Organic or Mineral Fertilization

Effects on Tomato Plant Growth and Fruit Quality

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Abstract

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To meet Swedish environmental goals organic farming will play an important role in the development of a sustainable and high quality food production system. However, several conflicts exist, such as the achievement of high yields without the use of chemical fertilizers or pesticides and the minimization of nutrient losses.

In this thesis the effect of organic fertilizers compared to mineral fertilizers on plant product quality was investigated. Tomato yield and quality aspects such as taste and chemical composition were measured. Three greenhouse experiments were performed at the Swedish University of Agricultural Sciences in Uppsala during 2002 and 2004. Organic fertilizers, based on chicken manure or fresh grass clover mulch, were compared to mineral fertilizer nutrient solutions with ammonium or nitrate as the dominant nitrogen source. In both years yields from the mineral fertilized tomato plants were higher than from the organic fertilized ones. However, plant nitrogen status was similar for all treatments and the limiting nutrient in the organic grass-mulch treatment appeared to be sulphur, as the addition of mineral sulphur increased the yield. The effects on quality appeared to be more complex. While taste test scores and vitamin C contents in 2002 were highest in the organic or ammonium-fertilized tomatoes and lowest in the nitrate-fertilized tomatoes, in 2004 the results were contrary.

Taste is a complex quality aspect, affected by sugars, acids and other secondary compounds (aromatic flavour compounds), which apart from nutrient supply are affected by environmental conditions as sunlight and temperature that are beyond the grower's control. It was concluded that organic or mineral fertilizers are not the major factors affecting yield and product quality. A balanced nutrient supply is important for yield and quality, irrespective nutrient source. A combination of organic and mineral fertilizers should be considered in order to achieve a resource saving and balanced nutrient supply and a high quality tomato yield.

Keywords: *Lycopersicon esculentum*, metabolomics, nitrogen form, organic vs conventional production, product quality, taste, secondary metabolites

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Organisk- eller mineralgödsling - Effekter på tillväxt och fruktkvalitet hos tomat

För att uppnå de svenska miljömålen spelar ekologiskt odling en viktig roll i utvecklingen av uthålliga system som producerar livsmedel av hög kvalitet. Det finns dock flera målkonflikter, såsom att nå höga skördar utan användning av kemiska gödselmedel eller pesticider, och minimering av växtnäringsförluster.

I denna avhandling undersöks hur produktkvaliteten påverkas av organisk gödsel jämfört med mineralgödsel. Tre växthusförsök genomfördes under åren 2002 och 2004 på Sveriges Lantbruksuniversitet (SLU) i Uppsala. Organiska gödselmedel såsom hönsgödsel eller färskt gräsklipp jämfördes med näringslösningar som innehöll ammonium eller nitrat som huvudsaklig kvävekälla. Under båda åren blev skörden större i de mineralgödslade behandlingarna än i de organiskt gödslade. Plantornas kvävestatus låg i stort sett på samma nivå i alla behandlingar och det begränsande näringsämnet i det organiska gräsklippsledet visade sig vara svavel, eftersom en extra svavelgiva höjde skörden. Effekterna på kvaliteten visade sig vara mer komplexa. Under 2002 var poängen i smaktesten och halterna av C-vitamin högst för de organiskt eller de ammoniumgödslade tomaterna, medan de var lägst i de nitratgödslade tomaterna. Under 2004 var dock resultaten de motsatta.

Smak är en komplex kvalitetsaspekt som påverkas av socker- och syrahalter samt aromatiska ämnen. Dessa i sin tur påverkas av gödslingen och omgivande faktorer såsom solljus och temperatur, vilka ligger utanför odlarens kontroll. Valet mellan organisk eller mineralgödsel verkar därför inte vara den avgörande faktorn för kvantitet och kvalitet. En balanserad näringsförsörjning däremot är viktig för skörderesultat och produktkvalitet, oavsett näringskälla. En kombination av organisk och mineralgödsel borde därför övervägas för att uppnå en resursbesparande och balanserad näringsförsörjning och en tomatskörd av hög kvalitet.

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s fertig machä

& m Heidi & dä Hiltrud
füs Vorbild sii

Organische Düngung oder Mineraldüngung - Einfluss auf das Wachstum von Tomatenpflanzen und die Qualität der Früchte

Um die schwedischen Umweltschutzziele zu erreichen spielt die ökologische Landwirtschaft eine wichtige Rolle bei der Entwicklung nachhaltiger Systeme und um hochwertige Nahrungsmittel zu produzieren. Dabei entstehen jedoch Zielkonflikte, wie beispielsweise das Erreichen hoher Ernten ohne den Einsatz von Mineraldünger oder Pestiziden, oder das Minimieren von Nährstoffverlusten.

In der vorliegenden Arbeit wird der Einfluss organischer Dünger im Vergleich zu Mineraldüngern auf die Qualität pflanzlicher Produkte untersucht. Der Ertrag und Qualitätsaspekte wie der Geschmack und die chemische Zusammensetzung von Tomaten wurden gemessen. Drei Gewächshausversuche wurden während der Jahre 2002 und 2004 an der schwedischen Universität für Agrarwissenschaften in Uppsala durchgeführt. Hühnermist oder frisch gemähtes Gras und Klee wurden mit Mineraldüngern verglichen, die entweder Ammonium oder Nitrat als hauptsächliche Stickstoffquelle enthielten. In beiden Jahren waren die Erträge der mineralisch gedüngten Tomatenpflanzen höher als die der organisch gedüngten. Der Stickstoffgehalt der Pflanzen war jedoch bei allen Behandlungen ähnlich. Schwefel erwies sich als begrenzender Nährstoff für die mit Gras und Klee gedüngten Tomatenpflanzen, denn die Beigabe mineralischen Schwefels erhöhte den Ertrag. Der Einfluss der verschiedenen Dünger auf die Qualität der Tomaten scheint komplizierter zu sein: Im Jahr 2002 waren die Punktzahl in den Geschmackstests und der Vitamin C Gehalt am höchsten in den organisch- oder mit Ammoniumstickstoff gedüngten Tomaten, und am tiefsten in den mit Nitratstickstoff gedüngten Tomaten. Im Jahr 2004 waren die Resultate jedoch gegenteilig.

Geschmack ist ein komplizierter Qualitätsaspekt, der durch den Zucker- und Säuregehalt, sowie von aromatischen Pflanzeninhaltsstoffen beeinflusst wird. Diese werden ihrerseits auch durch Sonnenlicht und Temperatur beeinflusst, was die Produzentin nicht kontrollieren kann. Organische Dünger oder Mineraldünger scheinen demzufolge nicht die Hauptfaktoren zu sein, die Ertrag und Qualität beeinflussen. Vielmehr scheint eine ausgewogene Versorgung der Pflanze mit allen Nährstoffen wichtig zu sein für den Ertrag und die Qualität, ungeachtet der Düngerart. Eine Kombination von organischem und Mineraldünger sollte in Erwägung gezogen werden um eine ausgewogene Versorgung mit Nährstoffen und einen qualitativ hochwertigen Ertrag zu erzielen.

Contents

Abbreviations

A = number of principle components in a PCA or PLS-DA model
$ABTS^{\bullet +}$ = 2,2´azinobis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt)
BER = blossom-end rot
BSE = bovine spongiforme enzephalopathy
C-… = carbon based, carbon containing
Cl^- = chloride
EC = electric conductivity
GC/TOFMS = gas chromatography/time-of-flight mass spectroscopy
HPLC = high-performance liquid chromatography
K = potassium
N = nitrogen
NaCl = sodium chloride
NH_3 = ammoniac
NH_4^+ = ammonium
NO_3^- = nitrate
P = phosphorus
PC1, PC2 = first, second principle component
PCA = principal component analysis
PLS-DA = partial least squares discriminant analysis
Q^2 = cumulative predicted variation
R^2X = cumulative modelled variation in X
R^2Y = cumulative modelled variation in Y
ROS = reactive oxygen species
S= sulphur
SO_4^{2-} = sulphate

Appendix

Papers I - IV

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Heeb, A., Lundegårdh, B., Ericsson, T. & Savage, G.P. 2005. Effects of nitrate-, ammonium-, and organic-nitrogen-based fertilizers on growth and yield of tomatoes. *Journal of Plant Nutrition and Soil Science* 168, 123-129.

II. Heeb, A., Lundegårdh, B., Ericsson, T. & Savage, G.P. 2005. Nitrogen form affects yield and taste of tomatoes. *Journal of the Science of Food and Agriculture* 85, 1405-1414.

III. Toor, R.K., Savage, G.P. & Heeb, A. 2005. Influence of different types of fertilisers on the major antioxidant components of tomatoes. *Journal of Food Composition and Analysis*, ***in press***.

IV. Heeb, A., Lundegårdh, B., Savage, G.P. & Ericsson, T. The nutrient balance of both organic and inorganic fertilizers affects yield, taste and nutritional quality of tomatoes, ***submitted to*** *the Journal of Plant Nutrition and Soil Science*.

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Responsibilities

In Papers I & II, Lundegårdh and Heeb set up the aim and the experiment, and Heeb was responsible for the practical work, the data collection, analysis and writing, guided by Savage and Ericsson.

In Paper III, Kaur-Toor was responsible for the chemical analyses of the tomatoes provided by Heeb, and writing was performed by Kaur-Toor, assisted by Savage and Heeb.

In Paper IV, Ericsson and Heeb were responsible for the set up of the aim and the experiment, Heeb was responsible for the practical work, the data collection and the analysis, Savage carried out the analysis of vitamin C, lycopene, pH and titratable acidity. Writing was performed by Heeb, supported by Ericsson, Savage and Lundegårdh.

Introduction

Organic or conventional production

The aim of organic farming is to develop a long term sustainable production of food by working with natural processes (e.g. the fixation of nitrogen by symbiotic bacteria in leguminous plants, which are used as manure), by reducing negative effects on the environment and by minimizing the use of non-renewable resources (LRF & Ekologiska Lantbrukarna, 1999). In Sweden the use of chemical pesticides and easily available mineral fertilizers is forbidden in organic production. The Swedish Government has set up an aim that 20% of the production area should be managed according to organic principles by year 2005 (Jordbruksverket, 1999). Organic farming as such is regarded as one of several measures that will help to reach some of the Swedish environmental goals (www.miljomal.nu), such as a varied agricultural landscape, non-toxic-environment, zero eutrophication and good quality groundwater (Jordbruksverket, 2001). Organic farming aims at e.g. improve soil fertility, enhance resource recycling, improve the production environment for farmers and enlarge the freedom of choice for consumers by supplying products of good quality at reasonable price. However, within these goals several conflicts exist, e.g. to achieve reasonably high yields at acceptable prices without the use of chemical fertilizers, or to obtain good quality products without the use of pesticides are goals that are not easily achievable. Knowledge about different effects of organic production compared to conventional production on environment and product quality is scarce or contradictory and needs more investigations within several different disciplines. For consumers, the promise for environmental friendly production is one of the important motives for buying organic products, and the consumers' demand for these products is the major precondition for a long term sound and sustainable organic production. Organic products therefore need to live up to the consumers' expectations and demands (Jordbruksverket, 2001).

The consumers' demands

During the 1990's consumers' interest in organically produced food has increased in Sweden (Jerkebring, 2003). Negative reports from intensive conventional production systems, distrust in genetically modified food and the BSE crisis in Europe were areas of major concern (Grunert, 2002). Even though consumers have a positive attitude towards organically produced foods (Koivisto Hursti & Magnusson, 2003), the most important criteria for buying a product is still good taste (Magnusson, Arvola & Koivisto Hursti, 2001). Consumers who buy organic food are characterized as health oriented (Tjärnemo & Ekelund, 2004), whereas consumers who do not buy organic food are more meal oriented and taste and price are considered more important than environmental aspects or organic production methods (Rozin, 1999; Magnusson, Arvola & Koivisto Hursti, 2001). Many consumers value the origin of local or Swedish produced higher than the production method itself (Ekelund & Tjärnemo, 2004), and ideals, price considerations, social context and knowledge have been identified as factors that influence the consumers food choice and thereby the food production systems through consumer demand (Furst *et al.*, 1996).

Swedish tomato growers compete with low price imported tomatoes from southern Europe and during 2004 the production area in Sweden decreased by 100 000 m^2 (Myrsten, 2005). More effective marketing of Swedish tomatoes is therefore needed. Consumers ask for a large variety of colours, sizes and shapes and good taste (Wildmark, 2004). Taste is known to vary between cultivars (Stevens, 1979), but how it is affected by the production method has not yet been clearly established (Adams, 1986; Grierson & Kader, 1986; Stevens & Rick, 1986). Does fertilization with organic or mineral nutrient-sources affect the taste and other quality aspects of tomatoes?

Contradictory results

Research results on the effects of organic vs conventional production on quality are contradictory. In terms of quality, some studies report better taste, higher vitamin C contents and higher levels of other quality related compounds for organically grown products (Haglund, 1997; Weibel *et al.*, 2000; Worthington, 2001; Asami *et al.*, 2003; Caris-Veyrat *et al.*, 2004), whereas several other studies have found the opposite or no differences in taste or other measured quality characteristics between organically and conventionally grown fruits and vegetables (Nilsson, 1979; Hansen, 1981; Finesilver, Johns & Hill, 1989; Montagu & Goh, 1990; Woese *et al.*, 1997; Colla *et al.*, 2000; Cushman & Snyder, 2002; Mitchell & Barrett, 2003; Felsot & Rosen, 2003; Gissén, 2004; Caris-Veyrat *et al.*, 2004).

Authorities and growers organisations are cautious when they give their comments on quality aspects related to organic or conventional production: "*..apart from differences in pesticide residuals there are no general differences in quality between organic or conventional vegetables…*" or "*..based on todays knowledge it cannot be said that organic vegetables generally are more healthy than conventional products..*" or "*.. organic vegetables contain among other more vitamin C and less water..*" (Drake & Björklund, 2002; Dahlin, 2003; Ekologiska Lantbrukarna, 2003; Ivarson & Albertson Juhlin, 2005). This reflects the fact that even though several scientific studies have compared conventional and organic products, so far no conclusive results have been obtained.

One major problem in comparative studies might be that genuine organic and conventional production systems differ in many factors and that a simple measurement of food composition does not reflect its quality. It has been emphasised that a comparison of organic and conventional production needs to consider all differences in the two systems in order to draw valid conclusions (Kirchmann *et al.*, 2004). Other scientists have argued that a valid comparison of nutritional quality would, for example, require that the same cultivars are grown at the same location, in the same soil and with the same amounts of nutrients, conditions which all normally differ between the two systems (Magkos, Arvaniti

& Zampelas, 2003). Factors such as amount of yield, maturity stage, transport, storage, processing of the product and bio-availability of the nutrients all affect the quality as well (Lövkvist, 2000; Dumas *et al.*, 2003).

Literature Review

Tomato fruit quality

Quality can be characterized by functional and nutritional values that can be measured or analysed (Hauffman & Bruce, 2002). In this thesis focus was set on yield and appearance of the tomato fruit, the size, form, structure and colour, as well as the perceived taste by the consumers, dry matter content, pH, and chemical composition, such as macronutrients, sugars, acids and other nutritionally important substances.

However, quality is more than this and can be defined as the sum of all characteristics that make a consumer satisfied with the product (Harker, Gunson & Jaeger, 2003). Apart from functional and nutritional characteristics, quality can include aspects of production method, environment or ethics, as well as availability of and information about a product (Hauffman & Bruce, 2002; Gissén, 2004). Food quality is even characterized by so-called credence qualities, which means that these characteristics are not measurable for the consumer, but the consumer believes in or expects these qualities (e.g. health promoting properties) and depends on the product information provided (Grunert, 2002). Quality then becomes a question of communication. Advertising has a low credibility and consumers desire accurate and reliable labels and clear-cut information on environmental effects or health-related claims (Grunert, 2002).

Why does product quality differ? - Some theories

High dry matter or low water content of the tomato has been reported to affect fruit taste positively because the major components of tomato taste, sugars and acids, are more concentrated (Hobson, 1988). In organic fruit and vegetable production increased dry matter contents have been reported and were related to a slower growth due to a lower availability of the organically bound nutrients in the fertilizer (Haglund *et al.*, 1997; Woese *et al.*, 1997; Weibel *et al.*, 2000). However, higher dry matter content can be achieved in conventional production as well, when water uptake is reduced (Guichard *et al.*, 2001) e.g. by increasing the electric conductivity (EC) of nutrient solutions through high fertilization rates (Satti, Al-Yhyai & Al-Said, 1996) or by addition of NaCl (Auerswald *et al.*, 1999; Dorais, Papadopoulos & Gosselin, 2001). In this thesis organic fertilization was therefore compared to fertilization with mineral nutrient solutions, in which different concentrations (EC-levels) and salt compositions (Cl^- vs SO_4^{2-} anions) were used.

Varying effects on taste have been observed in different cultivars (Auerswald *et al.*, 1999) and at different times of the year (Auerswald, Drews & Krumbein, 1996). To avoid an interaction with these effects, the same cultivar was grown in the experiments described in this thesis and tomato fruits were harvested during the same season in two different years.

Ammonium (NH_4^+) fertilization has been reported to enhance yield (Pilbeam & Kirkby, 1992; Gill & Reisenauer, 1993) and improve taste (Siddiqi *et al.*, 2002), but to reduce ascorbic acid contents compared to nitrate (NO_3^-) fertilization (Mozafar, 1993). Negative effects of NH_4^+ nutrition in tomato production have often been connected with blossom-end rot (BER) damage of fruits (Pilbeam & Kirkby, 1992; Siddiqi *et al.*, 2002), which is regarded to be caused by calcium deficiency (Kinet & Peet, 1997). Reasons for different effects of ammonium fertilization compared to nitrate fertilization can be due to reduced energy needs for the assimilation of NH_4^+ compared to NO_3^- (Raven, 1985; Ullrich, 1992), but also due to effects on pH (Raven, 1985; Lea & Morot-Gaudry, 2001) and differences in counter ions taken up when balancing the uptake of either NH_4^+ or NO_3^- in the plant roots (Jungk, 1970; Gerendás & Sattelmacher, 1990; Mozafar, 1993). Organic fertilizers release nitrogen (N) as organic-N compounds, which are mineralised to ammonium and can be nitrified to nitrate. All these different N-forms can be available and taken up by plants, but the exact proportions in the soil are difficult to determine (Näsholm & Persson, 2001; Persson & Näsholm, 2001). The organic fertilizers used in this thesis were therefore compared to mineral fertilizers with different ratios of NH_4^+ and NO_3^- as N-source and different counter ions in the nutrient solutions, and the macronutrient contents in the plants were measured in order to get an indication about the N-form taken up.

What determines the nutritional quality of tomatoes?

The chemical composition and content of nutrients that are important for the human diet determine the nutritional quality of a product. This can be mineral nutrients, (essential) amino acids, proteins, fatty acids, carbohydrates and vitamins (Hauffman & Bruce, 2002) or other secondary metabolites about which little is known (Brandt & Molgaard, 2001). Tomatoes, as well as other fruits, are especially important for the human diet because of their content of vitamin C, carotenes, lycopene and phenolic compounds (Fig.1) (Davey *et al.*, 2000; Dumas *et al.*, 2003). Many of these compounds have antioxidant properties. The question is, to what extent a certain production method would affect the content of different nutritionally important compounds.

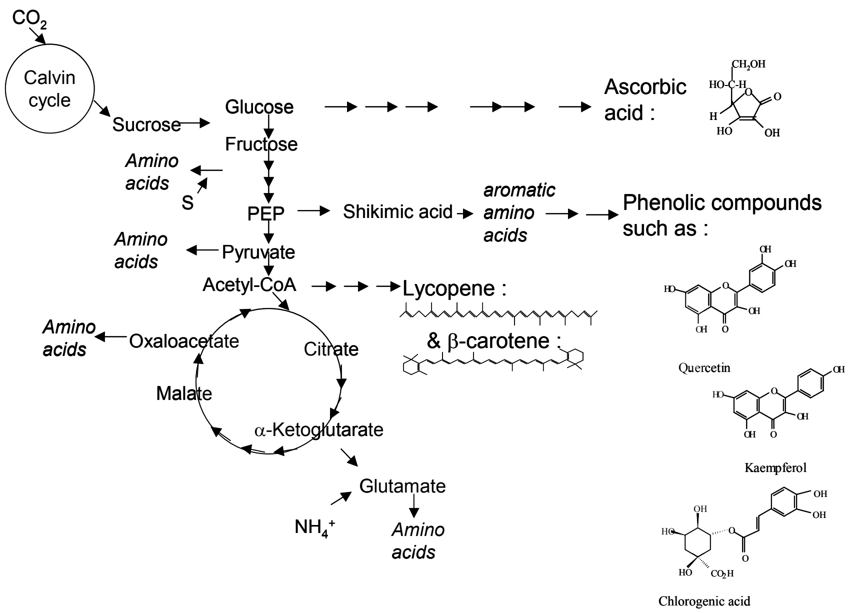

Fig. 1. A schematic overview over the biosynthetic pathways for some important metabolites determined in the tomato fruit tissue.

Antioxidants

All organisms have some kind of defence and repair systems to minimize oxidative damage caused by free radicals, which can initiate cascade reactions and destroy macromolecules and cellular structures (Lurie, 2003). Antioxidants are compounds that are able to inactivate these so called reactive oxygen species (ROS). There are several kinds of antioxidants: a) membran-associated (e.g. α-tocopherol/vitamin E and β-carotene/lycopene), b) water-soluble (e.g. ascorbic acid), c) enzymatic and d) other plant compounds such as phenols, flavonoids, or specific amino acids (e.g. cysteine and methionine) (Lurie, 2003). Several of these antioxidants act together through reduction/oxidation reactions in order to regenerate their function.

In order to achieve a positive effect on health the combined effect of several different antioxidant compounds is needed and the consumption of whole fruits rather than single isolated compounds is recommended (Riso & Porrini, 2001; Wise, 2001; Olsson *et al.*, 2004). Humans are not able to synthesize ascorbic acid and are therefore dependent on the intake of this important antioxidant through fruit and vegetable consumption (Wheeler, Jones & Smirnoff, 1998). A balanced diet rich in fruit and vegetables regardless of how it is produced has therefore been recommended (Magkos, Arvaniti & Zampelas, 2003). Rozin (1999) points out that the discussion mainly centres around positive or negative effects depending on production method, but the level of intake, the total daily consumption of fruits and vegetables, is often forgotten.

Benefit or harm for human health?

Brandt and Molgaard (2001) discuss the nutritional value of organic and conventionally produced vegetables and argue that differences in health effects are not likely, since none of the important parameters such as minerals, vitamins, proteins and carbohydrates are deficient in human diets in developed countries. An argument often given by organic producers is that plants grown without chemical fertilizers and pesticides contain higher levels of defence related minerals, vitamins and secondary metabolites and therefore are beneficial for human health (Brandt & Molgaard, 2001). Conventional producers argue that lack of protection and not-optimal fertilizer supply result in plant products with lower levels of minerals, vitamins and possibly the production of harmful secondary metabolites (Brandt & Molgaard, 2001). Additionally, some medical studies emphazise that an extra antioxidant intake is not always beneficial in e.g. cancer treatment, since the ROS are related to mechanisms responsible for the elimination of cancer cells (Lopaczynski & Zeisel, 2001). However, there exists a general consensus that the consumption of fruits and vegetables is positive for human health (e.g. Olsson *et al.*, 2004) and that secondary metabolites present in plants might be the reason for it, but so far little is known about them (Brandt & Molgaard, 2001).

What affects the antioxidant content in tomatoes?

The effect of variety, season, harvest time, maturity, as well as environmental factors such as light, water and nutrient supply on the antioxidant content of tomatoes are reviewed by Dumas *et al*. (2003). Results from different experiments are contradictory and incomplete. Elevated vitamin C contents have been measured in relation to poor yields or high light intensity, whereas high nitrogen applications decreased vitamin C contents (Mozafar, 1993). The latter might be an indirect effect as an increased plant growth causes self-shading of the fruits (Dumas *et al.*, 2003). Mozafar (1993) also reports that the form of nitrogen supplied could affect the content of vitamin C. Ammonium fertilization would according to the cited studies result in lower vitamin C contents than nitrate fertilization, but the mechanisms behind this are not yet clearly understood. Mozafar (1993) suggests that the different counter ions present in the respective fertilizers may play an important role as e.g. application of potassium (which is a possible counter ion to NO_3^-) has been observed to increase ascorbic acid contents, whereas sulphate or chloride (possible counter ions to NH_4^+) have been observed to reduce them (Mozafar, 1993, 1994). Organic fertilization was reported to result in low yields with high vitamin C content, whereas mineral fertilization gave higher yields with lower vitamin C content (Dumas *et al.*, 2003).

Lycopene has been reported to be a good indicator for fruit maturation, but correlations with red colour of tomato fruits have not always been found. Light affects the synthesis of lycopene and soluble phenols positively, but interaction with high temperatures (above 26 ºC) decreases the lycopene content (Grierson & Kader, 1986; Dumas *et al.*, 2003). Contents of phenolic compounds have been reported to increase under low temperature and under nutrient limiting conditions, especially under nitrogen limitation, and to decrease under water deficient conditions (Parr & Bolwell, 2000). However, contradictory results have been obtained in different varieties of wine grapes (Parr & Bolwell, 2000), and for different types of phenols (Haukioja *et al.*, 1998). Haukioja *et al.* (1998) showed that a negative correlation between growth and the synthesis of secondary carbon containing metabolites (C-compounds) only holds true if the proteins needed for growth and the secondary compounds directly compete for the same precursor (e.g. phenylalanine). This was confirmed by Riipi *et al.* (2002) who observed an increase of certain phenolic compounds at the same time as growth of young birch leaves and shoots was most intensive. Obviously, apart from fertilizer supply several environmental factors such as light, temperature or water supply interact and affect plant growth and stores of C-compounds, but plant responses are complex and can vary for specific metabolites.

Objectives

The overall objective of this thesis was to investigate differences in the nutritional quality of an organic- or a mineral-fertilized tomato crop. The underlying hypothesis was that organic fertilizers would supply nitrogen mainly as organic-N or NH_4^+-N, which would affect tomato quality differently than a conventional, NO_3^--based mineral fertilizer. Furthermore, a growth limitation was expected to result in higher contents of C-compounds, and thereby a higher nutritional quality and possibly a better taste. The specific questions addressed were:

- Does the nitrogen application rate affect tomato growth, fruit yield and quality differently with organic or mineral fertilizers?
- Does the nitrogen form supplied with the fertilizer affect yield, taste and nutritional quality of tomatoes?
- Is a nutrient limitation correlated with low yields and good taste?

Materials and Methods

The papers in the thesis are based on data from three greenhouse tomato production experiments, carried out at the Swedish University of Agricultural Sciences (SLU) in Uppsala (59 49´N, 17 43´E) during summers 2002 and 2004 (**Papers I-IV**). Greenhouse production was chosen due to the size and design of the experiments (no. of treatments, yield requirements for taste tests and analysis), despite the drawbacks regarding light and climate control. Furthermore, results obtained under greenhouse conditions may more easily be compared and adapted to commercial tomato productions in contrast to results obtained in climate chamber experiments. Tomatoes in all experiments were grown in sand in order to use the same substrate for both organic and mineral treatments. Two organic fertilizers were used: a year round commercially available product based on chicken manure (**Papers I-III**) and an only seasonally, but free and locally available fresh cut grass clover mulch (**Papers II-IV**). The same variety 'Armada' (Enza Zaden), a medium size red salad tomato, was used in all experiments and plants were grown for at least 120 days to yield mature fruits.

The design of the first experiment in 2002 was based on experiences from a greenhouse tomato production system developed at the Horticultural station (SLU), Uppsala, during 1990-1995 (Haglund, 1997; Gäredal, 1998; Johansson *et al.*, 1999) and during 1999-2000 (Lundegårdh, pers.comm.). Gäredal and Lundegårdh had obtained tastier tomatoes in the organic treatments compared to conventional treatments and observed low occurrence of blossom-end rot (BER) damaged tomatoes despite high levels of ammonium and chloride in the substrate of organically grown plants (Lundegårdh, pers.comm.).

The 1st experiment

In order to test whether the nitrogen form and rate applied would affect plant growth, fruit yield and selling quality, tomatoes were fertilized with either an organic mixture based on chicken manure or with mineral nutrient solutions with different ratios of NH_4^+ and NO_3^- combined with different chloride levels. All treatments were given at four application rates of nitrogen. Plants and fruits were harvested when the first fruits in all treatments were mature and weights and marketable yield were recorded (for details see **Paper I**).

The 2nd experiment

The effect of the type of fertilizer on fruit quality aspects such as taste and contents of nutritionally important compounds were investigated in a second experiment in 2002. NH_4^+ or NO_3^- dominated mineral nutrient solutions with different chloride and sulphate levels were compared with two organic fertilizers, one based on chicken manure (as in the first experiment) and the other consisting of fresh cut grass and clover mulch (for details see **Paper II**). Fruits were harvested for four weeks and used for taste tests as well as for chemical analysis (**Paper II**). Contents of ascorbic acid, lycopene and soluble phenolics were analysed. The total antioxidant capacity was measured as radical scavenging capacity by use of the $ABTS^{\bullet+}$ radical decolourisation assay (for details see **Paper III**).

The 3rd experiment

The impacts of nutrient limitations, as often observed in organic production systems, on plant growth, fruit yield, taste and nutritional quality were investigated in 2004. To study the effects of a general nutrient limitation all treatments were given at *low* and *high* fertilizer application rates, similar to the two intermediate levels used in the first experiment in 2002. The effects of a specific sulphur limitation were studied in the organic grass mulch treatments by an additional supply of K_2SO_4 to one of the treatments (*plus S* treatment) (for details see **Paper IV**).

Results and Discussion

Did the nitrogen application rate and different N-forms in organic or mineral fertilizers affect tomato growth, fruit yield and quality?

Yield

In all experiments, tomato yield and plant shoot biomass tended to be higher in the mineral treatments than in the organic treatments, although in 2002 this was not significant for the yield of red tomatoes (**Papers I, II, IV**). An increase in the total nitrogen application rate did increase plant shoot biomass, but not the marketable fruit yield (**Paper I**). Yields from the NH_4^+-dominated treatments in 2002 were slightly higher than those from the NO_3^--dominated treatments (**Paper II**), but in 2004 this trend was contrary (**Paper IV**). Different levels of chloride and sulphate in the nutrient solutions did not affect yield (**Papers I & II**). However, fruit appearance was seriously affected at high nitrogen application rates in the NH_4^+-dominated treatments with high Cl^--levels: elevated amounts of BER damaged fruits resulted in a decrease of marketable fruits (**Paper I**). These results suggest that increased levels of NH_4^+ in the fertilizer would only have negative effects at high fertilizer application rates (**Paper I**), but not at low rates (**Papers I, II, IV**). In organic fertilizers, where elevated NH_4^+ levels are expected, it is possible that these negative effects, expressed as BER damaged fruits, would not occur due to the slow release of nutrients. However, yields in the organic treatments in these experiments were lower than in the mineral treatments, and with increased applications of organic fertilizers, BER damage might arise. An addition of mineral sulphur to one of the organic grass-mulch treatments increased growth and yield (**Paper IV**). Analysis of the nutrient status of the plants also confirmed that the lower yields and plant growth in the organic treatments were not due to a nitrogen limitation, but a sulphur limitation. Furthermore it revealed that when this S-limitation was alleviated (*plus S* treatment) phosphorus appeared as a new limiting factor. As nutrients from organic material are released slowly this limitation was expressed in the initial lower yield of red tomatoes, but not in the yield of green tomatoes and shoot-biomass (**Paper IV**).

Overall these results indicated that yields in the organic treatments did not reach the levels of the mineral treatments, due to an insufficient or delayed release of nutrients, mainly sulphur and phosphorus. In commercial organic production it is common to combine different organic fertilizers in order to improve the nutrient balance. However, the risk for excessive nitrogen with consequent nutrient losses (especially nitrate leaching or ammonium volatilisation) or BER damaged fruits is high, if the amount of fertilizer needs to be increased to meet the demands of the limiting nutrient (here sulphur and/or phosphorus). If, on the other hand, the nutrient balance could be improved, for instance by a combination of organic and mineral fertilizers, the same yield could be achieved with minimal nutrient losses.

Did the nitrogen form affect the taste of tomatoes or was a sulphur limitation responsible for low yields and good taste?

Taste 2002

Tomatoes that were given organic fertilizers or NH_4^+-dominated nutrient solutions in 2002, ranked higher in firmness, sweetness, acidity, flavour length and intensity as well as overall acceptance than those given NO_3^--dominated fertilizer. Reduced N-forms (NH_4^+-N, org-N) could therefore be related to an improved taste as observed by Siddiqi *et al*. (2002) (**Paper II**).

The tomatoes fertilized with grass and clover mulch in 2002, ranked highest for acidity and flavour length. The nutrient status of the plants from all treatments was very similar, apart from limiting sulphur levels in the plants given the grass-clover mulch treatment. Elevated levels of starch in the grass-clover mulch treatment can be due to the sulphur limitation from this fertilizer. Under S-limiting conditions assimilated carbon cannot be used for structural growth (protein synthesis), but could be used for the synthesis of carbon-containing primary and secondary metabolites. These compounds could affect the acidity of the fruit (**Paper II**).

Taste 2004, sugars and acids

Tomatoes fertilized with NO_3^--dominated nutrient solutions (at *high* application rates) as well as those fertilized with grass mulch *plus S* (at both *low* and *high* application rates) ranked highest for sweetness, typical tomato flavour and overall acceptance. As the non-reduced-N NO_3^--treatment and the non-S-limited grass mulch *plus S* treatment were perceived to have a better taste than the NH_4^+- and the pure grass treatment in 2004 (**Paper IV**), neither the reduced N-form, nor the S-limitation of the pure grass mulch treatment could be directly related to an improved taste as observed in 2002 (**Paper II**). Analysis of sugars (glucose and fructose) and acids (citric and malic acid) in these fruits showed that both inorganic treatments had higher sugar contents, (which was not reflected by the perceived sweetness in the taste test), whereas both organic treatments had higher acid contents, which agreed well with the perceived acidity in these treatments in the taste test. However, this did not explain the preference for tomatoes fertilized with the NO_3^- and the organic *plus S* treatments (**Paper IV**).

When the results of the two years taste tests are considered together it can be concluded that flavour, sweetness and acidity are complex characteristics of taste, which cannot be defined by analysis of sugars and acids alone. A conclusive relationship with the nutrients supplied or the nutrient status of the plant was not found in these experiments. It can be expected that other substances, such as volatiles have a greater influence on the aroma of the tomato fruit and that the production of these compounds is also influenced by variations in sunlight and temperature (Krumbein & Auerswald, 1998). During the experimental period there was more sunlight in 2002 compared to 2004, and the first half of the experimental period was also warmer in 2002 than in 2004 (Per Nyman, pers. comm.).

Nutritional quality of the tomatoes

Ascorbic acid

The ascorbic acid content in 2002 was highest in tomatoes from the two organic and the mineral NH_4^+-dominated treatments, and lowest in the tomatoes grown with NO_3^--dominant fertilizer (**Paper III**). This pattern of higher levels in the reduced N-form treatments, and lowest level in the NO_3^- treatment, agreed well with the results obtained in the taste tests (**Paper II**) and ascorbic acid might contribute to the perceived taste. The elevated ascorbic acid levels in the organic treatments agree with results reported from organic treatments in combination with low yields (Dumas *et al.*, 2003). It has also been reported that high N application decreased ascorbic acid levels (Mozafar, 1993). At high N supply it could be expected that the plant metabolism would shift to N containing metabolites and/or increased growth and therefore contain lower amounts of carbohydrates and other C-metabolites such as ascorbic acid (Mozafar, 1993). An additional explanation could be a self-shading effect through the high biomass of these plants (Dumas *et al.*, 2003). In our experiment however, N contents in the plants from all treatments were similar and plant biomass of the NH_4^+ fertilized plants was similar to the NO_3^- fertilized plants (**Paper II**). Furthermore, the high levels in the NH_4^+- and the low levels in the NO_3^--treatments are contradictory to the report that ammonium fertilization resulted in lower ascorbic acid levels than nitrate fertilization (Mozafar, 1993).

In 2004, ascorbic acid contents did not differ significantly between treatments (**Paper IV**), but the same trends as in 2002 were observed, with highest contents in the *pure* organic treatments and higher contents in the NH_4^+ fertilized plants than in the NO_3^- fertilized plants. No agreement with the pattern of the taste test scores was found in 2004. However, these results agree with high ascorbic acid levels observed in the treatments with low yield (pure organic) and could be explained with the indirect effect of self-shading as reported by Dumas *et al.* (2003). But they do though not agree with the N fertilization effect reported by Mozafar (Mozafar, 1993), as N contents in plants of the pure organic treatments were higher than in the other treatments. It seems as if N availability was no major problem in the organic treatments in both 2002 and 2004 and reached similar levels as in the mineral treatments. The growth-limiting factor in the organic grass-mulch treatments was revealed to be sulphur, which could act in a similar way as a nitrogen limitation would, as proteins for structural growth could not be built and metabolism therefore would shift to C-metabolites. Nitrogen however would be taken up in excess and instead of structural growth stored in simple N-compounds. This would also explain why ascorbic acid contents in the organic *plus S* treatment in 2004 were similarly low as in the mineral treatments (**Paper IV**).

Lycopene

Lycopene contents in 2002 were highest for the NO_3^--dominated treatment, intermediate for the NH_4^+ & SO_4^{2-}, the chicken manure and the NH_4^+ & Cl^--treatment, and lowest for the organic grass mulch treatment. These results agree with observations by Lopez *et al*. (1996) and suggest that lower sulphur levels and/or higher chloride levels, which characterize the NH_4^+ & Cl^-- and the organic grass mulch treatments, could decrease the content of lycopene in the fruit (**Paper III**). It was also reported that exposure to direct sunlight would decrease the development of lycopene if interacting with unfavourably high temperatures (Dumas *et al.*, 2003). This could possibly explain the lower contents in the organic grass mulch treatment, but would not agree with the slightly lower levels in the NH_4^+ & Cl^-- levels than in the chicken manure treatment, as biomass (and thereby eventual self-shading) was higher in the NH_4^+ & Cl^--treatment than in the organic treatments (**Paper II**). Increasing phosporus and potassium levels have been associated with increased lycopene contents (Dumas *et al.*, 2003), which does not agree with the relatively high levels of P, the highest levels of K, but low lycopene content observed in the NH_4^+ & Cl^--treatment in 2002 (**Paper III**).

Lycopene contents in 2004 were not significantly different between treatments (**Paper IV**), which would neither agree with the above outlined effect of direct sunlight and self-shading theory, nor with the chloride/sulphur explanations. In some reports lycopene has been seen as a good indicator for fruit maturity stage, (Dumas *et al.*, 2003) and in the case of 2004, this would confirm that the fruits from all treatments were picked at the same developmental stage. On the other hand it would also mean that the maturity stages in 2002 might have been different between treatments, which was not identified by the colour-chart assessments made when picking. Red colour and lycopene content of tomatoes could however not always be correlated as reported by Dumas *et al.* (2003). Differences in sunlight and temperature between the two years might be a cause for the contradictory observations.

Phenols

Soluble phenolics of tomatoes measured in 2002 showed slightly higher levels in the two organic and the NO_3^--treatment than in the two NH_4^+- treatments (**Paper III**). Higher light levels due to lower self-shading could be a reason for the elevated levels of phenols in the organic treatments. A restricted plant growth due to slow nutrient availability in the organic treatments could also be responsible for an increased production of phenolic C-metabolites. However, this would not explain the intermediate levels in the NO_3^--treatment. A direct effect of growth on phenols contents would only be observed if the secondary compounds compete for the same precursors as are needed for plant growth (Haukioja *et al.*, 1998; Riipi *et al.*, 2002). Other differences between the fertilization treatments could affect the synthesis of phenolic compounds through many pathways.

Total soluble antioxidant activity

The total soluble antioxidant activity was significantly higher in the NO_3^--treatment and the two organic treatments than in the two NH_4^+- treatments (**Paper III**). This might be due to the increased levels of soluble phenolics, which have been reported to be major contributors to the antioxidant capacity of tomatoes (Toor, Savage & Lister, 2005). Due to a slightly better growth of the plant with the use of NH_4^+ fertilizers in 2002, the production of secondary metabolites involved in plant defence may be reduced and this may have resulted in the lower antioxidant activity, however differences in yield of NH_4^+ treatments compared to NO_3^- treatments were not significant.

Main findings

- Ammonium dominant nutrient supply at moderate levels of total nitrogen supply, as used in the experiments of this thesis, does <u>not</u> lead to negative effects on tomato fruit quality (BER damage) (**Papers I, II & IV**)
- Different nitrogen forms in the fertilizer do affect yield, taste and nutritional quality, but the results from two different years are contradictory and the effects <u>cannot</u> solely be explained by the supply of reduced nitrogen forms (**Papers II, III & IV**)
- The supply of nutrients from organic fertilizers tested in this thesis resulted in lower yields, but this was not due to a nitrogen limitation, but rather a sulphur or/and phosphorus limitation in the grass mulch treatments and a delay of nutrient release in the chicken manure treatments (**Papers I, II & IV**)
- A nutrient limitation resulting in lower yield could <u>not</u> be proved to be the major cause for improved taste (**Papers II & IV**)
- Elevated levels of ascorbic acid in the pure organic grass mulch treatment can be explained with a) a shift towards C-containing metabolites due to S-limitation in this treatment, and b) higher sunlight radiation reaching the fruits as a lower biomass results in lower self-shading through the leaves (**Papers III & IV**)
- Higher ascorbic acid contents in NH_4^+- than in NO_3^--treatments are contradictory with previous findings. A possible cause might be found in differences in counter ions taken up and needs further investigations (**Papers III & IV**)
- Lower contents of lycopene were observed in 2002 in fruits from treatments with higher levels of chloride or lower levels of sulphur, but in 2004 no difference in lycopene content was found between treatments (**Papers III & IV**)
- Phenols make a major contribution to the total antioxidant activity of the fruits, but the reasons for differences in phenol contents between treatments are not yet clear. A shift towards the production of phenolic C-metabolites could be due to slower growth in organic treatments, but this cannot explain the higher levels in NO_3^--dominant treatments compared to NH_4^+-dominant treatments, where other factors seem to be responsible for the different responses (**Paper III**).

Future Work

The use of *metabolomics* to find plant metabolites that cause the variation between samples and correlate with organic or mineral treatments

Metabolomics

A so-called *metabolomics* analysis (Fiehn, 2002) was used to detect relative amounts of low-molecular weight compounds in the tomato samples. Tomato samples from the second and third experiment were extracted, derivatised and analysed by GC/TOFMS (coupled gas chromatography and time-of-flight mass spectrometry) according to Gullberg *et al.* (2004) and Jonsson *et al.* (2004, 2005). *Metabolomics* (or *metabolite fingerprinting*) is a non-biased method, which simultaneously determines metabolites present in both the primary and secondary metabolism of the plant. As the method detects metabolites independent of predefined interest, this method is used as a complement to specific target analyses, which only analyse compounds already known to be of interest. The *metabolomics* technique has only recently been introduced as an alternative way to investigate large amounts of chemical data. However, it should be emphasized that the number of metabolites occurring in a plant tissue is very high and *metabolomics* analysis can today only detect and identify relatively few of those metabolites (Fiehn *et al.*, 2000; Fiehn, 2002; Fernie, 2003).

Multivariate data analysis

The number of variables obtained in a GC/TOFMS output (i.e. the peak areas of chemical compounds) exceeds by far the number of tomato samples analysed. Multivariate data analysis is used because within large amounts of variables the variables can correlate to each other (collinearity). Classical *univariate* statistical methods assume independency of variables. In multivariate data analysis variables are analysed simultaneously in order to find structures in the data set that group or separate the analysed samples. Therefore multivariate data analysis and modelling were performed using projection methods such as *principle components analysis* (PCA) and *partial least squares discriminant analysis* (PLS-DA). The idea of PCA is to project a summary of all measured variables (here over 300 for each sample), in order to find any systematic variation between the samples (Fig. 2). The obtained plot enables an easier interpretation of the data. The task is to find out the physiological and ecological based reasons for the grouping or separation of the samples on the *principal component* axes (PC1, PC2). This is done by interpretation of the so-called *loading plots* (not shown), which give the corresponding overview with all measured compounds.

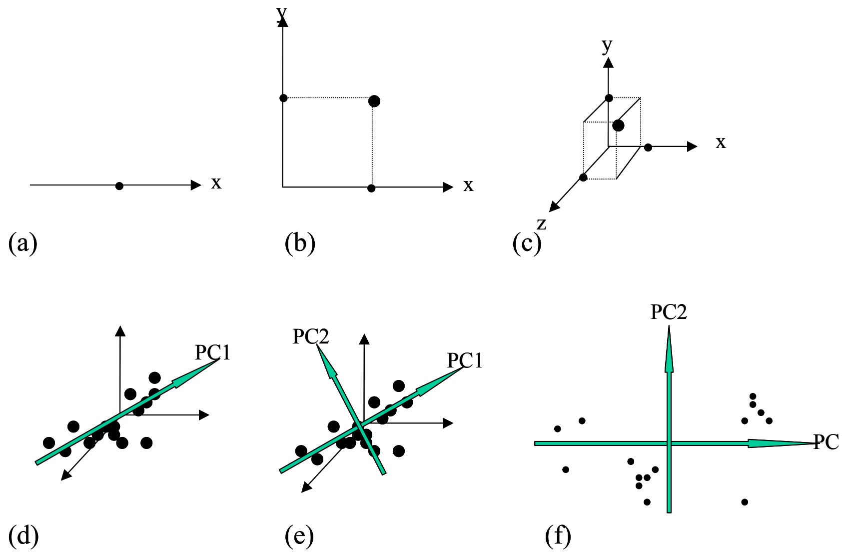

Fig. 2. A simplified geometrical explanation of the principles of multivariate data analysis. For each sample, the relative amount of a detected compound no.1 is marked on the x-axis (a), compound no. 2 on the y-axis (b), compound no.3 on the z-axis (c), and so on. Mathematically, this is feasible for hundreds of compounds in a multi-dimensional space (for details see Eriksson *et al.*, 2001), but to visualize this, a three-dimensional space is used here. A sample will then get a unique point in the three-dimensional space, which represents the summary of all measured compounds for this sample. If this is repeated for each sample, a "swarm" of points is obtained (d). If measurements give similar values the points in the space will be close together, but if there are large differences in the relative amounts of the measured compounds, the points will be more outspread. Mathematically the data points are mean-centred (i.e. the variables average is subtracted from the data points), and usually scaled to unit variance (i.e. divided by the standard deviation of each variable), which moves the point swarm with the average on the origo (Eriksson *et al.*, 2001). A vector, the so-called *first principle component* (PC1), can then be laid through this point swarm in the direction of its largest extension (d). A second vector, PC2, is laid right-angled to the first (e). All points in the three- (or multi-) dimensional space can then be projected onto the resulting plane (f). This plane shows a so-called *score plot*, which represents a visual overview of systematic differences between samples based on mathematical modelling.

Principle component analysis (PCA), is an unsupervised method, which means that it does not demand any prior knowledge of the sample treatments. It was used first to find structures in the data set. *Partial least squares discriminant analysis* (PLS-DA), is a supervised method where the knowledge about different treatments or experimental years is included in the mathematical modelling. It is used to maximize the (visualized) separation of the samples (Figs. 3-5) and to relate chemical compounds (X-matrix, not shown), which cause the variation between the classes of samples, to e.g treatment (Y-matrix) (Eriksson *et al.*, 2001). In the mathematical model, cross-validation (Wold, 1978) was used throughout to determine the number of principle components (A). The following values describe the validity of the mathematical model: R^2X is the amount of relevant information in the measured compounds for explaining the behaviour of the responses (i.e. the explained modelled variation in the X matrix), R^2Y is the goodness of fit (i.e. the cumulative modelled variation in Y) and Q^2 is the goodness of prediction (i.e. the cumulative predicted variation in Y, according to cross-validation). The range of these parameters is 0-1, where 1 indicates a perfect fit. All multivariate data analysis and modelling were performed using SIMCA 10.5 software (Umetrics, Umeå, Sweden).

Preliminary results and discussion

2002 samples

The data obtained in the *metabolomics* analysis of 2002's samples was subjected to a PLS-DA analysis to separate the organic from the mineral treatments (Fig. 3). With A = 4 components, the R^2X value of 0.615, R^2Y value of 0.985 and the Q^2 value of 0.86 indicate a valid model. Phosphoric, malic and citric acids and several unidentified metabolites appeared to be the major components causing the separation of the organic and mineral treatments (data not shown). Phosphoric acid was correlated with the mineral treatments (probably in agreement with the good P-status of these plants), whereas malic and citric acid correlated with the organic treatments (in agreement with the higher acidity observed in the taste tests (**Paper II**).

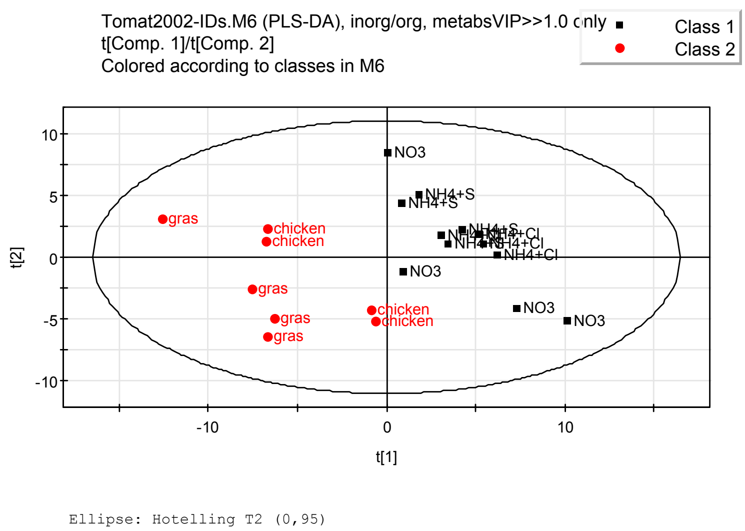

Fig. 3. The score plot of the 2002 data set subjected to a PLS-DA analysis shows a clear separation of the two classes (1=inorganic treatments, 2=organic treatments). With A=4 components, $R^2X = 0.615$, $R^2Y = 0.985$, $Q^2 = 0.86$ indicate a valid model.

2004 samples

The PCA analysis of the 2004's *metabolomics* data set gave a distinct separation of the organic from the mineral treatments, but within these two groups, further separation of the data points was not as clear (data not shown). PLS-DA was then used, including the knowledge about different treatments in the analysis, which resulted in a maximum separation of the mineral treatments as one group from the two organic treatments as two distinct groups (Fig. 4). With A = 6 components, the R^2X value of 0.766, R^2Y value of 0.928 and the Q^2 value of 0.747 indicate a valid model. The P-containing compounds phosphoric acid and glucose-6-phospate and the S-containing amino acid cysteine correlated with the mineral treatments, which corresponds well to the non-limiting conditions in these treatments (data not shown). Aspartic and glutamic acid as well as citric and malic acid, correlated with the organic treatments, which agreed well with the high acidity scores in the taste tests (**Paper IV**). In 2004 both citric and malic acid contents were also determined by a specific target analyses using HPLC (**Paper IV**) and the results agree well with the *metabolomics* results.

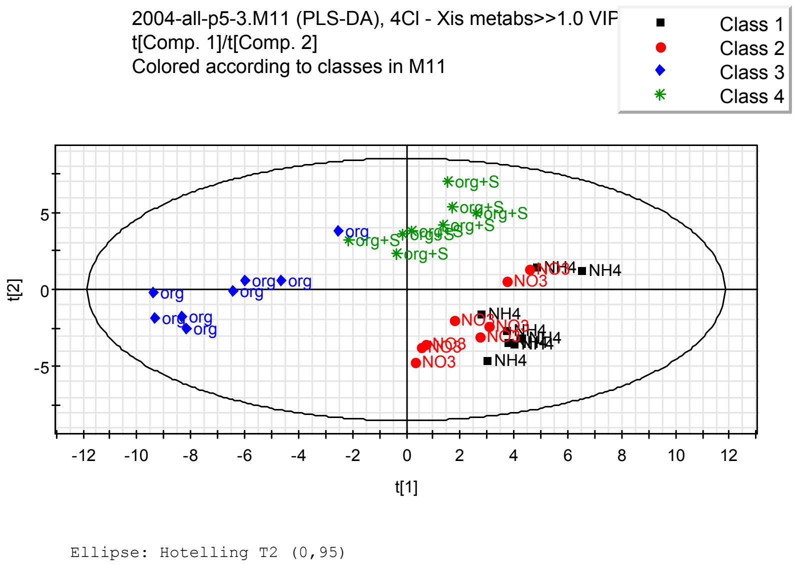

Fig. 4. The score plot of the 2004 data set subjected to a PLS-DA analysis, shows a distinct separation of the inorganic treatments (Class 1: NH_4^+, Class 2: NO_3^-) from the two organic treatments (Class 3: org, Class 4 org+S), the latter separating clearly into the pure organic and the organic *plus S* treatments. With A= 6 components, $R^2X = 0.766$, $R^2Y = 0.928$, $Q^2 = 0.747$ indicate a valid model.

Prediction

The *metabolomics* data obtained from the experiments in 2002 and 2004 were also used to compare the NO_3^-- and the pure grass-mulch treatments between the two years. A PCA analysis indicated a distinct separation of the two treatments, independent from the year, and this separation was maximized in a PLS-DA analysis (data not shown). The model of the 2002 data was then used to predict the 2004 data (Fig. 5). With A= 2 components, the R^2X value of 0.559, R^2Y value of 0.957 and Q^2 value of 0.892 indicate the strong validity of the model. Good correlation between the samples of the NO_3^--treatments and the S-containing amino acid cysteine was probably due to the good S-status of these plants (data not shown). Good correlation with fructose corresponded well with the higher scores for sweetness in the taste tests, and the higher levels of fructose measured in the specific target analysis (**Paper IV**). On the other side, good correlations between the samples of the organic grass mulch treatments and citric and malic acid agreed with the high acidity scores (**Papers II & IV**) and the high levels of these acids analysed specifically (**Paper IV**). Several other amino acids e.g. glutamine, asparagine or glutamic acid were also found to correlate with the pure organic treatment, which could possibly be related to the good N-status of the samples in this treatment due to S-limitation and thereby limited growth (data not shown).

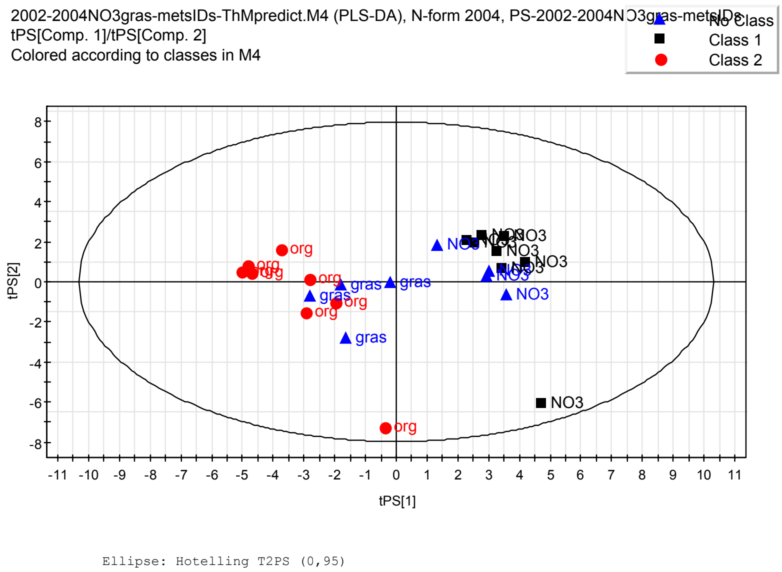

Fig. 5. The T-predicted plot shows that it was possible to predict the 2004 data by using the 2002 data as a prediction set. The NO_3^- and the pure-organic-grass-mulch treatments are clearly separated. 2002 data: grass & NO_3 = triangles; 2004 data: org = circles, NO_3 = squares. With A= 2 components, R^2X = 0.559, R^2Y = 0.957, Q^2 = 0.892 indicate a valid model.

The biochemical reasons underlying the observed variations between the tomato samples are not yet fully understood and need further investigations. The above-described results reveal complex interactions of nutrient availability and environmental factors. They also reveal that so far, it is not clearly established which chemical compounds affect taste and nutritional quality of plant products. For the discussion of taste and taste affecting factors, a shortcoming of the *metabolomics* method is that volatile compounds, which are thought to make a major contribution to taste factors, are not recorded by the GC/TOFMS method used in this study. However, with the use of other extraction methods it could be possible to include such compounds. This would help to detect and identify further important metabolites that relate to a variation between samples, e.g. those responsible for the separation of good or bad taste, high or low yield or other differences that could affect the product quality. An integration of the biochemical pathways for different metabolites would then be necessary to reveal the competition for common precursors or the accumulation of certain metabolites in one treatment and their breakdown and re-circulation in another treatment.

For future experimentation to reveal possible differences between organically or mineral fertilized plants, the achievement of similar plant nutrient status is a prerequisite in order to avoid variation caused by growth limiting conditions. In order to elucidate the effects of nutrient limitations more extreme differences between *low* and *high* nutrient supply than used in the above-described experiments seem to be necessary. It could then be possible to see if eventual differences in plant product quality were connected to a not yet revealed difference between organic or mineral fertilization or to some kind of nutrient limitation as often observed in organic production.

Concluding Remarks

The results of the experiments described in this thesis showed that under the same growing conditions, at the same place, in the same substrate, with the same tomato variety, different years result in different outcomes. The fertilizer-source itself, organic or mineral, cannot solely and definitely determine the quality of the product and probably interacts with sunlight and temperature, which are beyond the grower's control. This is in agreement with contradictory results earlier reported in the literature. Organic production has been and still is at the forefront in pushing conventional production towards more environmental friendly thinking, especially regarding the amount of fertilizers and pesticides used, the use of natural processes such as N-fixation, resource recycling and locally adapted varieties. However, organic production does not by definition guarantee a clean environment and a high quality product. Therefore, the advantages of both mineral and organic fertilizers, or rather production methods, should be used in commercial systems, in order to achieve the product the consumers demand: good tasting, healthy, environmental friendly and locally produced. To achieve high yields (not extreme, but reasonably high yields, as consumers still pay per kilo, and not for taste), a proper balance of available nutrients is crucial. If, as in this study, nitrogen could be supplied from an (locally produced) organic material (e.g. fresh cut grass-clover mulch) it is recommended that this resource is used prior to a mineral fertilizer. But if, at the same time, this organic fertilizer lacks a proper nutrient balance that satisfies the demands of the crop, a complementary mineral salt could easily supply the lacking or limiting nutrients. This, today, is not accepted in organic production in Sweden. However, rules and regulations, and ideologies can change with time and increased knowledge. Regarding the fact that certain mineral fertilizers that are forbidden in organic production in Sweden are allowed in Norway (e.g. K_2SO_4 used in the third experiment in this thesis in the organic *plus S* treatment; Michel Verheul, pers.comm.) this thought deserves some further consideration. Another aspect of combining organic and mineral fertilizers is the *environmentally friendly production* factor. If an unbalanced organic fertilizer is supplied at levels that satisfy the demands of the limiting nutrient (here S or P), a large surplus of other nutrients, especially nitrogen, would be supplied, with a probable risk for leaching (NO_3^-) or volatile losses (NH_3), and thereby negative effects on the environment. As concerning the health aspect of a product supplied with organic fertilizer higher ascorbic acid levels were obtained in the *pure* organic grass mulch treatment, which was linked to the restricted growth due to a sulphur limitation. The levels of other compounds regarded as health-related were contradictory in this study and could not be explained solely by the type of fertilizer used. However, as discussed above, it has been shown that a health promoting effect of certain substances was primarily connected to the consumption of fruits and vegetables in general, and an increase of fruits and vegetables in everyone's daily diet is therefore recommended regardless of the production method used.

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