. *Source*: reproduced with permission of Lyngved (1999).

Trial field	**Dragvoll**						**SELVA**		
Fertilization	**Mineral**			**Organic**			**Organic**		
Nitrogen (N/ ha)	0	34	68	0	34	68	0	34	68
hexyl butanoate	17.9	16.7	23.0	18.6	20.2	21.6	15.0	11.1	14.9
2-hexenyl butanoate	16.8	24.1	24.7	24.6	22.0	24.7	16.7	15.1	15.0
hexyl hexanoate	7.1	8.3	8.4	7.4	7.9	8.2	7.8	7.3	7.4
2-hexenyl hexanoate	11.4	13.6	12.2	10.4	12.1	11.7	8.8	8.2	8.2
octyl butanoate	1.4	0.8	0.8	0.9	0.8	0.8	0.9	0.7	0.7
γ-decalactone	17.8	16.5	12.6	16.1	14.8	14.8	12.2	12.6	11.3
γ -dodelactone	3.7	3.4	2.3	2.6	2.8	2.8	2.3	2.5	2.0
nerolidol	1.4	1.0	3.7	0.9	1.0	0.7	2.0	1.4	1.5
Sum Esters %	54.7	63.6	69.1	61.9	63.1	67.0	49.2	42.4	46.1

although no general trend could be observed for the three studied varieties. PCA-based studies of within-seasonal variation over a harvesting period of 4 weeks (July and August) (Lyngved 1999) revealed, at least for mid-seasonal and late picking time point, a relatively strong grouping of samples (**Fig. 6**). Effects of decreasing berry size and physiological processes (translocation) might be considered, but the obvious overall-impact of temperature and other environmental factors on aroma volatile levels and composition as discussed above, play a major role.

Geographic Factors – Microclimate (temperature, light, precipitation), soil factors (edaphic, humic levels, field capacity) and location (exposition, inclination) strongly contribute to the variability in quality of strawberry samples from different growing locations – also in terms of aroma compounds. Based on the total of 10 selected metabolites, PCA of strawberry aroma volatiles (var. 'Korona') cultivated in Mid-Norway was carried out (**Fig. 5B**) showing a clear distinction between the 2 chosen locations (Lyngved 1999). Confirming results were also obtained in other investigations (Rohloff *et al*. 2004) and by other research groups (e.g. Hakala *et al*. 2002). In general, the degree of variability in aroma composition and levels is genotypically determined and can be described as the metabolic plasticity which is affected by seasonal, geographic and environmental parameters. However, the occurrence of distinct aroma-impact compounds as discussed earlier form the basis to distinguish between characteristic strawberry aromatypes and varieties.

Fertilization, growth regulation and cultivation systems

Already in the 1930s, a positive effect of mineral fertilizer (super phosphate plus nitrogen) on strawberry aroma was observed (Darrow 1931). Aroma compound biosynthesis is believed to be dependent on the presence of assimilates and thus precursors, and is directly linked to quality traits in berries such as soluble solids content and titratable acidity. Sarooshi and Creswell (1994) demonstrated that aroma was improved at lower levels of electrical conductivity of the applied hydroponic solution. Moreover, adjusting the potassium to nitrogen ratio (K:N) from 1.7:1 to 1.4:1 had also a positive effect on strawberry aroma without decreasing berry yield. In recent studies, the direct and partly enhancing effect of N-fertilization on the formation of selected esters was shown (Ojeda-Real *et al*. 2009); however, the aroma volatile analysis was not comprehensive enough to make clear conclusions about allover effects of mineral nutrition. Another study investigated the aspect of increased salinity (NaCl) in the soil substrate. Higher NaCl-levels generally led to lower sugar contents and simultaneously, enhanced levels of both citric acid and the volatile acetic acid and thus, limited sensory acceptance (Keutgen and Pawelzik 2007). Based on own investigations by Lyngved (1999) carried out in 1997 and 1998 (**Table 3**), a clear increase of volatile esters could be observed at higher N-nutrient levels, at least in two variants of the study. However, these effects might be overlaid by seasonal and geographic factors as can be seen from **Fig. 5D**.

Potential quality differences of agricultural products derived from conventional (mineral fertilization) or organic (organic fertilizers) farming have been a major issue of scientific investigations and debate throughout the last decades. In terms of aroma composition and sensory properties, strawberry quality might be affected based on the chosen cultivation system – provided that environmental and agricultural conditions in such comparative studies were carefully chosen and controlled. Using organoleptic parameters, conventional and organically grown strawberries (var. 'Chandler') were assessed throughout the harvest season (Cayuela *et al*. 1997), resulting in higher scores for organically-produced berries in terms of the observed odour (aroma). Also in more recent studies, applying sensory analyses on organically and conventionally grown strawberries and purées, differences were revealed based on sensory profiles and PCA analyses (Kovačević *et al*. 2008); however, no chemical analyses were carried out regarding levels of responsible aroma volatiles. Reganold and co-workers (2010) assessed different parameters of strawberry quality (mineral composition, nutritional quality, phenols) in combination with sensory properties and found only one organically grown variety, 'Diamante', to be superior above conventional samples, while in 2 other varieties ('Lanai', 'San Juan') no difference could be observed with regard to fertilizer type. In a comprehensive study by Hakala *et al*. (2002), both cultivar, geographical, and cultivation systems (organic vs. conventional) were investigated based on the assessment of aroma volatile profiles of strawberries. Although the total area of detected volatile peaks was higher in almost all organically-grown samples, the effect was overlaid by varietal factors. Also results from our lab from a 2-years study (Lyngved 1999), comparing mineral fertilizer and cattle liquid manure did not yield conclusive results regarding the effect on aroma volatile composition of strawberries (**Table 3, Fig. 5C**). In general, the variety of studies on cultivation systems carried out, emphasize that at least several simultaneous analytical approaches should be carried out to assess both chemical and sensory properties of strawberry aroma.

The production of agricultural crops and strawberries in particular in marginal regions often requires the utilization of modified and/or adapted cultivation systems. The use of high tunnels might increase the temperature in the plants' environment, and simultaneously keep plants dry and reduce the spreading of air-/waterborne diseases such as *Botrytis cinerea*. In many industrialized countries, the growing season has been extended through greenhouse cultivation of certain crops and thus, enables agricultural production also in cold seasons. In both cases, plastic cover or glass alters light intensity and quality as observed when cultivating strawberries under rain roofs made of plastic canvas (Rohloff *et al*. 2004). Thus reduced light might directly lead to metabolic changes in ripening fruit. Unpublished results (**Fig. 7**) from the same study however show that the marketable flavour of strawberries in terms of aroma quality

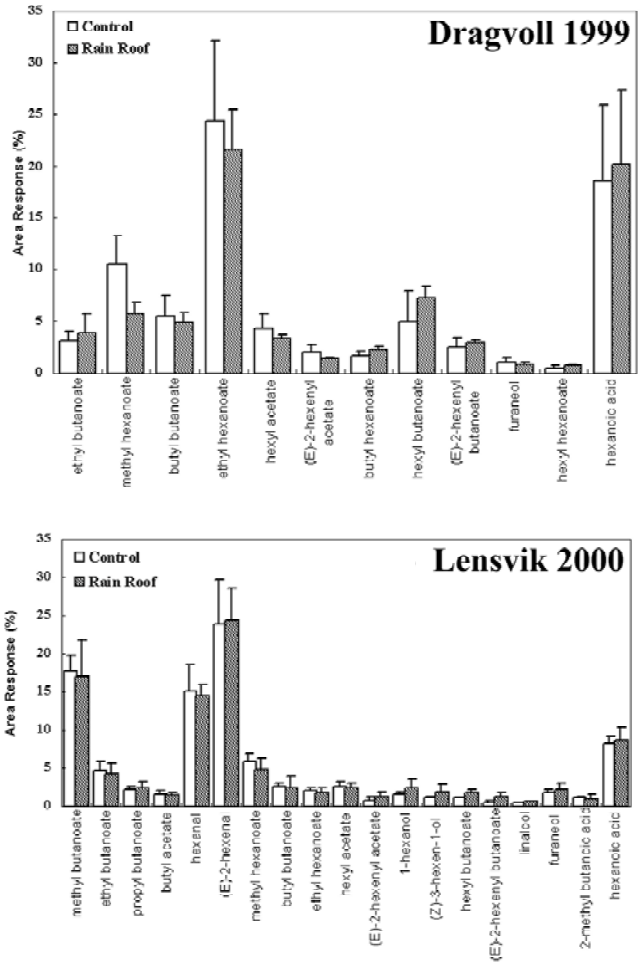

Fig. 7 Effect of rain roof cultivation on aroma volatile composition of 'Korona' strawberries at the locations Plant Biocentre/Dragvoll in 1999, and Lensvik in 2000. *Source*: Rohloff 1999 and 2000 (unpublished results).

seemed to be only slightly affected, since little variation in aroma volatile composition between control and covered strawberry plants could be observed. In the case of greenhouse vs. field cultivation of strawberries (**Table 4**) (Holt 1999), marked compositional differences could be detected as exemplified by clearly lower levels of terpenes (linalool and (*Z*)-nerolidol). A specific aspect of strawberry plant growth and metabolism was addressed by Wang and Bunce (2004) who studied the effect of enhanced CO_2-levels on both berry morphology, taste parameters and aroma volatile composition. They showed that increased CO_2 concentrations in the air drastically increased total and single aroma volatiles, indeed based on a dry weight basis in contrast to most other studies (e.g. Kafkas *et al*. 2005). However, when calculating the water content of fresh fruit, only slight enhanced levels could be theoretically estimated for several of the detected aroma-impact compounds.

Another aspect of agricultural production practice is the purposeful application of so-called growth regulators in order to modify physiological processes and defence responses in plants. Methyl jasmonate (MeJA) and jasmonic acid (JA) are considered as plant-hormonal active compounds, which derive from the 13-LOX pathway being involved in plant stress responses as signalling compounds. Numerous studies with MeJA and/or JA have been carried out in order to investigate plant defence mechanisms through application of an "artificial" trigger, but also to study the chemicals' potential to modify and increase levels of desirable secondary metabolites in crop plants. MeJA has been detected in developing strawberry fruit (Gansser *et al*. 1997) and thus established the basis to investigate effects of externally-applied MeJA on growth, nutritional value and

Table 4 Aroma profiles (area %) of 'Bounty' strawberries cultivated in an open field (**F**) and under greenhouse conditions (**G**). *Source*: reproduced with permission of Holt (1999).

Bounty	F	G	Aroma description
ethyl acetate		1.9	ethereal fruity
methyl butanoate	3.1	0.1	ethereal fruity
ethyl butanoate	1.1	11.2	fruity sweet, apple
ethyl 2-methyl butanoate		1.1	fruity apple, strawberry
(*E*)-2-hexenal	2.5	1.5	green fruity
methyl hexanoate	7.1	1.9	fruity pineapple
ethyl hexanoate	9.6	66.0	fruity pineapple
hexyl acetate	11.9	3.9	fruity green
(*E*)-2-hexenyl acetate	31.3	2.8	sweet green, apple
linalool	3.9	1.1	floral citrus
benzyl acetate	0.2	0.4	sweet floral fruity
hexyl butanoate	3.6	0.4	green waxy fruity
(*E*)-2-hexenyl butanoate	9.4	0.3	green fruity apple
ethyl octanoate	0.5	3.7	fruity waxy
hexyl hexanoate	0.9	0.7	green herbal, fruity
octyl butanoate	3.4		fruity, green waxy
(*Z*)-nerolidol	6.2	0.7	mild floral

levels of health-beneficial antioxidants and aroma compounds under field and post-harvest conditions (for the latter, see section "Post-harvest quality". Levels of several aroma volatiles were increased after MeJA treatment, among others furaneol (Moreno *et al*. 2010a). MeJA treatments might be used to receive a more uniform berry aroma quality in the post-harvest-period (Moreno *et al*. 2010c), possibly already through pre-harvest application. The utilization of *betaines* in agricultural production has been intensively studied in recent decades and led to commercial products for growth stimulation purposes, e.g. added as root application (Rohloff *et al*. 2002). The most prominent betaine in plants is glycine betaine (GB), but also other structures, derived from amino acids, have been found. Betaines function as osmolytes and protect plants against osmotic stress under unfavourable temperature, salinity and drought conditions. Large-scale studies with GB and valeric acid betaine (VAB) were aimed at investigating plant growth effects, yield, berry quality and aroma in strawberries harvested at different locations in Mid-Norway (**Table 5**) (Folkestad 2006). Treatments with GC/VAB and VAB alone obviously changed aroma volatile compositions and increased esters formation, e.g. levels of aroma-impact compounds such as methyl and ethyl butanoate/ hexanoate. The fact that GB and VAB were root-applied at µM concentrations through drip irrigation during the growing season, underscores the potential of growth regulators in strawberry production toward modification of berry quality.

Post-harvest quality

Strawberry quality in terms of firmness, taste, aroma and microbial contamination is affected during the post-harvest period, i.e. from the field via intermediate storage, packing, transport and retailing chain. Due to the berries soft fruit character and thus perishability since marketed as fresh fruit, a high loss of strawberries might occur. Shelf-life is strongly influenced by inner factors such as respiration and transpiration, physiological breakdown and compositional changes in the "living" berry fruit. Handling and physical damage, and moreover, storage conditions (temperature, humidity, atmosphere conditions) and microbial contamination might further impact on shelf-life and cause deterioration.

Cooling of strawberries right after picking is a major issue in order to slow down respiration, retard microbial growth and thus, preserve the nutritional and sensory quality of strawberries. Pursuant to recommendations (e.g. Mitcham *et al*. 2009), strawberries should be kept at low temperatures (0-4°C) under high relative humidity (> 90%) and preferably increased CO_2-levels (10-15%) based on

Table 5 Aroma volatile patterns (% value) of strawberries treated with the potential plant growth regulators glycine betaine (GB) and valeric acid betaine (VAB) harvested at locations Lensvik, Tornes and Dragvoll in 2000. *Source*: compiled with permission of Folkestad (2006).

LENSVIK - var. KORONA						
Treatment:	**Control**	**stdev**	**GB/VAB**	**stdev**	**VAB**	**stdev**
methyl butanoate	17.82	2.00	20.39	3.81	20.19	4.54
ethyl butanoate	4.68	1.11	4.82	1.49	5.56	2.13
propyl butanoate	2.30	0.36	2.33	0.20	2.55	0.75
butyl acetate	1.67	0.39	1.07	0.22	1.10	0.43
methyl hexanoate	5.74	1.21	5.73	1.68	5.12	1.01
butyl butanoate	2.55	0.57	1.95	0.35	2.15	1.47
ethyl hexanoate	2.03	0.46	2.89	1.71	2.76	1.07
hexyl acetate	2.62	0.69	1.62	0.53	1.78	0.73
(*E*)-2-hexenyl acetate	0.86	0.38	0.66	0.18	0.70	0.17
(*E*)-2-hexenyl butanoate	0.54	0.25	0.44	0.06	0.81	0.21
linalool	0.46	0.06	0.50	0.10	0.62	0.21
furaneol	1.84	0.40	2.00	0.51	1.74	0.63
2-methyl butanoic acid	1.07	0.19	1.07	0.17	0.85	0.10
hexanoic acid	8.21	0.93	7.35	1.90	7.20	0.45
SUM Esters	40.81		41.90		42.72	
TORNES - var. BOUNTY						
methyl butanoate	2.93	0.33	2.66	0.72	2.87	0.61
ethyl butanoate	4.85	1.88	7.90	4.84	7.70	5.14
methyl hexanoate	8.53	0.74	8.79	3.00	8.81	1.83
ethyl hexanoate	22.78	7.43	28.34	15.26	28.51	15.97
hexyl acetate	1.58	0.74	2.06	1.32	1.71	1.01
(*E*)-2-hexenyl acetate	2.08	1.30	1.36	0.58	1.55	0.54
(*E*)-2-hexenyl butanoate	1.19	0.46	0.85	0.27	1.14	0.15
linalool	30.90	2.71	21.77	4.46	24.87	12.16
furaneol	0.62	0.16	0.70	0.18	1.11	0.52
2-methyl butanoic acid	0.99	0.37	0.87	0.13	1.36	0.57
hexanoic acid	12.43	3.55	9.41	2.96	12.74	5.02
SUM Esters	43.94		51.96		52.29	
DRAGVOLL - var. KORONA						
methyl butanoate	9.01	1.49	8.15	1.68	8.80	1.87
ethyl butanoate	3.44	1.15	6.18	2.89	7.23	2.55
propyl butanoate	1.20	0.15	0.89	0.00	1.20	0.00
butyl acetate	1.25	0.14	1.08	0.32	1.21	0.23
methyl hexanoate	3.69	0.64	3.68	1.31	3.61	0.68
butyl butanoate	2.59	0.77	2.88	0.66	2.54	0.72
ethyl hexanoate	2.09	0.60	3.91	2.68	5.41	2.89
hexyl acetate	4.12	0.62	4.27	1.24	3.99	0.87
(*E*)-2-hexenyl acetate	2.02	2.50	2.02	0.66	1.43	0.86
hexyl butanoate	2.66	0.57	2.58	1.18	3.17	1.75
(*E*)-2-hexenyl butanoate	2.34	0.37	1.36	0.72	1.76	0.54
linalool	0.65	0.12	0.54	0.15	0.62	0.12
furaneol	1.06	0.19	1.07	0.16	0.85	0.29
2-methyl butanoic acid	0.87	0.08	0.83	0.18	0.73	0.14
hexanoic acid	14.83	2.15	12.79	2.32	10.57	3.23
SUM Esters	34.41		37.00		40.35	

controlled atmosphere (CA) or modified atmosphere (MA) conditions. Metabolic activity in berries does not stop even under cold storage conditions, as indicated in **Fig. 8A** and **Fig. 8B**. Refrigeration at higher temperatures (4°C) obviously lead to stronger metabolic changes compared to 0°C storage over a 9-10 days period, which is in accordance with findings by Ayala-Zavala and co-workers (2004), who compared post-harvest storage at 0, 5 and 10°C. In general, the acceptable post-harvest sensory quality might end after 8-10 days as pointed out by Koyuncu (2004). Levels of aroma-impact furanones (furaneol, mesifurane) can be used as indicators of berry ripeness, changing significantly during the post-harvest period dependent on the storage temperature (Pérez *et al*. 1996). In terms of ripeness, metabolic activity and volatile production was generally higher in red compared to pink strawberries (Miszczak *et al*. 1995), thus underscoring the importance of harvest time point for post-harvest aroma quality.

Aroma-related aspects of berry quality in the post-harvest period include the induction of so-called *off-flavour* (or off-odour), i.e., the development of undesirable volatile compounds which negatively affect sensory perception. Storage under anaerobe conditions might enhance levels of ethanol, acetaldehyde, and ethyl acetate (Boschetti *et al*. 1999), which are responsible for off-flavour effects (Ke *et al*. 1991; Whitaker 2008). Fungal or bacterial contamination during pre-harvest or picking period might result in extended microbial growth and decay, if berries are not appropriately stored and handled. Fungal growth might also lead to the production of off-flavour phenolic compounds as in the case of *Phytophthora cactorum* infection (Jeleń *et al*. 2005), C_6-fermentative products by yeast (Ragaert *et al*. 2006), and 1-decanol and indole produced by coliform bacteria (Yu *et al*. 2003).

Another aspect is the occurrence of natural aroma volatiles from strawberries showing *biological activity* against phytopathogenic microorganisms. C_6-esters and -aldehydes (Hamilton-Kemp *et al*. 1996), and aromatics (Ntirampemba *et al*. 1998) are naturally produced by strawberry fruit. The strong inhibition of post-harvest decay fungi (*Alternaria alternata*, *Botrytis cinerea*, and *Colletotrichum gloeosporioides*) by aliphatic (1-hexanol, (*E*)-2-hexenal, 2-nonanone) and aromatic volatiles (benzaldehyde) had already been reported earlier (Vaughn *et al*. 1993). Enhanced levels of 2-nonanal might also be artificially applied to prolong the shelf-life of woodland strawberry fruit (Almenar *et al*.

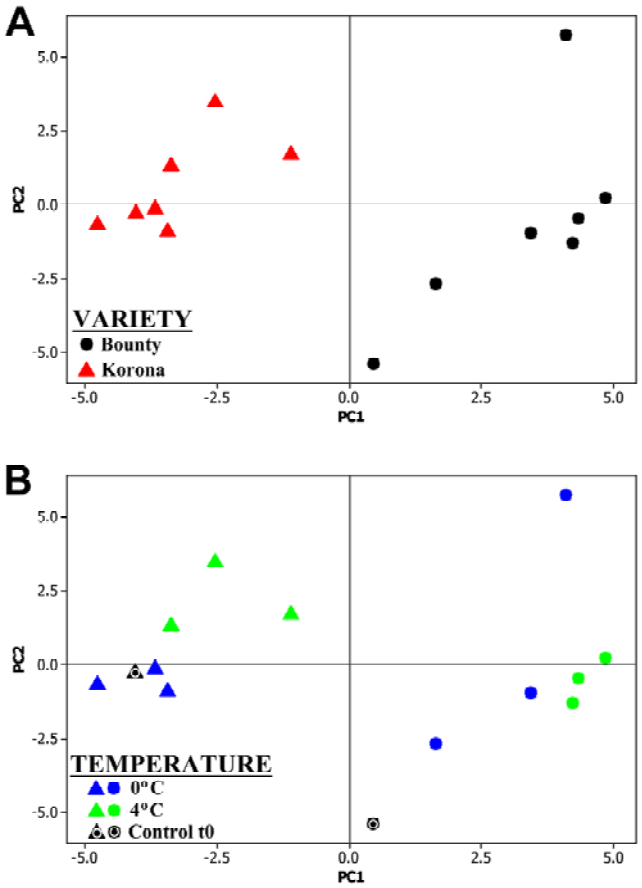

Fig. 8 Principal component analysis (PCA) of aroma profiles of ripe strawberries (var. 'Korona' Δ, and 'Bounty'○) stored at 0° and 4°C for a period of 9 to 10 days. The principal components PC1 and PC2 were computed based on the total of 31 aroma volatiles, which were detected in at least 50% of all samples. PC1 and PC2 explain 49.6% and 18.8% of variation, respectively. (**A**) PCA based on variety; (**B**) PCA based on treatment (temperature). *Source*: Holt and Rohloff 1996 (unpublished results).

2009). C_6-aldehydes are induced in strawberry fruit upon wounding (Myung *et al*. 2006). Their mode of action on *Botrytis cinerea* proteins and thus retarded fungal growth has been shown in recent studies (Myung *et al*. 2007). Also other volatiles such as terpenes (linalool, nerolidol) and aromatic compounds (eugenol, vanillin) potentially contribute to the natural antimicrobial activity of strawberry fruit, and underscore the significance of genotypically-determined aroma volatile patterns in innate defence responses.

CA and MA storage are frequently applied to extend the shelf-life of perishable fruits and vegetables. CA conditions imply that the gas composition of the atmosphere is kept constant and agricultural products are stored in closed environments. Normally, elevated CO_2-levels are utilized, which in turn might lead to off-flavour production as reported for cultivated strawberries (Pelayo *et al*. 2003) and woodland strawberries (Almenar *et al*. 2006) due to anaerobic conditions. Moreover, high-O_2 and simultaneously high-CO_2 conditions were shown to be most effective in suppressing fungal growth, but unfortunately also here led to enhanced off-flavour production (Pérez and Sanz 2001). If applicable, strawberries should be subjected to fast cooling upon harvest; in combination with short-term (2 h) treatment with 100% CO_2, shelf-life might be successfully improved without inducing fermentative and undesirable metabolites (Hwang *et al*. 1999). MA storage in closed or semi-closed containers or under packaging film has been shown to improve shelf-life due to naturally enhanced CO_2-levels upon respiration in strawberry fruit (Guichard *et al*. 1992). Although the nutritional value and aroma volatiles might be better preserved compared to storage under air, off-flavours will be induced also when using perforated packages (Sanz *et al*. 1999) as already pointed out for CA storage. The negative effect of off-flavour induction in CA storage after high CO_2 exposure might be reversible after 3 days through re-acclimation of berries at 20°C; exceeding storage time by one week might lead to persistent sensory deterioration (Larsen and Watkins 1995).

Several other post-harvest treatments have been investigated in order to find new ways of preserving strawberry fruit quality and shelf-life. Ozone application for several days was shown to be effective against *Botrytis* fungal growth. Although off-flavour production occurred (Pérez *et al*. 1999; Kappert *et al*. 2006), aroma volatile production might be reversed when keeping the strawberries at room temperature in air after ozone treatment (Nadas *et al*. 2003). This effect generally also applies for CA and MA storage. More recently, Allende *et al*. (2007) used a combined approach with UV-C, ozone, O_2, and/or CO_2 together with MA storage conditions. Sensory properties were generally lower compared to the untreated controls probably due to induction of off-flavour. Also in the case of ultrasound treatment (40 kHz), the shelf-life of strawberries could be prolonged due to delayed microbial growth (Cao *et al*. 2010); however, the effect on aroma volatile composition has yet to be investigated. Irradiation including γ-rays is frequently used for the preservation of food stuffs, and does not necessarily change strawberry aroma profiles. However, in the case of electron beam irradiation, an enhancing effect on off-flavour production after 6 and 8 days of storage could be observed (Yu *et al*. 1995).

The utilization of spraying reagents, coatings and dipping solutions represent yet another way of post-harvest strawberry treatment. The application of MeJA might induce defence pathways in plants and has been shown to positively affect flavonol and antioxidant levels, aroma volatiles and prolong shelf-life (Ayala-Zavala *et al*. 2005; Moreno *et al*. 2010b, 2010c). Phenylethyl alcohol treatment also improved post-harvest storage of strawberries up to 15 days due to suppressed fungal growth. Although volatile patterns did not seem to be significantly affected, no quantitative data about compound levels and thus, possible off-flavour effects were provided (Mo and Sung 2007). The use of a coating with the linear polysaccharide chitosan obviously suppressed off-flavour induction and simultaneously enhanced aroma production of certain volatile compounds (Almenar *et al*. 2009b), but did not necessarily improve the sensory properties of strawberry fruit as pointed out by Vargas *et al*. (2006). Potential H_2O-dipping heat treatment of strawberry fruit up to 46°C delayed *Botrytis* growth and did not enhance off-flavour levels (Garcia *et al*. 1996); however, this method might be too costly and less applicable compared to other treatments.

Freezing and processing

The long-term storage of fresh strawberries can be attained through different processes and handling including freezing, drying, and canning. Fruit nutritional and sensory qualities in particular might be strongly affected. The levels of aroma-impact volatiles such as furaneol and mesifurane did not change upon strawberry freezing, while concentrations of esters generally decreased (Ueda and Iwata 1982; Doulliard and Guichard 1990). On the other hand, levels of off-flavour compounds were shown to decrease again when storing berries for more than one week. Earlier studies by Schreier (1980) using 3 varieties also concluded that important aroma volatiles were strongly decreased, while furanone concentrations were enhanced. Freezing approaches using normal and immersion chilling freezing were shown to preserve aroma qualities of strawberries, while more advanced osmodehydration clearly led to the formation of off-flavour metabolites (Blanda *et al*. 2009). Own studies on aroma volatiles of fresh and frozen 'Korona' strawberries showed compositional changes (**Table 6**) and the quantitative depletion (data not shown) of several aroma

Table 6 Aroma composition (area %) of various strawberry products: **A** – Fresh berries, **B** – Frozen at -20°C for 30 days, **C** – Fluid-bed freeze-dried at -10°C, **D** – Strawberry jam, **E** – Sweets (strawberry-flavoured liquorrice). Samples **A**-**D** are based on the variety 'Korona'. *Source*: Rohloff 2006 (unpublished results).

	A	B	C	D	E
ethanol					8.75
methyl butanoate	1.05	4.86		6.39	
ethyl butanoate	1.55	12.09		13.67	9.34
butyl acetate	0.11	3.13	14.71		
hexanal	0.21				
(*E*)-2-hexenal	1.69				
2-heptanoate				0.66	
methyl hexanoate		5.45		2.36	
butyl butanoate	1.19	8.82		0.25	
ethyl hexanoate	3.03	7.82		15.36	11.13
hexyl acetate	3.51	1.12		0.98	
(*E*)-2-hexenyl acetate	3.67				
hexyl propanoate	0.47				
nonanal			2.29		
(*Z*)-3-hexen-1-ol					10.34
(*E*)-2-hexenyl propanoate	0.33				
isoamyl isobutanoate				1.53	2.55
hexyl butanoate	17.46	13.5	0.20	0.62	
ethyl octanoate			0.08	0.30	
(*Z*)-3-hexenyl butanoate	0.28				
(*E*)-3-hexenyl butanoate	0.21				
(*Z*)-2-hexenyl butanoate	32.62	8.15			
linalool				1.36	
decanal			14.55		
furaneol	0.26	2.02			
hexyl hexanoate	3.62		0.77	0.12	
octyl butanoate	0.92				
benzyl acetate					2.40
(*E*)-2-hexenyl hexanoate	2.58				
pentadecane		2.07	6.54	1.05	1.17
(*E*)-anethole					1.33
octadecane	0.22	3.01	25.46	2.03	2.82
hexanoic acid	0.88	1.86	7.94	1.54	
benzyl alcohol					7.38
nonadecane	0.38	1.68	12.14	0.89	3.00
maltol					0.88
(*E*)-nerolidol	0.55			1.51	
octanoic acid	0.07	0.32	0.61	0.37	
methyl cinnamate					5.70
γ-decalactone	4.15	3.50	4.39		
γ-dodecalactone	0.78	0.33			
sorbic acid				34.97	

compounds, while levels of furaneol were increased. Larsen and Poll (1995) could show that not freezing, but the process of thawing was responsible for the increase of certain aroma volatiles.

In order to test the reliability of aroma profiling by GC/MS method, both fresh and frozen fruits (-20°C, -80°C and liquid N_2) were investigated after 1 day and 1 week using either natural or water bath-forced thawing (Modise 2008). While 1-day storage minimally affected aroma volatiles in all treatments, freeze storage for 7 days generally enhanced levels of off-flavour volatiles. However, natural and forced thawing of -20°C samples resulted in lower concentrations of acetaldehyde, (*Z*)-3-hexenal and hexanal. Off-flavour production of hydrogen sulfide (H_2S) had already been reported earlier by Deng *et al*. (1996); also this study showed the positive effect of storage at -20°C compared to deep freezing at -40 and -80°C. The same group (Deng and Ueda 1993) had earlier shown that deep freezing (-40 and -80°C) and long-term storage for 6 months had a better preservation effect on aroma esters compared to freezing at -20°C.

High-pressure treatment of food is frequently applied to preserve food quality while simultaneously suppressing microbial growth. In contrast to thermal treatment and ultra-high pressure (800 Mpa), low and medium-pressure treatment (200 and 500 MPa) excellently preserved important aroma volatiles in strawberry coulis (Lambert *et al*. 1999). Heat treatment under jam processing changes the aroma volatile composition of existing compounds (**Table 6**) regarding levels of the important volatiles ethyl butanoate and ethyl hexanoate. For comparison reasons, SPME analyses of strawberry-flavoured liquorice were included in **Table 6** in order to present artificial aroma mixtures with a typical "strawberry-like flavour" being added to food. Thermal treatment might further lead to the formation of new volatile structures e.g. due to Maillard reactions (Barron and Etiévant 1990). Compositional changes in a strawberry drink were assessed by chemical and sensory analyses and showed that both increased (dimethyl sulfide, 2-ethylhexanoic acid) and decreased aroma volatile levels (ethyl butanoate, linalool) were responsible for poorer sensory impression (Siegmund *et al*. 2001). Also α-terpineol has been reported as potential off-flavour metabolite in strawberry juice, while the abundance of furaneol and mesifurane strongly contributed to "fresh strawberry" sensory attributes (Golaszewski *et al*. 1998).

In many food preservation processes, quality of the final product is maintained based on the addition of sugars. It has been shown that the addition of trehalose is advantageous over the use of sucrose or maltodextrin, and better preserves aroma properties of freeze-dried strawberry purée (Komes *et al*. 2003; Galmarini *et al*. 2009). Sometimes the product consistency is playing a role for aroma release and consumer perception. Adding an artificially-produced mixture of strawberry aroma to fat-free yogurt resulted in decreased aroma volatile release when yogurt samples changed from watery to a higher viscous phase after several days of refrigerated storage (Lubbers *et al*. 2004). Another important aspect of food storage is the sorption of aroma compounds in packaging material. It was shown that polyvinyl chloride (PVC) had a higher affinity towards aroma volatiles from strawberry syrup compared to polyethylene-based flasks (Ducruet *et al*. 2007); however, the total amount of sorbed compounds was quite low (< 0.1%).

STRAWBERRY FLAVOUR AND AROMA - FUTURE IMPLICATIONS AND PERSPECTIVES

Considering flavour as the combination of taste and olfaction based on the perception of the food's chemicals from primary and secondary metabolism, thus also including aroma volatiles, it becomes obvious that strawberry aroma *per se* is a quite complex trait. As earlier discussed, the intensity of aroma perception is strongly related to taste factors and can not be considered as independent of other important fruit traits. Agricultural and environmental factors strongly impact on strawberry aroma, but pre-harvest and post-harvest parameters in particular are to some extent controllable in order to influence biologically-determined aroma volatile production and breakdown in ripening and fully mature fruits. Available technology include the purposeful application of pre-harvest triggers for unified fruit ripening and increased homogeneity, adjustment of refrigerated storage conditions (CA, MA) and the utilization of protecting and metabolism-modifying coatings. Important biosynthetic pathways involved in strawberry aroma metabolism have first been uncovered in recent years. Either flavourful and soft fruits, or strawberries of superior firmness with long shelf-life and little aroma – the crux of plant carbon and energy balance in primary and secondary metabolism during berry ripening and thus, downstream gene expression might be overcome through a better understanding of regulatory metabolic networks. Traits to come include, beside improved health beneficial phytochemicals and antioxidants, strawberry fruit characteristics demanded by the food and processing industry related to e.g. colour but also DHF and DMF content, overall aroma and tastiness.

In the context of *Fragaria* breeding for new varietal plant material, the trait "aroma" and its significance in inhe-

ritance, has received much attention both in the cultivated strawberry (Olbricht *et al.* 2008, 2011) but also other closely-related fruit crops within the Rosaceae family such as Virginia strawberry (Carrasco *et al*. 2005), blackberry (Du *et al*. 2010c), and apple (Dunemann *et al*. 2009; Rowan *et al.* 2009). The necessity to address the combination of nutritional traits for strawberry flavour breeding, has been pointed out by Olbricht and co-workers (2008). Comprehensive approaches by integrating the strawberry's metabolome with other important traits (texture, firmness, shape, berry size, etc.) are likely to be carried out in future breeding programmes. QTL mapping and marker-assisted breeding (MAB) will be the necessary tools as demonstrated by USDA-ARS' on-going project "RosBREED: Enabling Marker-Assisted Breeding in Rosaceae". Moreover, developments will not only be based on available varietal material in the *F. x ananassa* genetic background, but potentially also employ species at lower ploidy levels such as the hexaploid *F. moschata* Duch. (Ulrich *et al*. 2007) and diploids such as *F. nilgerrensis* Schltdl. ex J.Gay (Noguchi *et al*. 2002). The most promising results, however, can be expected when utilizing the parental octoploid species *F. virginiana* (Carrasco *et al*. 2005) and *F. chiloensis* (González *et al*. 2009), in order to reconstruct today's cultivated strawberry through interspecific hybridization (Hancock *et al*. 2010; Stegmeir *et al*. 2010). Such efforts will certainly lead to the development of octoploid strawberry varieties with improved berry characters regarding yield, nutritional quality and flavour.

Furthermore, gene modification has high potential for the successful development of novel plant material with altered nutritional traits and health-beneficial properties. Most functional genomics studies in *Fragaria* in recent years have focused on specific traits related to fruit ripening, firmness, polyphenols, pathogen resistance, allergens, vitamins, sugars and acids, also included flavour aspects. Thus, the basis for the potential production of GM strawberries has been established using modification strategies as currently applied in other crops. However, consumer acceptance toward GM food is rather reluctant, at least in the European countries. Although new technologies for the quality assessment and assurance of food have been introduced to shed light on the substantial equivalence of conventional vs. GM food, uncertainties about product quality and outcrossing from GM strawberries to wild *Fragaria* species have a strong impact on consumer attitudes, and it seems rather unlikely that GM strawberries will hit the markets in the next decades – not least due to promising MAB strategies in molecular breeding, and the utilization of hitherto unexploited *Fragaria* germplasm.

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