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Effects of storage and heat treatment on metabolism and quality of fresh tomato and potato

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Abstract

Potato and tomato are two highly consumed vegetables in Norway as well as throughout the rest of the world, with high consumer demands of sensory and nutritional quality. In addition to being sold fresh, a large use for vegetables are found in processed food for convenient preparation at home. All plant products continue to live and undergo metabolic processes after harvest, and these processes are affected by environmental conditions such as temperature and light as well as processing conditions. This study was designed to address how the nutritional content in tomato and potato change over time due to these metabolic processes when the plant products are stored at different temperatures as well as how processing by heat treatment affect the nutritional content compared to the fresh produce.

Potatoes were stored at 4 °C and 20 °C over 14 days and tomatoes in the same temperatures over 10 days. Each sampling day samples were taken from storage at 4 °C and dried at 40 °C and 60 °C for approximately 18 hours. The biochemical composition of the samples was then analysed to find total soluble solids content (°Brix), total phenolic content (Folin-Ciocalteu assay), antioxidant activity (FRAP) and relative concentrations of individual metabolites using gas chromatography-mass spectrometry (GC-MS).

The results showed a clear difference between fresh and dried samples in both potato and tomato. Fresh potatoes stored at 4 °C increased in reducing sugars indicating low temperature sweetening, whereas fresh tomatoes underwent less biochemical changes in low temperature storage compared to room temperature storage. Heat treatment resulted in significant decreases of metabolite levels, total phenolic content and antioxidant activity in both potato and tomato. Some metabolites were however found to increase, including glucose, fructose, chlorogenic acid and several fatty acids in potato, and glucose, fructose and α-tocopherol in tomato. The dried samples generally followed the trends seen in the samples stored at 4 °C over the storage period.

A large number of studies assessing quality in potatoes and tomatoes have been published, but all with different cultivars that have been grown, stored and processed under different conditions. Large-scale studies that monitor all aspects of growth, storage and processing could therefore be of importance, as well as thorough literature reviews that can provide a collected source of information for researchers in the food industry.

Sammendrag

Potet og tomat er to av grønsakene som forbrukes i størst grad i Norge og i resten av verden, med høye forbrukerkrav til kvalitet. I tillegg til å selges som ferske råvarer blir grønsakene i utstrakt grad brukt i prosessert mat for enkel tilberedelse i hjemmet. Alle plantematerialer fortsetter å leve og gå gjennom metabolske prosesser etter høsting, og disse prosessene påvirkes av miljøforhold som lys og temperatur i tillegg til forholdene under prosessering. Denne studien er designet for å undersøke hvordan næringsinnholdet i tomat og potet endres over tid som en effekt av disse metabolske prosessene når grønsakene lagres ved ulike temperaturer, og hvordan prosessering ved temperaturbehandling påvirker næringsinnholdet sammenlignet med ferske grønsaker.

Poteter ble lagret ved 4 °C og 20 °C i 14 dager og tomater ved samme temperaturer i 10 dager. På dagene med prøveopparbeiding ble prøver lagret ved 4 °C tørket på 40 °C og 60 °C i 18 timer. Den biokjemiske sammensetningen til prøvene ble deretter analysert for å finne total mengde løselig tørrstoff (°Brix), totalt fenol-innhold (Folin-Ciocalteu assay), antioksidant aktivitet (FRAP) og relative konsentrasjoner av individuelle metabolitter ved bruk av gass kromatografi-gass spektrometri (GC-MS).

Resultatene viste en klar forskjell mellom ferske og tørkede prøver i både tomat og potet. Det ble funnet et økt innhold av reduserende sukker i prøver av ferske poteter lagret ved 4 °C som indikerte sukkerinnholdakkumulering ved lave temperaturer. I ferske tomater ble det funnet mindre endringer i metabolitt-sammensetningen ved prøver lagret i lavere temperaturer samenlignet med prøver lagret i romtemperatur. Varmebehandling resulterte i signifikant reduserte metabolittnivå, totale fenoler og antioksidant aktivitet i både potet og tomat. Noen av metabolittene økte ved varmebehandling, blant disse var glukose, fruktose, klorogensyre og flere fettsyrer i potet, og glukose, fruktose og α-tokoferol i tomat. De tørkede prøvene fulgte generelt tendensene sett i prøvene lagret ved 4 °C gjennom lagringsperioden.

Mange studier som vurderer kvalitet av potet og tomat har blitt publisert, men alle med ulike sorter som har blitt dyrket, lagret og prosessert under ulike omstendigheter. Studier i storskala som overvåker alle aspekter rundt dyrkning, lagring og videre prosessering vil kunne være av stor verdi for videre forskning i tillegg til grundige litteraturgjennomganger som kan gi samlet informasjon for forskere i matindustrien.

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Abbreviations

°Bx	=	Degrees Brix
AMDIS	=	Automated Mass Spectral Deconvolution and Identification System
ANOVA	=	Analysis of variance
AOX	=	Antioxidant activity
D40	=	Samples dried at 40 °C over night
D60	=	Samples dried at 60 °C over night
DM	=	Dry matter
DW	=	Dry weight
F-C	=	Folin-Ciocalteu
FC	=	First principal component
FRAP	=	Ferric reducing antioxidant power
GABA	=	4-Aminobutyric acid
GAE	=	Gallic acid equivalents
GC-MS	=	Gas chromatography-mass spectrometry
LC-MS	=	Liquid chromatography-mass spectrometry
MeV	=	MultiExperiment Viewer
MSTFA	=	N-Methyl-N–(trimethylilyl)-trifluoroacetamide
NIST	=	The National Institute of Standards and Technology
P0	=	Potatoes extracted on day of harvest
P3	=	Potatoes extracted 3 days after harvest
P7	=	Potatoes extracted 7 days after harvest
P14	=	Potatoes extracted 14 days after harvest
PCA	=	Principal component analysis
RT	=	Samples stored in room temperature $\approx$ 20 °C
SC	=	Second principal component
SS	=	Soluble solids
ST	=	Samples stored in 4 °C
T0	=	Tomatoes extracted on day of harvest
T3	=	Tomatoes extracted 3 days after harvest
T7	=	Tomatoes extracted 7 days after harvest
T10	=	Tomatoes extracted 10 days after harvest
TPH	=	Total phenolic content

Chapter 1

Introduction

Food quality can be described as the standard of food that is acceptable to consumers. There are several aspects that combine to determine overall food quality in agricultural products. Sensory quality including visual appeal, texture, flavour and aroma are attributes induced by chemical composition. Nutritional quality is an aspect that also relates to chemical composition, with high quality plant products being high in nutrients. Other quality aspects of plant products are demands of handling, storage and properties when processed. If a product has a low handling, storage or processing quality it will affect the final sensory and nutritional quality when it reaches the consumer. Food quality can be influenced by many factors including light conditions, temperature, soil quality, choice of cultivar, and time of harvest when grown, as well as conditions when the products are being handled, stored and processed. To optimise the quality it is important to identify the chemical composition and metabolic processes contributing to sensory and nutritional quality and assess how these parameters change during handling, storage and processing.

1.1 Potato

The potato (*Solanum tuberosum* L.) is a tuber root vegetable that is used extensively throughout the world. It is rated as the largest non grain crop with 373 million tons produced worldwide in 2011 (FAO, 2011). The potato is versatile and can be cooked in many different ways as well as processed into french fries, chips and dehydrated products. It is not well suited to be eaten raw due to the indigestibility of ungelatenised starch (Burton, 1989). Cultivation was thought to begin in the Andes mountains in Peru and Bolivia around 8000 years ago, and from there brought to Europe by Spanish travellers. The crop then spread to the rest of the world with Asia and Europe being the largest crop producers of this popular vegetable today (Po and Sinha, 2011).

The potato plant starts out in development and growth as a lateral seed in soil. Under favourable conditions a shoot forms and grows to become a plant. Roots form from the lateral seed and stems develop underneath the soil. These stems swell out forming tubers that store the glucose formed by photosynthesis as starch. As the mother plant dies the plant

reproduces asexually forming new lateral buds on the tubers. The potato is a temperate climate crop with different varieties being well adopted to a large range of climates. Soils rich in organic matter, well aerated and with good drainage are preferred for cultivation.

Classification of the potato is done according to size, shape, flesh or skin colour, texture, taste, cooking characteristics or early/mid-season/late maturity. Specific cultivars are grown for specific purposes including household cooking, bio-ethanol production, industry starch production and processed food production (chips, potato crisps, mashed potatoes, potato flour, potato starch). There are approximately 5000 known varieties of potatoes worldwide. Most belong to the species *Solanum tuberosum* as well as around 200 wild species recorded. Variety is believed to be one of the most significant factors affecting nutritional content (Toledo and Burlingame, 2006). For instance Navarre et al. (2009) found a 15-fold difference in phenolic compounds when comparing hundreds of potato genotypes, where white-fleshed potatoes were reported to contain significantly less phenolics than purple-fleshed wild species. The different metabolite compositions of cultivars make them suitable for different uses and processing methods (Finotti et al., 2006).

The potato provides dietary energy through starch which is a source of glucose; the primary substrate for cell metabolism. More than 95 % of the energy in raw potato comes from its carbohydrate content (Burlingame et al., 2009). In addition to starch, potatoes contain small molecules and secondary metabolites that contribute in plant defence, prevention of enzymatic browning and possess anticarcinogenic, antiglycemic, antimutagenic and antioxidative beneficial properties (Friedman, 1997). Secondary metabolites in potatoes include phenolics, flavonoids, folates, anthocyanins and carotenoids. Although the concentration of phenolics in potato is low, Chun et al. (2005) found that potatoes are the largest contributors of phenolics in the american diet due to the high consumption of potatoes. Phenolics and anthocyanins have shown a high positive correlation with antioxidant capacity in potatoes (Reyes et al., 2005).

When potatoes are heat processed at high temperatures there has been found an increase in Acrylamide production. Acrylamide has been classified as a probable carcinogenic compound in humans (IARC, 1994). It is formed as a result of the Maillard reaction between amino acids and reducing sugars. Asparagine, a major amino acid in potatoes, is a particularly suitable reactant (Mottram et al., 2002). The amounts of Asparagine and reducing sugars is therefore an important aspect to consider when choosing cultivars to use in heat processed food.

Being a vegetable with low respiration rates the potato can be stored for a long time under suitable conditions without spoiling. One of the key factors determining storage quality of potatoes is temperature. At high temperatures respiration, evaporation and sprouting (in combination with light) increases and the potatoes spoil faster, it is therefore important to keep a cool storage temperature. However, at lower temperatures low-temperature sweetening, as a result of starch degradation and sugar accumulation (Amir et al., 1977), and chilling injury can occur (Singh and Kaur, 2009). Spoilage by microorganisms such as bacteria and fungi can follow mechanical injury or chilling injury. As the potato is stored and starch is broken down resulting in higher glucose levels, microorganisms use this glucose as their source of carbon. Some products of carbohydrate decomposition by microorganisms can result in unpleasant odours and flavours with discolouration and softening of the tissue making it unsuitable as a food source for humans (Tournas, 2005).

1.2 Tomato

The tomato (*Lycopersicon esculentum* Mill.) of the family *Solanaceae* is a fruit vegetable (classified botanically as a berry) used extensively as a food product throughout the world. It is grown in home gardens and used largely in industial agriculture in fields/greenhouses with production world wide estimated to 159 million tonnes in 2011 (FAO, 2011). In addition to being sold fresh it is also produced and sold dried, in powder form, as a puree or paste, in sauces, soups, ketchup and as canned whole fruit.

The tomato originated from the Andes in South America evolving from the wild cherry tomato (*L. esculentum* var. cerasiforme A. Gray) (Peralta and Spooner, 2006). From here it spread throughout the world and cultivation began for food purposes after first being grown only as a curiosity believing that it was poisonous. Tomatoes need a warm climate both day and night for optimal germination, growth and flowering (Motamedzadegan and Tabarestani, 2011). Crops are therefore usually grown in greenhouses, in a controlled environment, in locations with colder climate or large variations in temperature.

There are thousands of different tomato varieties of different colours, sizes and shapes. They can be classified as heirloom or hybrid strains, as determinate or indeterminate plants or according to shape and size. Determinate plants grow to a certain height, and after pollination the entire crop ripens at around the same time. An indeterminate plant will grow as tall as it is able to. While it is growing it will flower and grow and ripen fruit. The fruits of an indeterminate plant will therefore ripen in smaller quantities throughout the growing season. Tomato plants are self-fertile but not self-pollenising, meaning the plant has to be shaken or vibrated to release pollen. This can be done by wind, by hand, using mechanics or by using bumble bees as pollinators (van Ravestijn and van der Sande, 1991).

There are several aspects to consider when assessing tomato fruit quality. For the consumer the sensory and nutritional quality is the most important aspect. However from the production and sales side it is equally important to consider resistance to abiotic stress, uniformity, appearance, firmness and to provide a longer shelf-life (Shewfelt, as cited in Beckles (2012)). Flavour arises from the interaction of aroma and taste. Sugars, acids, phenols and minerals are the main contributors to flavour with sugars being the main component balancing the acidity (Kader, 2008). Tomatoes contain many nutrients common in the human diet including sugars, amino acids, dietary fibre and minerals, as well as several secondary metabolites beneficial to human health including flavonoids, chlorogenic acid, carotenoids, tocopherols (vitamin E) and ascorbic acid (vitamin C) (de Vos et al., 2011). The concentration of the potentially healthy secondary metabolites vary considerably between tomatoes. This is mainly dependent on variety (George et al., 2004; Slimestad and Verheul, 2009), ripening stage (Obiadalla-Ali et al., 2004; Mintz-Oron et al., 2008), growth conditions (Semel et al., 2007), post-harvest storage (Slimestad and Verheul, 2005) and for processed tomatoes; the processing methods (Capanoglu et al., 2008). The metabolic content also differs largely between the different parts of the fruit (epidermis, pericarp, seeds etc.) (de Vos et al., 2011).

As a climacteric fruit an increase in respiration, CO_2 and ethylene production follows ripening in tomatoes. These changes lead to the degradation of starch and chlorophyll, accumulation of carotenoids (lycopene), an increased development of flavour as specific volatiles (alcohols, aldehydes and esters) and changes in the sugar-acid balance of the fruit

(Alexander and Grierson, 2002). These changes also make the tomatoes soften quickly and shorten shelf-life when edible, before it continues to ripen and spoil. To increase shelf life tomatoes are kept in a cool and controlled atmosphere to slow down the respiratory metabolism (Yanuriati et al., 1999). It is however important to store the tomatoes at a temperature high enough to avoid chilling injury which limits storage life and leads to a degradation of produce quality (Ding et al., 2001). Ripe tomatoes stored at low temperatures have also been descricribed as less sweet and aromatic than ripe tomatoes stored at room temperature in sensory descriptor ratings (Maul et al., 2000) and studies of chemical composition in low temperature storage (Raffo et al., 2012; Buttery et al., 1987), although some of the flavour might be regained after storage at room temperature before consumption.

1.3 Metabolism, nutrients and phytochemicals

Fruits and vegetables are major sources of vital nutrients in the human diet. Nutrients are often thought of as food components that the human body need, but cannot synthesise for itself (e.g. vitamin C), as well as food components that are not always readily available to be synthesised in the body (e.g. amino acids) (Hounsome et al., 2008). Phytochemicals like dietary fiber, flavonoids, sterols, phenolic acids and glucosinolates have later been regarded as nutrients that lower disease risks and promotes good health (Hounsome et al., 2008; Willett, 1994). Nutrients are used in cell metabolism for energy production and in other metabolic processes. Dunn et al. (2005) describes the metabolome as

> . . . the final downstream product of the genome and is defined as the total quantitative collection of small molecular weight compounds (metabolites) present in a cell or organism which participate in metabolic reactions required for growth, maintenance and normal function.

The metabolic process changes rapidly and can have a reaction time of less than one second (Dunn et al., 2005). To assess the nutritional quality at a given moment in time, metabolomics gives an instant picture of the cells metabolites. In plant biology metabolites are divided into primary and secondary metabolites.

Many primary and secondary metabolites have antioxidant activities. Dietary antioxidants are defined as food substances that significantly decrease the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1998). Reactive oxygen and nitrogen species cause oxidation, nitration, halogenation and deamination of all types of biomolecules becoming toxic and/or mutagenic products (Castro and Freeman, 2001). Antioxidants delay the formation or slow the rate of formation of free radicals. Antioxidant activity is found in many different compounds with different functional groups (Hounsome and Hounsome, 2011). Several studies have found that the additive and synergetic effects of phytochemicals in fruit and vegetables result in higher antioxidant activity than the sum of individual phytochemical antioxidant activity (Liu, 2003; Trombino et al., 2004).

1.3.1 Primary metabolites

Primary metabolites are compounds that are essential for plant growth and survival, development, respiration and photosynthesis, and synthesis of proteins and hormones. Primary metabolites include nucleotides, nucleic acids, carbohydrates, fatty acids, organic acids and amino acids. They are found in all species and are produced using the same or similar pathways (Hounsome and Hounsome, 2011).

Carbohydrates are a class of organic compounds consisting of oxygen, carbon and hydrogen. In plants they occur as monosaccharides, disaccharides, polysaccharides and sugar alcohols. Sugars contribute in controlling blood glucose levels, insulin metabolism and food fermentation. They can also be involved in cell signalling when attached to a protein or lipid molecule (Hounsome and Hounsome, 2011). Dietary fiber includes non-starch polysaccharides, lignin, resistant oligosccharides, resistant starch, waxes, cutin and suberin (De Vries, 2003). High dietary fiber foods reduce symptoms of chronic constipation. It has been reported that low fiber diets can decrease the risk of cardiovascular diseases and obesity (Threapleton et al., 2013; Slavin, 2005).

Amino acids are derived from the glycolysis pathway, pentose phosphate pathway and the citric acid cycle in plants. They are intermediates in metabolism and join to form proteins which provide structural material for the body as well as functioning as enzymes, hormones and antibodies. The human body can synthesise some amino acids, but nine amino acids must be from the diet. These include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan and valine, and are called essential amino acids. Some non-protein amino acids have specific functions in the human body e.g. aminobutyric acid (GABA) which is an inhibitory neurotransmitter in the nervous system and retina (Hounsome and Hounsome, 2011). Amino acids also contribute to the taste of vegetables. Glycine and alanine are sweet, valine and leucine are bitter, aspartic acid and glutamate have sour and savoury tastes (Solms, 1969).

Organic acids release protons in solution which gives them an acidic taste. They are flavour enhancers giving the vegetables tartness that balances sweetness (Kader, 2008). Plants contain many organic acids, highest in concentration is malic and citric acid. Succinic, fumaric and quinic acids are found in lower concentrations. Other acids can also be found in specific vegetables and fruits. Vitamin C includes ascorbic and dehydroascorbic acids. These are organic acids high in antioxidant activity. Vitamin C is involved in several processes in the human body including synthesis of neurotransmitters, collagen and steroid hormones; conversion of cholesterol to bile acids and absorption of iron and calcium (Hounsome and Hounsome, 2011). The content of vitamin C in vegetables is strongly affected by storage conditions and processing (Kabasakalis et al., 2000; Miglio et al., 2008).

Amines and polyamines synthesised in plants are essential in cell metabolic activity, blood pressure control and allergic responses (Silla Santos, 1996; Kalač and Krausová, 2005). Putrescine, spermidine and spermine play important roles in stress response (Groppa and Benavides, 2008). Accumulation of amines including histamine, putrescine, spermidine and tyramine is associated with spoilage of vegetables. Concentration of these can therefore be used as an indicator of freshness of food (Halász et al., 1994).

Fatty acids are components of fats, oils and waxes. The human body gets energy and structural material for cell membranes (phospholipids) from fatty acids. They are also involved in the absorption of vitamins A and D, blood clotting and the immune response (Hounsome and Hounsome, 2011). There are two essential fatty acids that must be provided through diet: linoleic acid and α-linoleic acid. Consumption of unsaturated fatty acids are reported to reduce risk factors associated with cardiovascular disease and type 2 diabetes (Kris-Etherton et al., 2003; Nettleton and Katz, 2005).

1.3.2 Secondary metabolites

Secondary metabolites are compounds that are not essential for plant growth and survival. They generally occur in low quantities and some can be specific to a certain family, genus or species. Secondary metabolites provide defence mechanisms for plants as well as attract seed-dispersing animals/pollinators, contributing to taste, colour and aroma of the plant (Crozier et al., 2006). The nutritional value of secondary metabolites have been more extensively researched in recent years as increasing evidence of how moderate intake of these metabolites might be involved in preventing incidents of cancer, cardiovascular disease and type II diabetes (Wink et al., 2012; Morton et al., 2000; Dembinska-Kiec et al., 2008). Secondary metabolites include phenolic compounds, terpenes and terpenoids, sulphur containing compounds and alkaloids.

Phenolic compounds include around 8000 metabolites in plants. The largest groups found in vegetables include phenolic acids, flavonoids, lignans, lignins and tannins. In plants phenolics are involved in cold acclimation and protection against UV radiation, high concentrations are associated with increased resistance to fungal pathogens (Nicholson and Hammerschmidt, 1992). Some phenolics determine colour and smell of plants to attract pollinators (Hounsome and Hounsome, 2011). Many factors affect the phenolic content of a plant; for instance plant maturity, mineral nutrition, temperature and light (Parr and Bolwell, 2000). Phenolic acids such as caffeic and chlorogenic acids have been reported of having strong antioxidant activity (Sroka and Cisowski, 2003; Cheng et al., 2007). Chlorogenic acid has also been reported of slowing down glucose absorption into the blood-stream (Bassoli et al., 2008). Flavonoids are a group of plant phenolics that include flavones, flavonols, flavonones, catechins, anthocyanidins, isoflavones and chalcones. Many are plant pigments, that determine the colour of vegetables and fruits. In humans flavonoids possess antiviral, antiinflammatory, antihistamine and antioxidant properties (Hounsome and Hounsome, 2011). Flavonoids generally have a bitter taste or a bitter taste with a sweet aftertaste (Drewnowski and Gomez-Carneros, 2000). Phenolics are a good source of antioxidants (Ainsworth and Gillespie, 2007). The free radical savaging properties provide several health benefits in the human body (see Manach et al. (2004)).

Terpenes and terpenoids are the largest class of secondary metabolites with over 20,000 different structures (Connolly and Hill, 1991). Terpenoids in plants include volatile oils, gibberellins, tocopherols, carotenoids , sterols, sapogenins, steroid hormones and quinones. They often have a strong smell that deters parasites, protecting the plant, or after damage from herbivores act as an indirect plant defence by attracting arthropods that pray on the parasitic herbivores, preventing further damage (Dudareva et al., 2004). They can also act as structural components of membranes (sterols), photosynthetic pigments (carotenoids),

electron carriers (quinones) and hormones (Hounsome and Hounsome, 2011). Carotenoids are pigments located in the chloroplasts of plants. In humans they can act as precursors for provitamin A and have been associated with inhibition of some chronic diseases (Paiva and Russell, 1999). They also act as biological antioxidants (Edge et al., 1997). Tocopherols and tocotrienols are known as vitamin E. In plants they protect chloroplast membranes from oxidation (Munné-Bosch and Alegre, 2002). In humans vitamin E is present in all cell membranes as a lipid-soluble chain-breaking antioxidant that protects DNA, lipoproteins and fatty acids from free radical-induced oxidation (Hounsome and Hounsome, 2011).

1.4 Methods

This section gives a short overview of the principles of the three main methods used in this study. The Folin-Ciocalteu assay is used to assess the total phenolic content, the method of ferric reducing antioxidant power assessing antioxidant activity and gas chromatography-mass spectrometry for metabolic profiling.

1.4.1 Folin-Ciocalteu assay

The Folin-Ciocalteu (F-C) assay is a method based on the reaction of phenolic compounds with a colorimetric reagent . It has been proposed as a standardised method for use in the routine quality control of food products (Prior et al., 2005), due to its simple, fast, reproducible nature. It is therefore an appropriate method for this study.

The assay is based on transferring electrons in an alkaline medium from phenolic compounds to form blue phosphomolybdic/phosphotungstic acid complexes that can be determined spectroscopically at 760 nm. The exact chemical nature of the F-C reaction is not known, but it is believed that several reversible electron reduction reactions lead to blue products (Huang et al., 2005).

An important aspect to keep in mind using this method is that the chemistry is non-specific and that other oxidation substrates in an extract sample can interfere in an additive, inhibitory or enhancing manner. For example ascorbic acid readily reacts with the F-C reagent producing an additive effect (Singleton and Rossi Jr., 1965). It is therefore important to keep in mind that this method measures total phenolics and other oxidation substrates.

1.4.2 Ferric reducing antioxidant power

Halliwell (1989) defines a biological antioxidant as "any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate." The oxidising species reacts with the antioxidant instead of the substrate, making the antioxidant a reductant. Using a redox-linked colorimetric method adding an easily reduced oxidant in excess is a simple way of assessing the reducing ability, and therefore the antioxidant activity of a substance.

The ferric reducing antioxidant power (FRAP) also known as the ferric reducing ability of plasma, is a method of assessing antioxidant activity based on this principle developed

by Benzie and Strain (1996). It uses a ferric to ferrous ion reduction at low pH that forms a coloured ferrous-tripyridyltriazine complex. By comparing the absorbance change at 593 nm of test reaction mixtures with mixtures containing known concentrations of ferrous ions, FRAP values can be obtained.

At low pH a ferric-tripyridyltriazine (Fe^{III}-TPTZ) is reduced to the ferrous (Fe^{II}) form that has an intense blue colour with an absorbance maximum at 593 nm. When an antioxidant (reductant) is present the test conditions favour a reduction of the complex and colour development (Benzie and Strain, 1996).

The FRAP assay gives fast and reproducible results for single antioxidants as well as mixtures of antioxidants. It is inexpensive, the reagents are simple to prepare and the procedure is straight forward. The test was originally designed to measure reducing power in plasma but lends itself to measuring reducing power in fluids in general, making it an appropriate choice of method for this study.

1.4.3 Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is a method for quantitative and qualitative detection of metabolites with an open approach allowing for unexpected changes in metabolite levels. The method is highly sensitive, specific and allows for high reproducibility (Roessner et al., 2000).

The gas chromatography separates components in a mixture in the gas phase. GC-MS analysis is therefore limited to the metabolites that are or can be made volatile. For instance lycopene, an important nutrient of tomato will not be identified because it is an apolar non-volatile compound. However the compounds that are volatile and released into the air are important for the taste and flavour of tomato. This makes GC-MS an appropriate method to assess fruit quality (de Vos et al., 2011).

Mass spectroscopy characterises the separated components individually. The mass spectra are recorded as intensity against m/z ratios. Identification of the unknown components can be done using Automated Mass Spectral Deconvolution and Identification System (AMDIS). AMDIS deconvolutes the GC-MS data and can be connected to mass spectral libraries like the National Institute of Standards and Technology (NIST) mass spectral database to help identify the separated components. For accurate mass calculations (quantification) of the identified components the MetAlign software can be used (Lommen, 2009).

1.5 Aim of study

This study aims to give an insight into how storage temperatures and heat processing affect food quality in tomatoes and potatoes. Plant products continue to live and undergo metabolic processes after harvest. This study is designed to assess how the nutrient content is changed due to responses in metabolic processes in tomato and potato when the plant products are stored at different temperatures, as well as how processing by heat treatment of the plant products affects the nutritional content compared to the fresh produce.

Chapter 2

Materials and methods

This chapter provides a description of the materials and methods used in plant product sampling, biochemical analyses and data analyses of this study. Supplementary figures to the methods are included in Appendices.

2.1 Sampling of plant products

The tomatoes (*Solanum lycopersicum* Mill.), cultivar 'Espero', were obtained from Skjetlein Videregående Skole, an agricultural school in Sør-Trøndelag, Norway. The Espero cultivar is from an indeterminate plant, it is a red fruit, with round normal shape and of medium size. They were transported to the laboratory where damaged, unripe and overly ripe tomatoes were discarded.

The potatoes (*Solanum tuberosum* L.), cultivar 'Lady Claire', was harvested from a farm in Skatval, Nord-Trøndelag, Norway. Lady Claire is a medium late variety with light yellow flesh colour, long dormancy, white oval and round tubers used increasingly in Norwegian chips industry since its approval in 2005 (Møllerhagen and Nybråten, 2006). They were transported to the laboratory where excess dirt was removed with a dry paper towel. Damaged, very small and very large potatoes were discarded.

The tomatoes and potatoes of similar size, color and without any damages were processed in the lab immediately after harvest or stored in room temperature ($\approx$ 20 °C) or storage temperature ($\approx$ 4 °C) prior to extraction. The dried samples were stored fresh in the storage temperature before drying on day 3, 7 and 10 and extracted the day after drying.

There were four extraction times for each plant product. The potatoes were extracted on the day of harvest (P0) as well as on day 3 (P3), 7 (P7) and 14 (P14) after harvest. The tomatoes were extracted on the day of harvest (T0) as well as on day 3 (T3), 7 (T7), and 10 (T10) after harvest. For each sampling there were made three replicas of every sample giving a sample size of n=3, where each replica consisted of three tomatoes or potatoes.

2.2 Biochemical analysis

All laboratory work was performed in labs for the Department of Biology, in Realfagbygget at NTNU. Degrees Brix (°Bx) was measured using a refractometer to estimate soluble solids (SS) content. Antioxidant activity (AOX) was measured using the FRAP assay. Total phenolic content (TPH) was measured using the F-C assay. Metabolite profiling was performed by GC-MS to identify and quantify metabolites relevant to the quality of the plant products.

2.2.1 Estimation of dry matter

Small containers were formed out of aluminium foil to prevent the plant material from moving, and the container was weighed. The plant material was rinsed and sliced into small cubes. The potato peel was discarded, nothing was discarded from the tomato. 10 g of plant material was placed on the aluminium foil and the foil was incubated at 105 °C over night. For the potatoes the aluminium foil was placed on a ceramic plate to prevent it from moving in the incubator. The aluminium container with the plant material was then weighed and dry matter estimated as a percentage of fresh material weight. The same method was used to estimate dry matter (DM) after drying over night at 40 °C and 60 °C.

2.2.2 Soluble solids content

Three tomatoes were rinsed and sliced into small cubes. The potatoes were homogenised into a paste using a kitchen hand blender. About 10 mL of plant sample was added into a 15 mL tube and centrifuged at 3 100 rpm at 4 °C for 10 min. A drop of the supernatant was placed on a refractometer (Atago Hand-Held Refractometer N-1E) to measure °Bx values expressed as SS content in %.

2.2.3 Total phenolic content

Three tomatoes were rinsed, sliced into small cubes using a scalpel, transferred into a beaker and mixed. The same method was applied for the potatoes, except the peel was removed and discarded before slicing the tubers into small cubes.

1 g of plant product was transferred into a 15 mL tube and 9 mL of 80% MeOH was added. The samples were incubated on ice on a shaker table at 120 rpm and centrifuged at 3 100 rpm at 4 °C for 10 min. The supernatant was transferred into 1.5 mL Eppendorf-tubes and stored at -20 °C until further analysis.

The analysis of total phenolic content followed the F-C assay (Ainsworth and Gillespie, 2007) with some modifications. The reagents were prepared to the same concentrations described in the protocol. 125 μL F-C reagent was added to a 96-well plate, followed by 25 μL of sample or standard. This order was used to prevent evaporation of the sample if added to an empty well. Three of the wells were blanks containing 250 μL of the F-C reagent. The plate was shaken for 10 sec and 100 μL of Na_2CO_3 was added after 2 min. The plates were covered with parafilm to prevent evaporation and incubated at

room temperature for 2 h. The samples were measured at 750 nm using the Thermo Scientific/Labsystems Multiskan MS micro plate reader and Ascent Software (Labsystems Multiscan MS, Helsinki, Finland).

Known concentrations of gaellic acid between 0 and 140 μg/mL were used for calibration. A standard curve shown in Figure A.1 in Appendices was made from the known concentrations and the total phenolics as mg gallic acid equivalents (GAE)/100 g DM calculated using the regression equation y = 0.006x. Equation 2.1 shows the calculations from raw data to total phenolic content

$$\text{mg GAE / 100 g DW} = \text{RD} / 0.006 \cdot (25 / 1000) / 1000 / \text{FW} \cdot 100 / \%\text{DM} / 100 \tag{2.1}$$

where RD is the measured absorbance raw data, FW is grams of fresh weight in the samples and %DM is the measured dry matter percentage from Section 2.2.1.

2.2.4 Antioxidant activity

The method of extraction followed the same procedure as the previous section (First two paragraphs of 2.2.3).

The analysis for antioxidant activity followed the FRAP method by Benzie and Strain (1996) with some modifications. The FRAP reagents were prepared in the same concentrations as described in the article and 155 μL FRAP reagent was transferred into wells on a 96-well plate.Three of the wells were blanks containing 155 μL of FRAP reagent. 5 μL of sample was added to each well, the samples were shaken for 10 sec and incubated at room temperature for 4 min. The plate was then shaken again for 10 sec and measured at 595 nm using the Thermo Scientific / Labsystems Multiskan MS micro plate reader and the Ascent software (Labsystems Multiscan MS, Helsinki, Finland).

Known concentrations of iron (III) sulfate heptahydrate ($FeSO_4 * 7H_2O$) between 0 and 3000 μmol / L were used for calibration. The standard curve shown in Figure A.2 in Appendices was made from the known concentrations and total antioxidant activity in mmol/100 g DM calculated using the regression equation y = 0.0003x. Equation 2.2 shows the calculations from raw data to antioxidant activity.

$$\text{mmol / 100 g DW} = \text{RD} / 0.0003 \cdot (5 / 1\,000\,000) / 1000 / \text{FW} \cdot 100 / \%\text{DM} / 100 \tag{2.2}$$

where RD is the measured absorbance raw data, FW is grams of fresh weight in the samples and %DM is the measured dry matter percentage from Section 2.2.1.

2.2.5 Metabolite profiling

The plant products were rinsed and sliced in the same way as in the sample preparation in the previous section. 1 g of plant product was then transferred into a 15 mL tube and 5 mL of 80 % MeOH was added. The samples were vortexed for 10 sec and incubated at 70 °C for 5 min. After incubation the lids were opened to release vapour before being placed in an ultrasonic bath (UltraSonik 57X, Elmsford, USA) for 60 min.

The samples were cooled to room temperature and the lids were opened to release vapour before centrifuging at 3 100 rpm at 4 °C for 10 min. 400 μL of the supernatant was transferred into round-bottomed 1.5 mL Eppendorf tubes. An extra lid was added to

the Eppendorf tube and five holes pierced trough the lid using a syringe needle. The tubes were placed in a Savant SpeedVac Plus (ThermoQuest, San Jose, California, USA) for drying over night. The lids with the holes were discarded, the Eppendorf tube sealed with its normal lid, and the samples stored in a -80 °C freezer prior to derivatization.

For the derevatisation the samples of dried residue was redissolved in 80 μL of 20 mg/mL methoxyamine hydrochloride in pyridine and derivatised at 30 °C for 90 min. If the residue was not completely dissolved after the 90 min, the tubes were transferred to an ultrasonic bath at 30 °C for 30 min, vortexed for 10 sec, and transferred back for incubation at 30 °C for 60 min. The last step of derivatisation was treating the samples with 80 μL of N-Methyl-N–(trimethylilyl)-trifluoroacetamide (MSTFA) at 37 °C for 30 min. The samples were then transferred into 1.5 mL autosampler vials with glass inserts and stored at -20 °C prior to GC-MS.

Metabolite profiling by GC-MS was performed using Agilent 6890/5975 GC-MS system (Agilent technologies, Santa Clara, California, USA) for all analyses. 1 μL sample volumes were injected with a split ratio of 25:1. GC separations were carried out on a HP-5MS capillary column with inner diameter 30 m x 0.25 mm and film thickness 0.25 μm. Injection temperature was 230 °C and He was used as a carrier gas at a constant flow rate of 1 mL/min. The GC temperature programme was held at 70 °C for 5 min, then increased to 310 °C at a rate of 4 °C/min, and held at 310 °C for 7 min, giving a total analysis time of 60 min per sample. The MS source was adjusted to 230 °C and the mass range of *m/z* 70-700 was recorded. The mass spectra were acquired in electron impact ionisation mode.

The peak area integration and chromatogram visualisation were performed using Agilent ChemStation software (Agilent Technologies, Waldbronn, Germany.) For peak identification and mass spectra evaluation, Automated Mass spectral Deconvolution and Identification Software (AMDIS) (version 2.71; National Institute of Standards and Technology, Boulder, CO, USA) was used. In addition to AMDIS the NIST05 database and a target TMS database (Max-Planck Institute for Molecular Plant Physiology, Golm, Germany) were used as supplements.

The detected metabolites were measured using peak area integration with the MetAlign software (see Lommen (2009)) and assessed quantitatively based on the internal standard ribitol and expressed as mg/100 g dry weight (DW). The MetAlign setup used is shown in Figure A.3 in Appendices. Equation 2.3 was used to calculate mg/100 g DW from the raw data of fresh samples from the GC-MS and MetAlign.

$$\text{mg / 100 g DW} = \text{RDM / RDIS} \cdot 80 \cdot 12.5 / 1000 \cdot 100 \cdot (100 / \text{DME}) \tag{2.3}$$

where RDM is the metabolite raw data in the sample, RDIS is the raw data of the internal standard (ribitol) in the sample, and DME is the dry matter estimate measured in section 2.2.1. The dried samples needed two more factors. Equation 2.4 was used to calculate mg/100 g DW from the raw data of dried samples.

$$\text{mg / 100 g DW} = \text{RDM / RDIS} \cdot 80 \cdot 12.5 / 1000 \cdot 100 \cdot (100 / \text{DME}) / (100 / \text{DMED}) \cdot \text{IS} \tag{2.4}$$

where RDM is the metabolite raw data in the sample, RDIS is the raw data of the internal standard (ribitol) in the sample, and DME is the dry weight estimate measured in section 2.2.1. DMED are the measured dry weight after the samples were dried at

40 °C (D40) or 60 °C (D60) over night and IS is a factor added to the dried samples to make the results comparable to the fresh samples.

The internal standard values after GC-MS in the dried samples were much lower than in the fresh samples. To get comparable results between fresh and dried samples the internal standard values should be in the same range. A factor was therefore calculated to be added to the dried samples. The IS factor was calculated from the raw data of the internal standard ribitol. The average of the dried samples were divided by the average of the control sample P0, expressing the dried samples as a fraction of P0. This fraction was subtracted by 1 to find the fraction of ribitol missing in the dried samples and then multiplied by the dried sample values to obtain values in a similar range for both fresh and dried samples.

2.3 Data analysis

The data from the biochemical analysis were analysed using Microsoft Excel and Minitab statistical software. All estimates are given as sample mean (n=3) and standard deviation.

A one-way analysis of variance (ANOVA) was used to test for significant differences between the SS content in the samples stored at room temperature at $\approx$ 20 °C (RT) and the samples stored at a storage temperature at $\approx$ 4 °C (ST), and in each treatment over the storage period. To test for significant differences between RT and ST at each sampling day a paired sample t-test was used on the sample means from each sampling day.

One-way ANOVA was performed on the results of total phenolic content and antioxidant activity to test for significant differences between treatments (RT, ST, D40 and D60) and over the storage period within treatments using Minitab statistical software. In addition to analysis of the four treatments the samples of fresh plant material (RT and ST) and the samples of dried plant material (D40 and D60) were analysed separately. The FRAP results from the dried potato samples were very low in concentration and the raw data samples were therefore tested for significant differences to the blank samples using a one-way ANOVA.

The identified metabolites from GC-MS tested for significant differences between treatments (RT, ST, D40 and D60) at each sampling day using one-way ANOVA in MultiExperiment Viewer (MeV). The metabolites selected for further discussion were tested for significant differences using one-way ANOVA between the fresh samples, the dried samples and over the storage period within each treatment.

All ANOVA results were analysed using a least significant difference of 5 %. Samples close to 5 % ($0.049 < p\text{-value} < 0.051$) were defined to have weak statistical differences.

Multivariate statistics for comparison of metabolite concentrations were performed using principal component analysis (PCA) and hierarchical clustering. PCA was performed using Minitab and visualised using score- and loading plots. PCA was completed for fresh and dried samples together and separately. Hierarchical clustering was performed on all samples creating a heat map using MeV. To construct the heat map in MeV the data points from the GC-MS were divided by the median of the samples for each metabolite, and changed to a logarithmic scale with base 2 using Microsoft Excel. The data set was then analysed in MeV using hierarchical clustering.

Chapter 3

Results

This chapter presents the results of this study displayed as tables and figures, with supplementary data included in Appendices. The results have been divided into two sections, the first section represent the results from the analysis of potato and the second is the results of the analysis of tomato. All estimates are given as sample mean (n=3) and standard deviation.

A total of 93 metabolites were identified in potato and 66 in tomato based on GC-MS analysis. Multivariate analysis gave an overall assessment of the quality changes in the plant products. In addition to the multivariate analysis 8 selected metabolites from each plant product were assessed individually. The selection of the metabolites was made to include both primary and secondary metabolites. The sugars fructose, glucose and sucrose, and organic acids malic and citric acid, were included, as sugars and acids make up a large part of the flavour quality, and were present in high concentrations in both potato and tomato.
4-Aminobutyric acid (GABA) was selected being an amino acid present in high concentration in both potato and tomato. Chlorogenic acid was chosen for it being a phenolic compound which can further be compared with the results from the F-C assay. Asparagine was only included in the section for potato. The amino acid was chosen due to it forming acrylamide with reducing sugars in potato. In the section for tomato α-Tocopherol was chosen, as it is an important component of vitamin E that was only identified in the tomato samples.

The results of the dry matter estimate, soluble solids content, total phenolic content and antioxidant activity will be presented first, followed by the metabolite profiling and multivariate analysis.

3.1 Potato

3.1.1 Dry matter estimate

The results of the DM estimate is summarised in Table 3.1 where % DM is the percentage of dry matter that remained after drying. These estimates were used when calculating the resulting values for total phenolic content, antioxidant activity and concentrations of metabolites from metabolite profiling. The results in Table 3.1 show that the percentage of dry matter were higher for D40 samples than for D60 samples, who were in turn higher than the samples dried at 105 °C.

Table 3.1: Dry matter estimate results of potatoes incubated over night at 40 °C, 60 °C and 105 °C. % DM is the percentage of dry matter that remained after drying.

Temperature	% DM	*Stdev*
40	27.88	*1.03*
60	26.55	*0.44*
105	23.57	*2.97*

3.1.2 Soluble solids content

The results from the estimation of soluble solids using a hand-held refractometer is shown in Table 3.2. SS content increased when stored at both room temperature and storage temperature, with a significant difference found between the samples of different sampling days ($p = 0.005$). The SS content seemed to be higher in the RT samples than the ST samples throughout the storage period, but there was found no significant difference between the treatments ($p = 0.642$). Using a paired t-test there was found a significant difference ($p = 0.034$) between the two treatments compared for each sampling day.

Table 3.2: Soluble solids (SS) content in potatoes measured in °Bx. Sampling day is the number of days in storage after harvest before sample extraction. RT are potatoes stored in room temperature ($\approx 20^{\circ}C$) and ST are potatoes stored in storage temperature ($\approx 4^{\circ}C$).

	RT		ST	
Sampling day	SS	*Stdev*	SS	*Stdev*
0	5.27	*0.15*	5.27	*0.15*
3	5.57	*0.06*	5.40	*0.40*
7	6.03	*0.45*	5.77	*0.21*
14	6.07	*0.21*	5.80	*0.17*

3.1.3 Total phenolic content

The result from the F-C assay is shown in Figure 3.1. The numerical values are presented in Table A.1 in Appendices. There was a much larger concentration of phenolics in the fresh samples compared to the dried samples, with a significant difference found between fresh and dried samples ($p = 0.000$). The fresh ST samples seemed to have higher concentrations than the RT samples, with a weak statistical difference between the two treatments ($p = 0.050$). Total phenolic content remained stable the first week at room temperature before increasing the last week, with no significant difference found in the RT samples over the storage period ($p = 0.617$). Total phenolic content in the ST samples increased the the first week before declining slightly the last week, and there was found no significant differences in the ST samples over the storage period ($p = 0.568$).

In the dried samples the phenolic content was relatively stable throughout the storage period, declining slightly in the D60 samples, but there was found no significant difference in the D60 samples over the storage period ($p = 0.260$). The phenolic content was found increase slightly in the D40 samples the first week before declining the last week, there was found no significant difference in the samples over the storage period ($p = 0.153$). There was found no significant difference in total phenolic content between the D40 and D60 samples ($p = 0.392$).

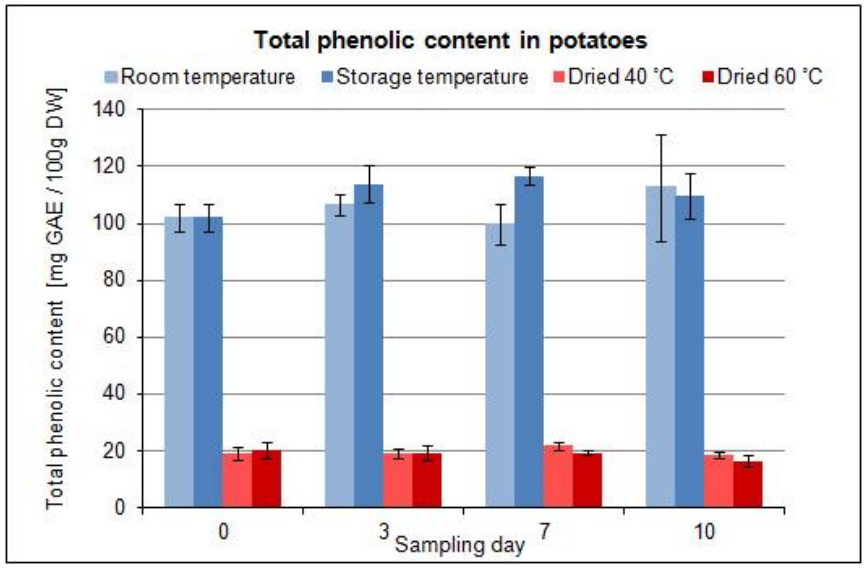

Figure 3.1: Measured total phenolic content in mg GAE/ 100 g DW in potatoes. The samples are colour grouped by treatment. Sampling day is the number of days after harvest the sample was extracted. The error bars show ± standard deviation.

3.1.4 Antioxidant activity

The results of the FRAP assay is shown in Figure 3.2. The numerical values are presented in Table A.2 in Appendices. The results show a very low antioxidant activity in the dried samples compared to the fresh samples. The fresh samples were found to be significantly different to the dried samples (p = 0.000). The antioxidant activity in the fresh samples decreased the first 3 days before increasing slightly again in the P7 samples. There was found no significant difference in the RT samples (p = 0.275) or in the ST samples (p = 0.176) over the storage period. The ST samples had higher antioxidant activity concentrations than the RT samples on all sampling days, however the two treatments were not found to be significantly different (p = 0.185).

The concentrations of antioxidant activity were very low in the dried samples varying between 0.02 and 0.05 mmol/100 g DW. Although the FRAP results were low the samples were found to be significantly different from the blank samples (p = 0.000). The dried samples were of a similarly low concentration in both treatments, there was found no significant difference in the D40 samples compared to the D60 samples (p = 0.062). Samples of both dried temperatures were increasing slightly the first week and decreased at the last measurement P3. There were found no significant differences in the D40 samples (p = 0.166) or in the D60 samples (p = 0.066) over the storage period.

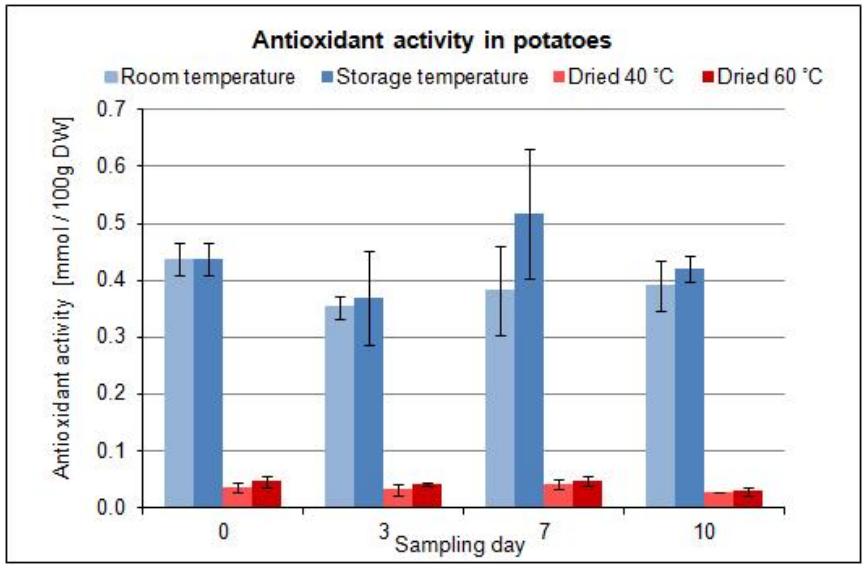

Figure 3.2: Measured antioxidant activity in mmol/ 100 g DW in potatoes. The samples are colour grouped by treatments. Sampling day is the number of days after harvest the sample was extracted. The error bars show ± standard deviation.

3.1.5 Metabolite profiling

Figure 3.3 shows selected metabolites from GC-MS, which are related to potato quality. The *p*-values of the 8 selected metabolites are presented in Table 3.3. The complete table of identified metabolites with *p*-values is presented in tables A.5 to A.8 in Appendices.

Table 3.3: *p*-values of the selected metabolites of potato in Figure 3.3. Fresh / dried are the fresh samples compared to the dried samples. ST / RT are the ST samples compared to the RT samples, D40 / D60 are the D40 samples compared to the D60 samples and SD RT, SD ST, SD D40 and SD D60 are the sampling days compared within each treatment.

	p-value						
Metabolite	Fresh / dried	ST / RT	D40 / D60	SD RT	SD ST	SD D40	SD D60
4-Aminobutyric acid	0.000	0.033	0.000	0.695	0.773	0.002	0.000
Asparagine	0.000	0.635	0.843	0.599	0.317	0.060	0.233
Malic acid	0.000	0.204	0.073	0.138	0.058	0.352	0.017
Citric acid	0.000	0.191	0.000	0.505	0.487	0.006	0.038
Fructose	0.345	0.002	0.348	0.553	0.001	0.000	0.000
Glucose	0.008	0.001	0.045	0.570	0.003	0.002	0.046
Sucrose	0.000	0.151	0.000	0.649	0.590	0.010	0.032
Chlorogenic acid	0.000	0.000	0.968	0.707	0.008	0.006	0.000

All metabolites except fructose were found to show significant differences between the fresh and dried samples. 4-Aminobutyric acid, fructose, glucose and chlorogenic acid had significantly different values in the RT samples to the ST samples. 4-aminobutyric acid, citric acid, glucose and sucrose had significantly different values in the D40 samples to the D60 samples. In the RT samples there were no metabolites with significantly different values over the sampling days. In the ST samples fructose, glucose and chlorogenic acid were found to be significantly different over the sampling days. In the D40 samples all metabolites except asparagine and malic acid were found to be significantly different over the sampling days, and in the D60 samples asparagine was the only metabolite that was not found to be significantly different over the sampling days.

4-aminobutyric acid (GABA) in Figure 3.3a) had a much lower concentration in the dried samples than in the fresh samples. The fresh sample treatments seemed to remain relatively stable over the storage period, where both the dried samples seemed to decrease slightly over the storage period. The RT samples were higher than the ST samples, and the D40 samples were higher than the D60 samples.

Asparagine in Figure 3.3b) had a much lower concentration in the dried samples than in the fresh samples. The concentrations in the fresh samples seemed to increase in the P7 samples before decreasing in the P14 samples, and the dried samples levels seemed to slightly decrease over the storage period. However, no significant differences were found between the RT and ST samples, the D40 and D60 samples or in any of the treatments over the storage period.

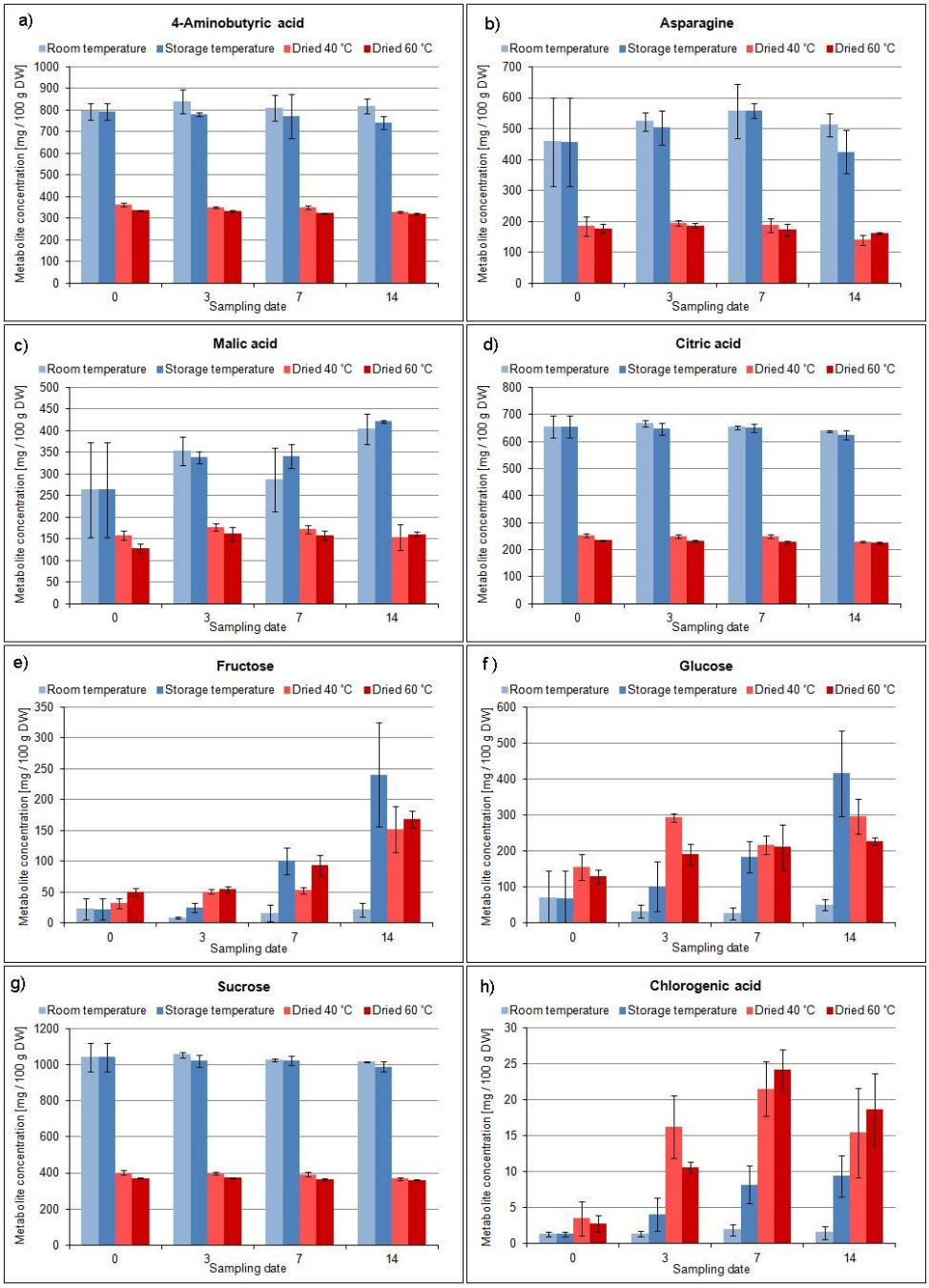

Figure 3.3: Concentrations of selected metabolites based on GC-MS in potatoes a) 4-aminobutyric acid, b) asparagine, c) malic acid, d) citric acid, e) fructose, f) glucose, g) sucrose and h) chlorogenic acid [mg / 100 g DW]. The samples are colour grouped by treatment. The error bars show ± standard deviation.

The malic acid in Figure 3.3c) was higher in the fresh samples compared to the dried samples. The RT samples and ST samples were in the same range with high variation in some samples. The D40 and D60 samples were of similar concentrations as well. Sample concentrations in both of the dried treatments seemed to increase in the P3 samples before stabilising and declining slightly by the P14 samples. There was only found significantly different concentrations over the storage period in the D60 samples.

The concentration of citric acid in Figure 3.3d) was much lower in the dried samples than in the fresh samples. The RT samples were similar to the ST samples with very little variation throughout the storage period. The dried samples seemed to decrease over the storage period with the D40 samples being slightly higher than the D60 samples throughout.

Fructose in Figure 3.3e) was the only metabolite with no significant difference between the fresh and the dried samples. The ST samples were much larger than the RT samples with ST samples increasing the most in the P7 and P14 samples. The RT sample concentrations remained stable throughout the sampling days. Both the dried sample treatments increased over the storage period, with a large increase in the P14 samples. The D60 samples seemed to be slightly higher than the D40 samples, but they were not found to be significantly different from each other.

Figure 3.3f) shows that the glucose concentrations were higher in the dried samples compared to the fresh samples except for the P14 ST samples. The RT samples had similar concentrations throughout the storage period but the ST samples increased each sampling day. The D40 samples had higher concentrations of glucose compared to the D60 samples, increasing in the P3 samples, decreasing in the P7 samples and increasing again in the P14 samples. The D60 samples increased steadily over the storage period.

The sucrose concentrations in Figure 3.3g) were much higher in the fresh samples compared to the dried samples. The fresh samples seemed to decrease over the storage period with RT samples being slightly higher than the ST samples. There were however found no significant differences found between the fresh treatments, or within the fresh treatments over the storage period. The D40 samples were found to be significantly different from the D60 samples with the D40 samples having higher concentrations throughout the storage period. Both the D40 and D60 samples decreased slightly over the storage period.

The chlorogenic acid concentrations in Figure 3.3h) were higher in the dried samples than in the fresh samples. ST samples were significantly different than the RT samples with RT samples remaining stable and ST samples increasing over the storage period. The D40 and D60 samples were not found to be significantly different to each other, but both were found to increase up to the P7 samples before decreasing slightly in the P14 samples.

3.1.6 Multivariate analysis

Two multivariate analyses were performed on the results from GC-MS to visualise data variation and relationships: Principal component analysis and a cluster analysis using hierarchical clustering on a heat map. The samples in the multivariate analysis were labeled according to sampling day (P0 = harvest date, P3= day 3, P7 = day 7 and P14 = day 14), sample treatment (RT = fresh sample stored at room temperature, ST = fresh sample stored at storage temperature, D40 = sample dried at 40 °C and D60 = sample dried at 60 °C over night) and sample number (n=3) (1, 2 and 3).

PCA

Principal component analysis was performed on all the samples together as well as on the fresh and dried samples separately. PCA showed patterns in variation of the data sets by orthogonal transformation creating principal components. The first principal component (FC) contributed to the largest variability in the data set, and the second principal component (SC) contributed to the largest variation that was uncorrelated with the first component. The figures in this section include a score plot and loading plot for all samples, and a score plot and loading plot for the fresh samples. The fresh samples were assessed separately to see how the RT and ST samples, and the samples at each sampling day grouped together. This was also done for the dried samples, but there was found no distinct pattern in the dried samples and therefore the results were omitted. The loading plot displays the relative significance of the metabolites in the samples. The loading plot can be superimposed on the score plot to assess which metabolites have an impact on the data pattern in the score plot.

The score plot of all the samples in Figure 3.4 showed the FC accounting for 44.3 % of the variation in the data set and the SC accounting for 13.6 % of the variation.There was a clear separation of the fresh and dried samples on the FC axis. The samples seemed to be evenly distributed on the SC, except from one outlier in the fresh samples.

The loading plot in in Figure 3.5 indicate that most of the metabolites were distributed along the principal component axes. The fatty acids, chlorogenic acid, glucose fructose and some of the nitrogen compounds contributed to the variation on the negative axis of the FC towards the dried samples. Phosphates, acids, amino acids, some nitrogen compounds and sucrose contributed to the variation on the positive axis of the FC towards the fresh samples.

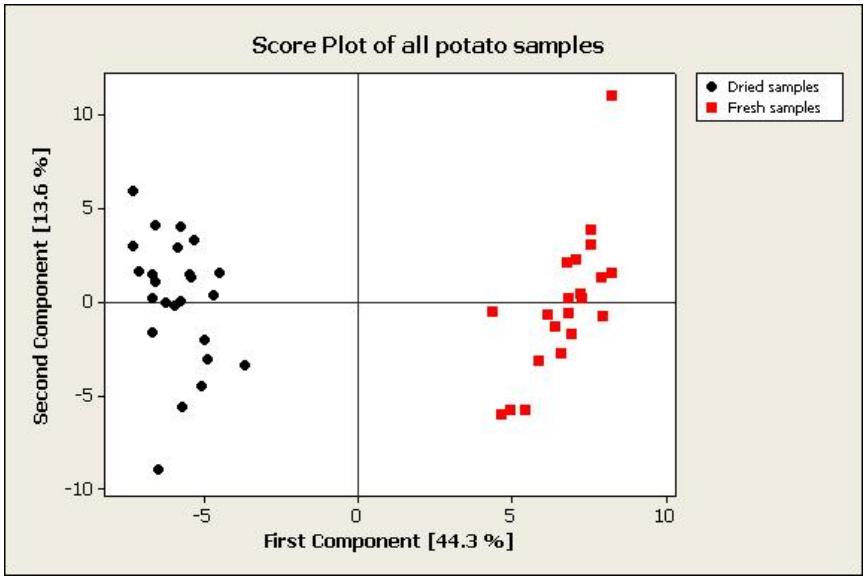

Figure 3.4: Score plot from PCA of all potato samples. Eigenvalue proportions of each component marked as a percentage on the axis label. The samples are colour grouped as fresh/dried samples.

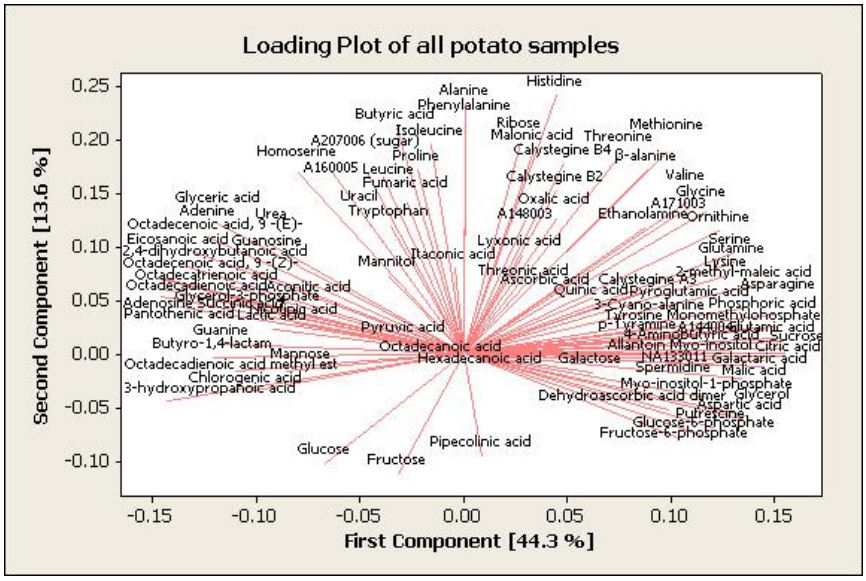

Figure 3.5: Loading plot from PCA of all potato samples. Eigenvalue proportions of each component marked as a percentage on the axis label. The metabolite names have been moved slightly to not overlap with each other.

Figure 3.6 shows the score plot of the fresh potato samples. In the fresh samples the FC accounted for 24.5 % of the variation in the data set, and the SC accounted for 13.9 %. The plot indicates a separation of ST samples and RT samples on the SC axis for all except one ST sample on the positive axis of SC, and all except one RT sample on the negative axis. The P0 control samples were located on the negative axis of the SC with the RT samples except the sample P0_1 which was located to the top far right corner of the plot. The sample was located so far off that it might be classified as an outlier. There was found no distinct patterns of grouping on the FC axis regarding treatment or storage period.

The loading plot of the fresh potato samples presented in Figure 3.7 showed that the metabolites that contributed to the variation on the negative SC axis included many of the amino acids and organic acids. Contributing to the variation on the positive SC axis were the metabolites glucose, fructose and chlorogenic acid. On the FC axis sucrose, citric acid, amino acids and nitrogen compounds contributed on the positive axis with phosphates, acids, glucose and fructose contributing on the negative FC axis.

Cluster analysis

The cluster analysis of the potato samples is shown as a heat map with hierarchical trees in Figure 3.8. The samples are listed on top of the figure with the clusters beneath them and the metabolites are listed to the right of the figure with the clusters displayed on the left side of the heat map.

Two main clusters were formed within the samples, one including the dried samples and one including the fresh samples. Within the cluster of dried samples 5 smaller clusters can be distinguished. Clusters 1 and 4 from the left included samples of both D40 and D60 samples. Cluster 2 and 3 from the left included only D40 samples, and the far right cluster included only D60 samples. The fresh sample clusters were all mixed clusters that seemed to group in no specific pattern regarding storage temperature or sampling day. The fresh samples were in general clustered closer together than the dried samples, except the dried cluster to the far right. This suggests that the fresh samples are more highly correlated than the dried samples.

The metabolites clustered together according to concentrations. A large cluster of metabolites with high concentrations in the fresh samples and lower concentrations in the dried samples formed (pyroglutamic acid-ethanolamine). Another distinct cluster was formed where the concentrations are low in the fresh samples and higher in the dried samples (aconitic acid-mannose). There were two more clusters formed with mixed concentrations across the dried and fresh samples and with many samples having a concentration around the median value. These two clusters included octadecanoic acid-pipecolinic acid and A148003-tryptophan. Within these larger clusters there were found two smaller clusters of metabolites with close correlation to each other. One of which was a mixed cluster of different primary metabolites relating to glycolysis and Krebs cycle (glycerol-A144004). The other showed a strong correlation between fatty-acid metabolism related compounds (octadecadienoic acid methyl ester-octadecenoic acid, 9-(E)-).

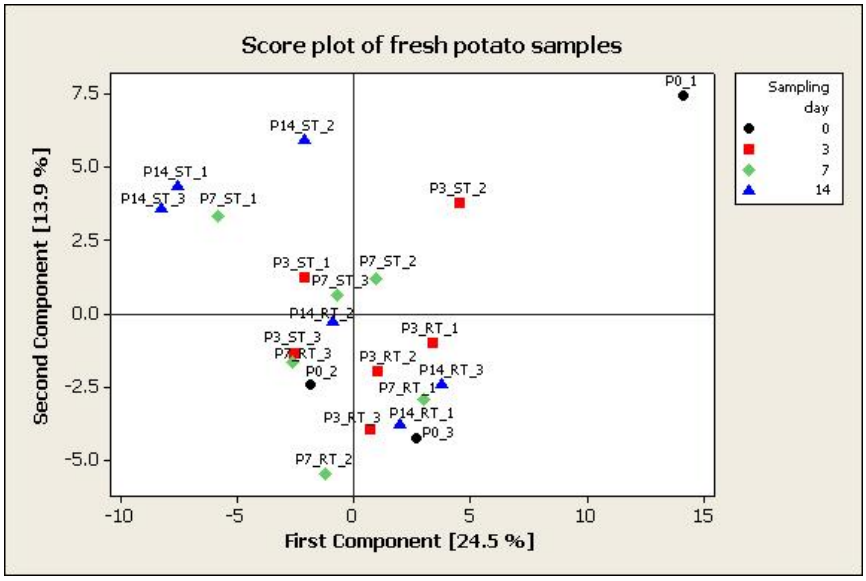

Figure 3.6: Score plot from PCA of fresh potato samples. Eigenvalue proportions of each component marked as a percentage on the axis label. The samples are colour grouped by sampling day.

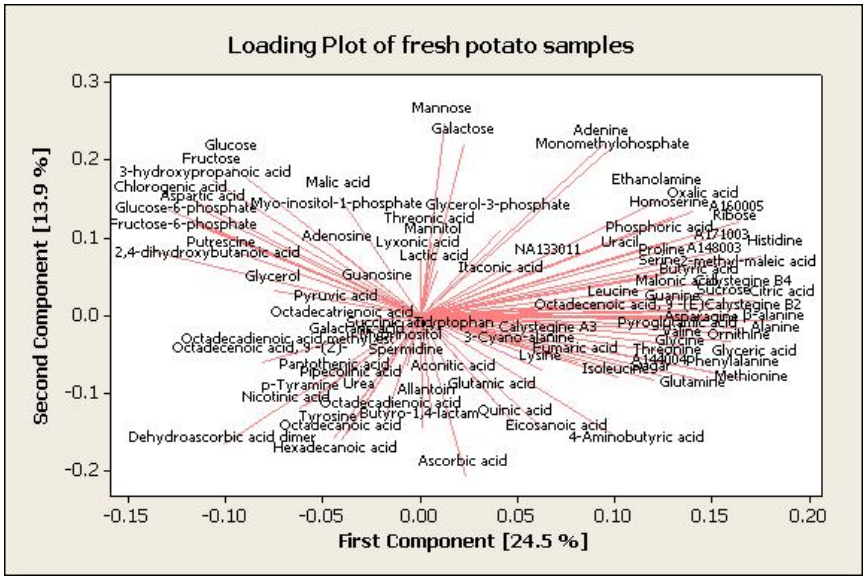

Figure 3.7: Loading plot from PCA of fresh potato samples. Eigenvalue proportions of each component marked as a percentage on the axis label. The metabolite names have been moved slightly to not overlap with each other.

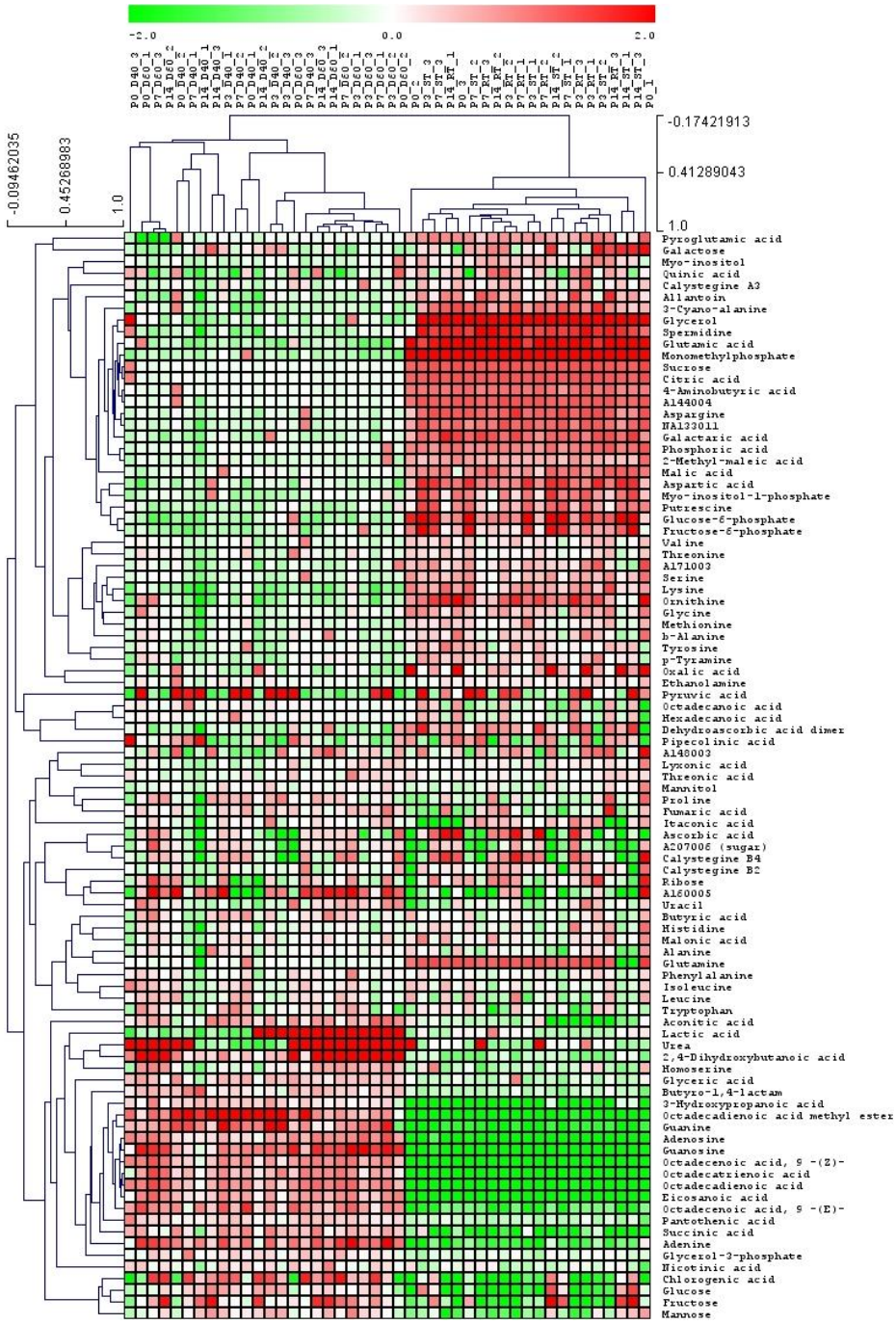

Figure 3.8: Hierarchical tree of the cluster analysis of metabolites in potato samples. Red colour indicates higher metabolite concentration than the median of the samples, green indicates a lower concentration. The metabolites are listed on the right side of the heat map with metabolite clustering on the left. All samples are listed on the top of the map as well as the sample clustering tree.

3.2 Tomato

3.2.1 Dry matter estimate

The results of the DM estimate is summarised in Table 3.4 where % DM is the percentage of dry matter that remained after drying. These estimates were used when calculating the resulting values for total phenolic content, antioxidant activity and concentrations of metabolites from metabolite profiling. The results in Table 3.4 show that the percentage of dry matter were higher in the D40 samples than in the D60 samples, who were in turn similar to the samples dried at 105 °C, indicating that samples dried at 60 °C over night had a dry weight resembling the dry matter content of tomato.

Table 3.4: Dry matter estimate results of tomatoes incubated over night at 40, 60 and 105 °C. % DM is the percentage of dry matter that remained after drying.

Temperature	% DM	*Stdev*
40	6.68	*0.15*
60	6.19	*0.08*
105	6.20	*0.50*

3.2.2 Soluble solids content

The results from the estimation of soluble solids using a hand-held refractometer is shown in Table 3.5. The SS content was higher in RT samples than in ST samples throughout the storage period, there was however found no significant difference between the two treatments ($p = 0.338$). The SS content in RT samples fluctuated over the sampling days decreasing by the end of the storage period. The SS content in the ST samples decreased over the storage period. There was found no significant difference in the samples over the storage period ($p = 0.648$). Using a paired t-test there was found a weak statistical difference ($p = 0.051$) between the treatments at each sampling day.

Table 3.5: Soluble solids (SS) content in tomatoes measured in °Bx. Sampling day is the number of days in storage after harvest before sample extraction. RT are tomatoes stored in room temperature and ST are tomatoes stored in storage temperature.

	RT		ST	
Sampling day	SS	*Stdev*	SS	*Stdev*
0	5.20	*0.20*	5.20	*0.20*
3	5.17	*0.15*	5.13	*0.23*
7	5.23	*0.21*	5.13	*0.23*
10	5.10	*0.30*	5.00	*0.20*

3.2.3 Total phenolic content

The result from the F-C assay is shown in Figure 3.9. The numerical values are presented in Table A.3 in Appendices. The phenolic content in tomato was almost doubled in the fresh samples compared to the dried samples, the fresh and dried samples were found to be significantly different ($p = 0.000$). In the fresh samples the phenolic content seemed to decrease by day T3 and T7 before increasing by T10, but there were found no significant differences in the RT samples ($p = 0.717$) or in the ST samples ($p = 0.457$) over the storage period. Samples stored at storage temperatures seemed to remain lower than samples stored in room temperature, but the RT samples were not found to be significantly different to the ST samples ($p = 0.110$).

The samples dried at 60 °C had higher concentrations of phenolic content than the samples dried at 40 °C, and D40 samples were found to be significantly different to D60 samples ($p = 0.000$). Levels in D60 samples seemed to decrease slightly in concentration in the T3 and T7 samples and increase in the T10 samples. The D40 samples remained stable throughout the storage period. There was found no significant difference in the D40 samples (0.451) or the D60 samples ($p = 0.149$) over the storage period.

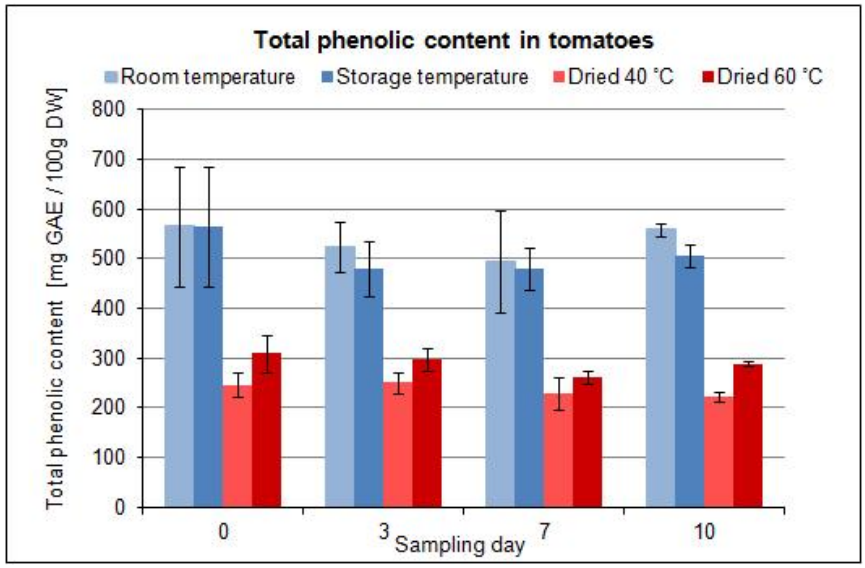

Figure 3.9: Measured total phenolic content in mg GAE/ 100 g DW in tomatoes. Samples are colour grouped by treatment. Sampling day is the number of days after harvest the sample was extracted. The error bars show ± standard deviation.

3.2.4 Antioxidant activity

The results of the FRAP assay is shown in Figure 3.10. The numerical values are presented in Table A.4 in Appendices. Antioxidant activity decreased by half in the dried samples compared to the fresh samples and a significant difference between the dried and fresh samples was found ($p = 0.000$). In the RT samples the antioxidant activity decreased throughout the ten days, and the concentrations were found to be significantly different over the storage period ($p = 0.025$). In the ST samples there was a drop in antioxidant activity in the T3 samples followed by an increase by T7, and a decrease in the T10 samples. The concentrations of the ST samples were found to be significantly different over the storage period ($p = 0.009$). The ST samples were lower or equal to the RT samples and the treatments were found to be significantly different ($p = 0.019$).

D40 samples seemed to have lower antioxidant concentrations than the D60 samples throughout the storage period, but the two treatments were not found to be significantly different ($p = 0.134$). The D40 samples had higher FRAP values than the D60 samples the first week of storage before evening out. Antioxidant activity in both the D40 and the D60 samples decreased the first week before increasing slightly in the T10 samples. The D40 samples were found to be significantly different over the storage period ($p = 0.028$), the D60 samples were not found to be significantly different over the storage period ($p = 0.106$).

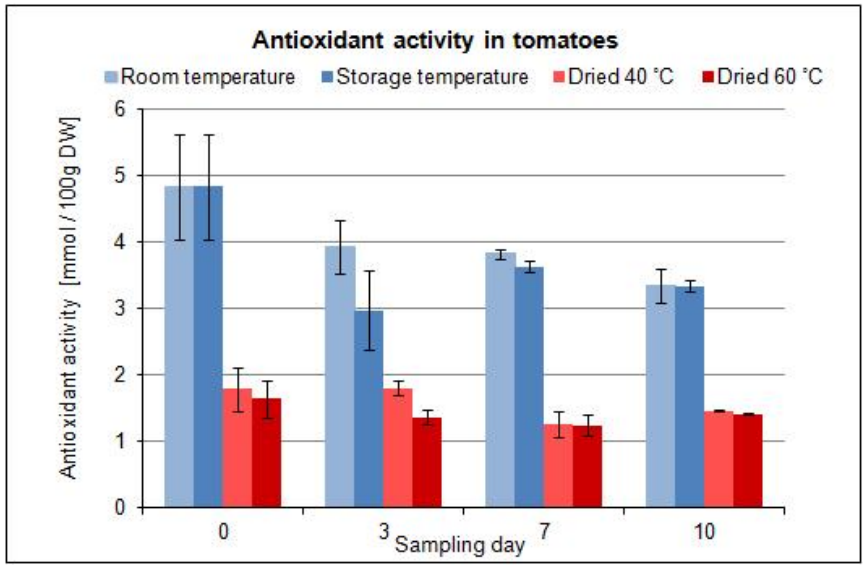

Figure 3.10: Measured antioxidant activity in mmol/ 100 g DW in tomatoes. Samples are colour grouped by treatment. Sampling day is the number of days after harvest the sample was extracted. Error bars show ± standard deviation.

3.2.5 Metabolite profiling

Figure 3.11 shows the selected metabolites which were to assessed in relation to tomato quality. The p-values of the selected metabolites are shown in Table 3.6. The complete table of identified metabolites and p-values are presented in tables A.9 to A.12 in Appendices. Samples T3_4_2 and T3_6_3 were omitted from analysis due to unsatisfying GC-MS values.

Table 3.6: p-values of the selected metabolites of tomatoes in Figure 3.11. Fresh / Dried are the fresh samples compared to the dried samples. ST / RT are the ST samples compared to the RT samples, D40 / D60 are the D40 samples compared to the D60 samples and SD RT, SD ST, SD D40 and SD D60 are the sampling days compared within each treatment.

	p-value						
Metabolite	Fresh/dried	ST/RT	D40/D60	SD RT	SD ST	SD D40	SD D60
4-Aminobutyric acid	0.000	0.977	0.008	0.105	0.425	0.237	0.514
α-Tocopherol	0.000	0.106	0.000	0.922	0.874	0.930	0.451
Malic acid	0.000	0.244	0.280	0.027	0.446	0.606	0.430
Citric acid	0.000	0.805	0.032	0.686	0.826	0.641	0.154
Fructose	0.005	0.565	0.001	0.749	0.799	0.402	0.142
Glucose	0.618	0.851	0.001	0.485	0.970	0.453	0.170
Sucrose	0.000	0.000	0.001	0.014	0.366	0.881	0.348
Chlorogenic acid	0.001	0.489	0.000	0.004	0.009	0.492	0.675

All metabolites except glucose were found to have significantly different values in the fresh samples compared to the dried samples. Sucrose was the only metabolite that was found to have significantly different values in the RT samples compared to the ST samples. All metabolites except malic acid were found to have significantly different values in D40 samples compared to D60 samples. Malic acid, sucrose and chlorogenic acid were found to have significantly different values in the RT samples over the sampling days. Only chlorogenic acid was found to have significantly different values in the ST samples over the sampling days. None of the metabolites were found to be significantly different in the D40 samples or the D60 samples over the sampling days.

Some of the concentrations measured were very small in some of the treatments compared to other treatments, and could not be interpreted well from the bar charts; these have been labeled with the numerical value above the bars in Figure 3.11.

GABA in Figure 3.11a) had very low values in the dried samples. The RT and ST samples were relatively similar and both seemed to decrease over the storage period, but there were found no significant differences in either treatment over the sampling days. The D40 samples showed lower levels than the D60 samples.

The α-tocopherol in Figure 3.11b) showed much higher values in the dried samples compared to the fresh samples, and the D60 samples were much higher than the D40 samples. The D60 samples seemed to increase throughout the storage period, but there was found no significant difference in the samples over the storage period.

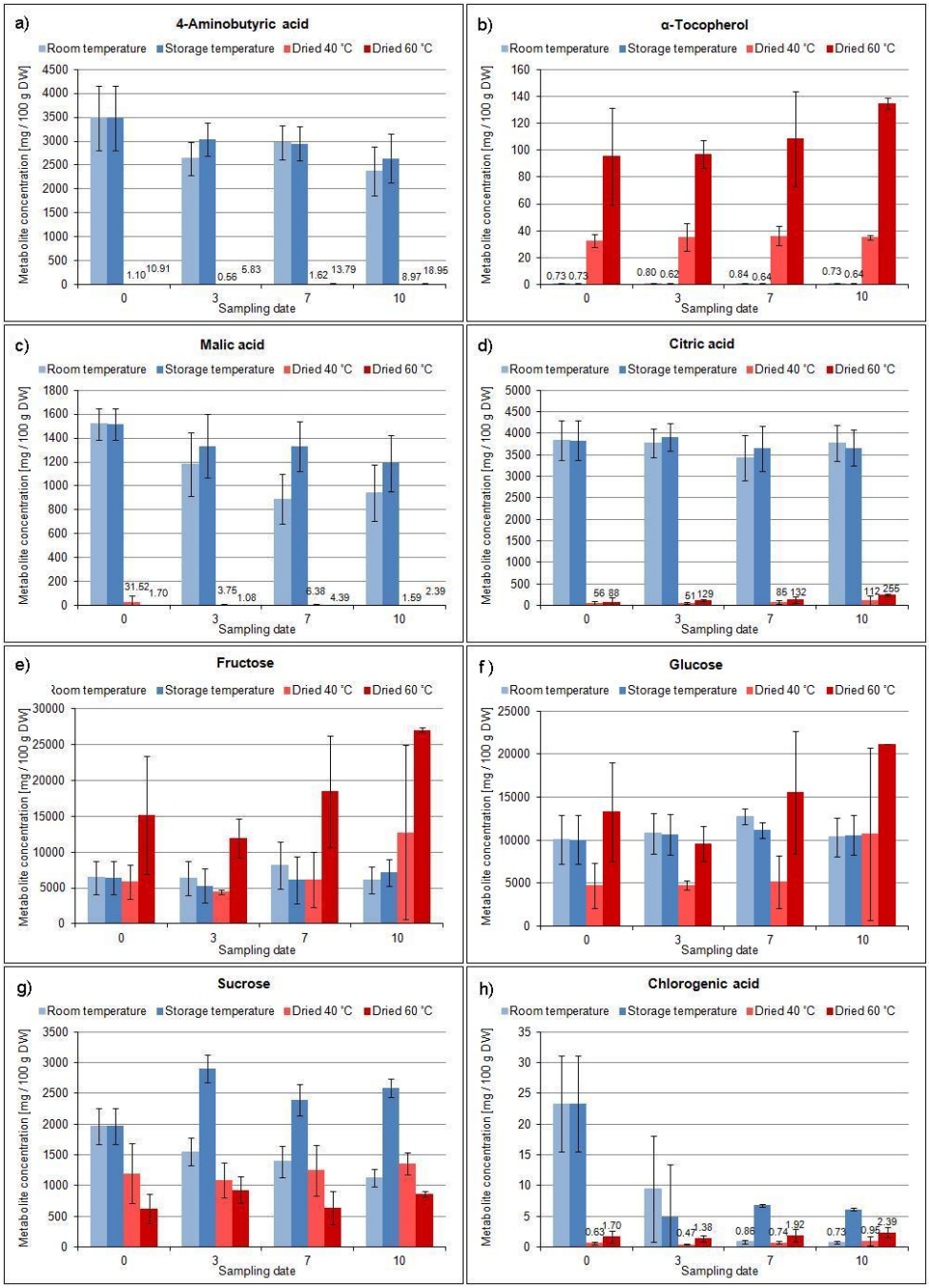

Figure 3.11: Concentrations of selected metabolites based on GC-MS in tomatoes a) 4-aminobutyric acid, b) α-tocopherol c) malic acid, d) citric acid, e) fructose, f) glucose, g) sucrose and h) chlorogenic acid [mg / 100 g DW]. The samples are colour grouped by treatment. The error bars show $\pm$ standard deviation.The smaller bars have the numerical value denoted above them.

Malic acid in Figure 3.11c) had significantly higher values in the fresh samples compared to the dried samples. The RT sample concentrations decreased during the storage period. The ST samples also seemed to decrease during the storage period but there was found no significant difference in the samples over the storage period. The D40 and D60 samples were not found to be significantly different to each other and sample concentrations of malic acid in both treatments remained stable over the storage period.

The citric acid in Figure 3.11d) had much higher concentrations in the fresh samples compared to the dried samples. The RT samples were similar to the ST samples and both treatments remained stable throughout the storage period. The D60 samples were higher than the D40 samples. Both dried samples seemed to increase over the storage period, but there were found no significant differences over the sampling days in samples of either treatments.

The fructose in Figure 3.11e) had significantly higher concentrations in D60 samples compared to the fresh samples, but the D40 samples had similar concentrations to the fresh samples. The RT and ST samples were of similar concentrations that remained stable over the storage period. The D60 samples seemed to increase over the storage period, and the D40 samples seemed to remain the same over the storage period. Neither of the dried samples were found to be significantly different over the storage period.

The glucose in Figure 3.11f) was the only selected metabolite showing no significant difference between the fresh and dried samples. The D40 samples were found to be lower than the D60 samples throughout the storage period. Both the RT, ST and D40 samples remained stable over the storage period with some samples having very high standard deviations. The D60 samples concentrations seemed to increase by T10, but there was found no significant difference in the D60 samples over the storage period.

The sucrose concentrations in Figure 3.11g) were higher in the fresh samples compared to the dried samples. The RT sample concentration decreased over the storage period. The ST samples seemed to increase over the storage period, but there were found no significant differences in the ST samples over the sampling days. The D40 samples were higher in sucrose concentration than the D60 samples.

Chlorogenic acid in Figure 3.11h) had higher concentrations in the fresh samples than the dried samples in T0 and T3 samples. The RT sample concentrations decreased in the T7 and T10 samples to lower levels than in the dried samples. The RT and ST samples were significantly different from each other with ST samples also decreasing from T0 to T3, but then stabilising as the treatment with highest concentrations in the T7 and T10 samples. The D60 samples had higher levels than the D40 samples. Both seemed to increase slightly over the storage period, but there were found no significant differences in either the D40 or the D60 samples over the storage period.

3.2.6 Multivariate analysis

Two multivariate analyses were performed on the results from the GC-MS to visualise the data variation and relationships: Principal component analysis and a cluster analysis using hierarchical clustering on a heat map of the results from GC-MS. The samples in the multivariate analysis were labeled according to sampling day (T0 = harvest date, T3= day 3, T7 = day 7 and T10 = day 10), sample treatment (RT = fresh sample stored at room temperature, ST = fresh sample stored at storage temperature, D40 = sample dried at 40 °C and D60 = sample dried at 60 °C) over night and sample number (n=3) (1, 2 and 3). Samples T10_D40_2 and T10_D60_3 were omitted from analysis due to unsatisfying GC-MS values.

PCA

Principal component analysis was performed on all the samples together as well as on the fresh and dried samples separately. PCA showed patterns in variation of the data sets by orthogonal transformation creating principal components. The first principal component (FC) contributed to the largest variability in the data set, and the second principal component (SC) contributed to the largest variation that was uncorrelated with the first component. The figures in this section include a score plot and loading plot for all samples, and a score plot and loading plot for the fresh samples. The fresh samples were assessed separately to see how the RT and ST samples, and the samples at each sampling day grouped together. This was also done for the dried samples, but there was found no distinct pattern in the samples and therefore the results were omitted. The loading plot indicates the relative significance of the metabolites in the samples. The loading plot can be superimposed on the score plot to help assess which metabolites have an impact on the data pattern in the score plot.

The score plot of all the tomato samples in Figure 3.12 showed that the FC accounted for 57.8 % of the variation in the data set and the SC accounted for 11.5 %. There was a distinct separation between fresh and dried samples on the FC axis with fresh samples on the positive axis, and dried samples on the negative axis. The dried samples displayed a larger variation on the SC axis as well, whereas the fresh samples showed very little separation on the SC axis.

The loading plot Figure 3.13 shows a large group of metabolites that were grouped on the positive FC axis along the centre of the SC axis, towards the fresh samples including all the amino acids, many of the organic acids and sucrose. Fewer of the metabolites contributed to the variation on the negative FC axis, these included the tocopherols, a few sugars and some acids. The metabolites contributing on the SC axis were mainly glucose, fructose and galacturonic acid on the negative axis and 1,4-lactonethreonic acid and itaconic acid on the positive SC axis.

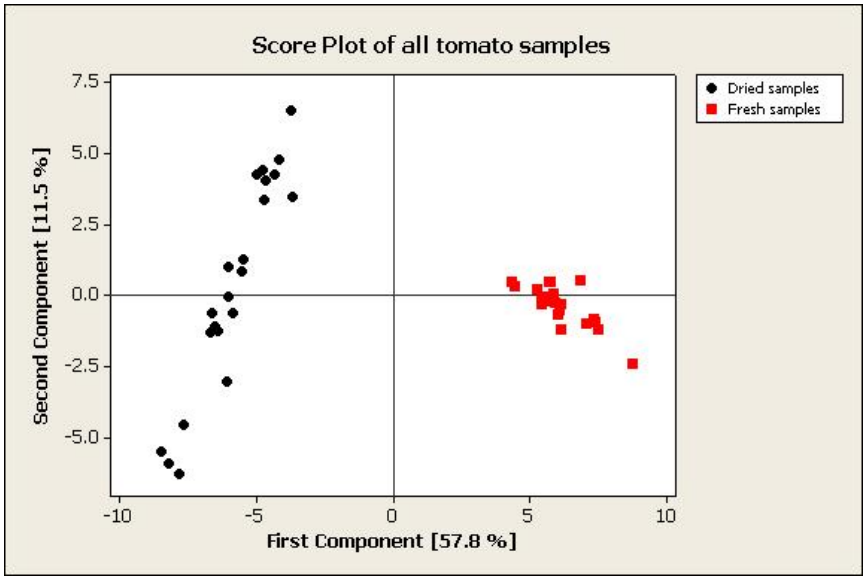

Figure 3.12: Score plot from PCA of all tomato samples. Eigenvalue proportions of each component is marked as a percentage on the axis label. The samples are colour grouped as fresh/dried samples.

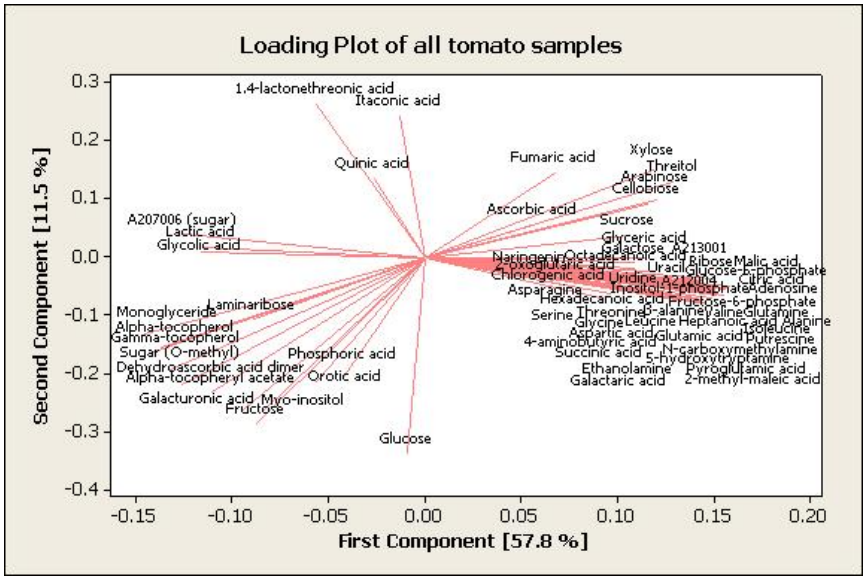

Figure 3.13: Loading plot from PCA of all tomato samples. Eigenvalue proportions of each component is marked as a percentage on the axis label. The metabolite names have been moved slightly to not overlap with each other.

The score plot from the fresh tomato samples is shown in Figure 3.14. The FC accounted for 22.8 % of the variation in the data set and the SC accounted for 14.9 % of the variation. There was found no pattern in the grouping of samples across the sampling days. The ST samples seemed to be primarily located on the negative axis of both the FC and SC with some exceptions. The RT samples seemed to be located primarily on the positive axis of both the FC and SC, also with some exceptions.

Figure 3.15 represents the loading plot of the fresh tomato samples. The metabolites that contributed to the variation on the negative axis of both the FC and the SC were mainly sucrose and malic acid. The metabolites that contributed to the variation on the positive axis of both the FC and the SC included threitol, adenosine, the tocopherols, alanine and laminaribose.

Cluster analysis

The cluster analysis of all the tomato samples is shown as a heat map with hierarchical trees in Figure 3.16. The samples are listed on top of the figure with the clusters beneath them, and the metabolites are listed to the right of the figure with the clusters displayed on the left side of the heat map.

There were two main clusters formed within the samples; one including the dried samples and one comprising the fresh samples. Within the dried samples D60 and D40 samples clustered mostly separately into two clusters. The cluster on the right included all of the D60 samples as well as 4 D40 samples. The cluster on the left contained the remaining D40 samples. The samples did not cluster in any specific order regarding sampling day. The fresh samples clustered in no specific order due to sampling day or treatment. The clusters of the fresh samples were spaced closely together indicating that they were highly correlated compared to the dried samples.

The metabolites clustered together according to concentrations. A large cluster of metabolites with high concentrations in the fresh samples and lower concentrations in the dried samples was formed by quinic acid-itaconic acid. Another distinct cluster was formed where the concentrations were low in the fresh samples and higher in the dried samples (dehydroascorbic acid dimer-A207006 (sugar)). There was one more cluster that formed with mixed concentrations across the dried and fresh samples and with many samples having a concentrations around the median value. This cluster included hexadecanoic acid-galacturonic acid. Within these larger clusters there was one cluster of metabolites that stood out and displayed high correlation between metabolites (succinic acid-citric acid). This cluster comprised many amino acids, amines and other primary metabolites related to glycolysis and Krebs-cycle.

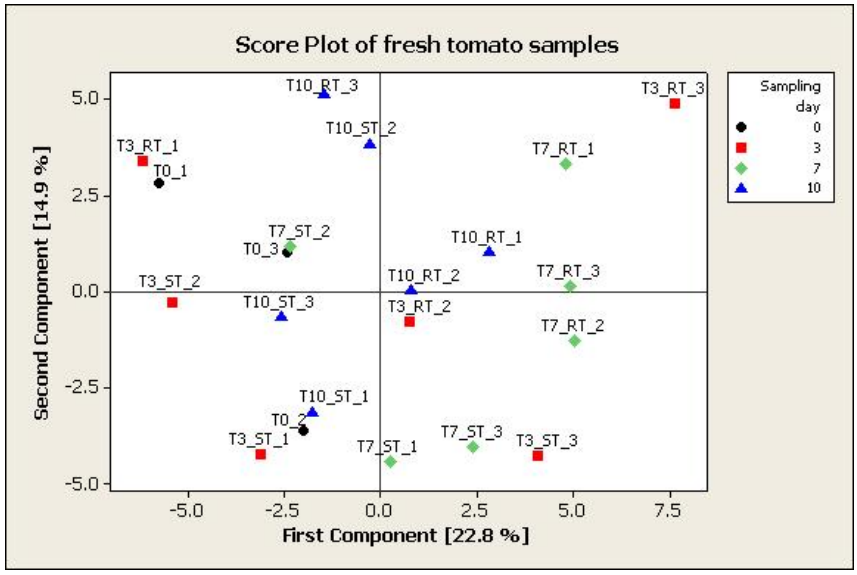

Figure 3.14: Score plot from PCA of fresh tomato samples. Eigenvalue proportions of each component marked as a percentage on the axis label. The samples are colour grouped by sampling day.

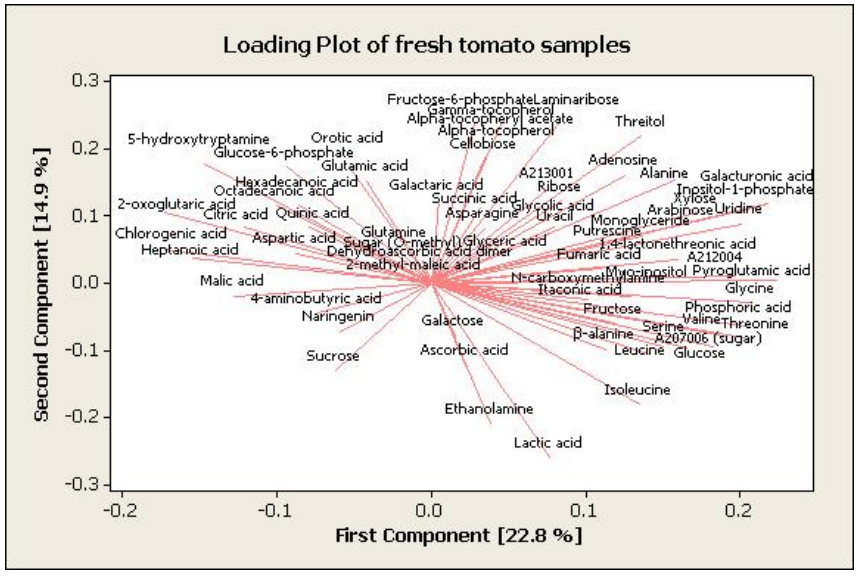

Figure 3.15: Loading plot from PCA of fresh tomato samples. Eigenvalue proportions of each component marked as a percentage on the axis label. The metabolite names have been moved slightly to not overlap with each other.

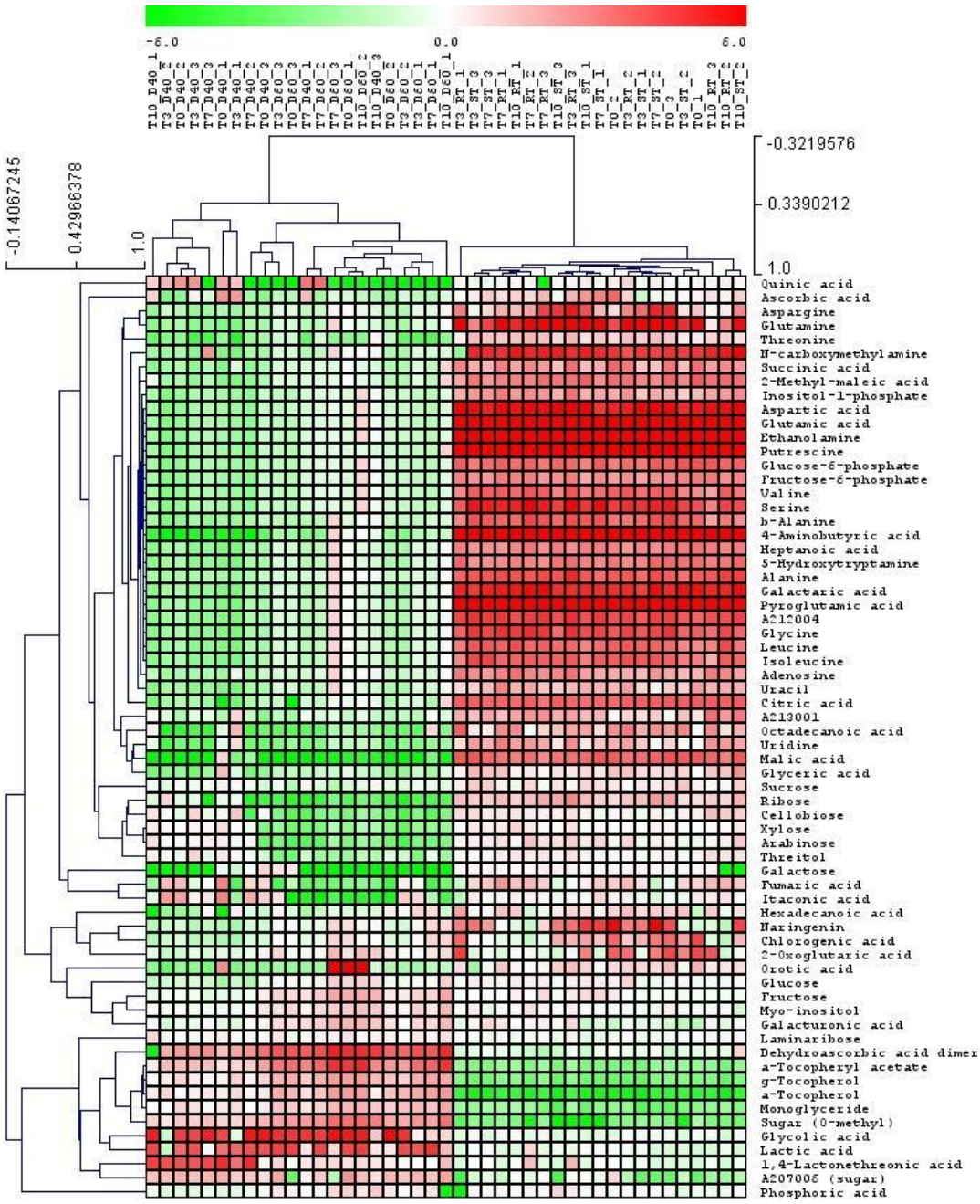

Figure 3.16: Hierarchical tree of cluster analysis of metabolites in all samples of tomato. Red colour indicates higher metabolite concentration than the median of the samples, green indicates a lower concentration. The metabolites are listed on the right side of the heat map with metabolite clustering tree on the left. All samples are listed on the top of the map as well as the sample clustering tree.

Chapter 4

Discussion

As in Chapter 3, this chapter is divided into two sections; a discussion of the results from the analysis of potato followed by a discussion of results from the analysis of tomato. These sections are further divided into two sub-sections, one discussing the the effects of storage, focusing on the results from the RT and ST samples, and the second focusing on the results from the D40 and D60 samples compared to the fresh control samples P0.

Before assessing the results it must be mentioned that the concentrations of metabolites measured by GC-MS in this study do not represent exact concentrations, due to using only one internal standard. Therefore the method is fit for semiquantitative analysis where the differences between samples are assessed reliably. The concentrations of this study might therefore not be directly comparable to concentrations in published literature and nutrient databases and tables. The literature cited is therefore only used to indicate if the concentrations from GC-MS are within a realistic range.

Some of the metabolites in the study showed high standard deviations which could be attributed to both method and human error. For instance there were only 3 replicas for each sample, giving each sample a large influence on the results. If a mistake was made during analysis of one sample it could have large consequences for the final result. This was the case for the two tomato samples discarded from the GC-MS results. A larger sample size or a rerun of the samples could have given more reliable results. Regarding the method of slicing the plant products, some tomato samples might have had more pericarp and others more seeds, thus affecting the metabolite concentrations. A possible solution to this problem might be to freeze dry the plant products and grind them to a paste, assuring a more equal distribution in the samples.

4.1 Potato

4.1.1 Effects of storage

The resulting SS content in fresh potato tubers of 5-6 % is similar to the results in a study including 18 cultivars of potato presented by Feltran et al. (2004). The paired t-test found significant differences in the RT and ST samples at each sampling day, with RT samples having higher SS content than ST samples. The SS content increased over the storage period in both RT and ST samples with significant differences found in both treatments. The increase in SS content might be a result of the continuing metabolic processes and transpiration during storage. Transpiration also explains why the RT samples have a higher increase than the ST samples, as respiration rates generally increase when plants are stored at higher temperatures.

The phenolic content in the fresh samples in the start of the study estimated to be 102 ± 4.86 mg GAE /100 g DW were in the same range as others have previously reported (Murniece et al., 2013; Brat et al., 2006). Studies have showed that phenolic content generally increases upon storage, but little change or a small decline have also been reported (Blessington et al., 2010; Stushnoff et al., 2008; Murniece et al., 2013). The contrasting results might be caused by different genotypes being used in each study. Ezekiel and Singh (2007) reported that changes in phenolic content was higher in samples stored at lower temperatures. This was indicated in the results of this study as well, but with a weak statistical difference between the RT and ST samples over the storage period ($p = 0.050$). The selected metabolite chlorogenic acid, which has been reported to be the principal phenolic compound in potatoes (Shakya and Navarre, 2006), indicated the same results in the GC-MS analysis as the total phenolic content in the F-C assay. The increase found in the ST samples as the RT samples remained stable has also been reported in previous studies (Hasegawa et al., 1966). This increase could arise from chlorogenic acid synthesis that has been found to be stimulated trough glucose, sucrose and fructose (Zucker and Levy, 1959), which accumulate trough low temperature sweetening.

The antioxidant activity of fresh potatoes on the day of harvest had a concentration of 0.44 ± 0.03 mmol / 100 g DW. This is a similar value to the antioxidant activity levels in the variety Beate (0.06 mmol / 100 g FW) found in the Antioxidant Food Table (Carlsen et al., 2010). Since many phenolic compounds also have antioxidant properties it might be expected that the antioxidant activity showed a similar pattern to the total phenolic content. There seemed to be a similar pattern in the results of the total phenolic content and antioxidant activity, but a simple correlation analysis in Excel showed no correlation between the total phenolic content and antioxidant activity of RT samples or ST samples ($r<0.3$). Other compounds that participate in antioxidant activity including nitrogenous compounds, carotenoids and ascorbic acid, might have contributed to the lack of correlation. The ST samples showed slightly higher levels compared to the RT samples in Figure 3.2, but there were found no significant differences in antioxidant activity between the RT and ST samples, or in each treatment over the storage period. An increase in antioxidant activity in potatoes stored at 4 °C have also been found in 4 other genotypes of potato (Blessington et al., 2010). Ascorbic acid is a metabolite with very high antioxidant activity. The measured ascorbic acid concentrations in this study provided unsatisfying results by GC-MS due to very high standard deviations. Being a polar water soluble compound,

ascorbic acid content is better suited for testing using liquid chromatography–mass spectrometry (LC-MS). Other studies have found that ascorbic acid content decreased with storage time (Dale et al., 2003) and decreased more rapidly at higher temperatures than lower temperatures (Nourian et al., 2003).

The amino acids GABA and Asparagine did not show any significant differences over the storage period in the RT and the ST samples. GABA has been reported to remain stable with no signignificant differences upon storage (Talley et al., 1984). A study including the Lady Claire cultivar in long term storage found that the asparagine content increased upon storage (Halford et al., 2012). The short term storage as used in this study might not have been sufficient to induce any significant changes. The same study of Halford et al. (2012) found that Lady Claire had low concentrations of sucrose, fructose and glucose compared to many of the other cultivars, and reported low acrylamide production in Lady Claire.

Spychalla and Desborough (1990) reported large variation in sugar concentrations in different cultivars. The concentrations in this study at harvest date are similar to the cultivars with lower concentrations of sucrose and glucose + fructose found in their study. The large increase of reducing sugar concentration in the ST samples and no significant increase in the RT samples, correlates well with the results of Spychalla and Desborough indicating low temperature sweetening due to chilling stress which has also been described by Amir et al. (1977). Sucrose concentrations remained stable over the storage period for both ST and RT samples, Spychalla and Desborough (1990) found a decline in sucrose concentrations in the cold stored potatoes between week 5 and 10 of storage.These results indicate that potatoes intended for the production of potato crisps and chips should be stored at a temperature higher than 4 °C to avoid low temperature sweetening in order to minimise acrylamide formation. Moreover, potatoes should only be stored over a short period of time before processed to minimise asparagine content. Choosing the right cultivar is also highly relevant as cultivars vary in both asparagine and sugar content.

Citric acid concentrations in this study remained stable upon storage and malic acid seemed to increase, but there were no significant differences over the storage period. This indicates that a shorter storage period in potatoes will not affect the organic acid content. A previous study found that citric acid concentrations increase, and malic acid concentrations decrease over longer storage periods, these changes were found to be smaller in potatoes stored at lower temperatures (Sweeney et al., 1969). Schwartz et al. (1961) however found that at 38 F (3.33 °C) citric acid first decreased before increasing, and the opposite in mallic acid. They also found that this pattern showed no change when the storage temperature increased.

The PCA and Hierarchical clustering gave no distinct results relating to the storage period in the samples, instead the samples seemed to cluster related to the different treatments. The PCA of fresh samples in Figure 3.6 showed a separation of RT and ST samples on the SC axis with many of the sugars (fructose, glucose, mannose, galactose), adenine, monomethylphosphate and chlorogenic acid amongst the metabolites contributing in the direction of the ST samples, and many of the organic and fatty acids contributing in the direction of the RT samples. This is in agreement with the results discussed above. With the SC and FC accounting for 38.4 % of the total variance in the data set, it does not fully show the complexity of the data pattern and must be analysed with caution.

The clustering formed according to treatments in the PCA and hierarchical clustering

indicates that the treatments had a larger impact on overall metabolite composition of the potatoes than the storage period. This is not surprising due to the good storage properties of the potato and the short storage period of this study.

4.1.2 Effects of heat treatment

The dry matter estimate of Lady Claire potatoes was found to be 23.57 % at 105 °C which is close to the dry matter estimate found in the same area of Norway of 23.0 % by Møllerhagen and Nybråten (2006). The percentage was higher for material dried at 60 °C, and higher still for material dried at 40 °C, indicating that some water content remained in the material after drying over night at these temperatures.

The total phenolic content in the samples after heat treatment decreased around 80 % from the fresh control samples P0. The phenolic content did not show a significant difference in the dried samples over the storage period, indicating that short term storage prior to heat treatment will neither increase or decrease the phenolic content after heat treatment. This was however not the case for the phenolic compound chlorogenic acid which increased over the storage period in both the D40 and D60 samples, with the dried samples having higher concentrations of chlorogenic acid than the fresh samples. Studies have found stable and increasing total phenolic content and chlorogenic acid with heat treatment (baking, boiling, steaming and stir-frying) (Navarre et al., 2010; Blessington et al., 2010). Another study reported that baking at 170 °C reduced the total phenolic content more than cooking in the microwave or boiling potatoes, and found an increase in chlorogenic acid after baking (Stushnoff et al., 2008). There has also been reported complete destruction (Dao and Friedman, 1992) and small losses (Im et al., 2008) of chlorogenic acid when baked. The different results may arise from each study baking at different temperatures over different time periods. Blessington et al. (2010) suggested that the increase in phenolics after baking is due to an increased extractability from the cellular matrix when starch changes textures in the cooking process. This is more likely to occur in potatoes heat treated at higher temperatures, and might provide an explanation to the decrease in phenolic content in this study.

The concentration of antioxidant values were very low in the dried samples compared to the fresh samples, and remained stable throughout the storage period indicating that short term storage prior to heat treatment will not effect the antioxidant activity of the dried product. Studies have reported antioxidant capacity to be higher in baked, boiled, microwaved and steamed potatoes than in uncooked potatoes (Navarre et al., 2010; Faller and Fialho, 2009; Blessington et al., 2010). The formation of compounds such as the products of Maillard reactions have been suggested as a possible reason for this increase in antioxidant capacity. There has also been reported declines in antioxidant activity after heat processing (baked, boiled, microwave) (Xu et al., 2009). In antioxidant capacity as well as in total phenolic content, the difference might arise from different temperatures used over different time periods. When drying over longer time periods it is also expected to find antioxidant reduction due to heavily oxidising conditions. To avoid oxidation drying should take place in a vacuum or by nitrogen purging (Chang and Liu, 2007).

A simple correlation test in Excel showed a high correlation between total phenolic content and antioxidant activity in both D40 and D60 samples ($r<0.9$), indicating that phenolic content is a large contributor to antioxidant activity in dried samples of potato.

Malic and citric acid content decreased in potato when heat treated, indicating degradation upon heat treatment. The D40 samples contained higher citric acid content than the D60 samples. This suggests that increasing the temperature when heat treating over night can significantly reduce the organic acid content in the potatoes. Malic acid concentrations increased slightly in both D40 and D60 samples over the storage period, where the citric acid concentrations decreased slightly over the storage period. These results followed the trends from the fresh ST samples, showing that storage period prior to heat treatment affects the organic acid content in processed potatoes.

Both of the amino acids GABA and asparagine concentrations decreased by more than half their concentration in the dried samples, indicating degradation upon heat treatment. The reduction of asparagine might be attributed to the acrylamide formation in Maillard reaction. Asparagine remained stable over the storage period in both D40 and D60 samples, where GABA decreased in both D40 and D60 samples, following the trend of the ST samples.

The dried samples showed increased levels of reducing sugars fructose and glucose over the storage period following the trends of the fresh ST samples, and might also be a result of starch being hydrolysed into sugars. In the first sampling days both fructose and glucose levels were higher in dried than fresh samples, with the ST samples being higher than the dried samples on the last sampling day. Sucrose concentrations were much lower in the dried samples compared to the fresh samples and remained stable over the storage period with D40 samples slightly higher than D60 samples.

The PCA and cluster analysis separated the dried and fresh samples distinctly with the two PCs accounting for 69.3 % of the variance, and therefore describing much of the pattern in the data set. The metabolites from the loading plot of the PCA contributing in the direction of the dried samples included all of the fatty acids, some of the organic acids, chlorogenic acid, some of the amines, glucose and fructose. This is in accordance with the results found in the selected metabolites above. The same metabolites clustered with high concentration in the cluster analysis. The consistent results from both PCA and hierarchical clustering indicate that the results are reliable.

4.2 Tomato

4.2.1 Effects of storage

The result of around 5% SS content is as expected in medium sized tomatoes (Luengwilai et al., 2010; Toor et al., 2006b). The results from the SS content showed no significant differences in tomatoes stored in neither room temperature or storage temperature over the storage period. These are similar results as found in tomatoes stored at 5 °C and 25-27 °C (Javanmardi and Kubota, 2006), 6 °C and 25 °C (Vinha et al., 2013) and 20 °C (Wills and Ku, 2002). This indicates that the transpiration and evaporation of the tomatoes over a 10 day storage period is very small. Continued metabolic processes post harvest may however change the composition of the SS content with regard to the ratio of metabolites such as sugars and organic acids.

The total phenolic content in fresh tomato samples were 480-565 mg GAE/100 g DW in this study. Previous studies range from 221-387 mg GAE/100 g DW in medium sized ripe tomatoes (Brat et al., 2006; Proteggente et al., 2002; Chun et al., 2005; Toor and Savage, 2006; Toor et al., 2006b). The slightly higher measured phenolic content could be due to differences in cultivar, growth stage and soil composition/fertilizer used (Toor et al., 2006a). There was found no significant differences between the RT and ST samples, and no significant differences over the storage period in either of the treatments. However samples of both treatments seemed to decrease the to P3 before slightly increasing by P14, and ST samples seemed to have slightly lower levels than RT samples. Vinha et al. (2013) performed a study with 4 tomato cultivars where the total phenolic content in samples stored at 6 °C increased over the storage period peaking at day 9. Samples of the same cultivars stored at 25 °C showed a decrease in phenolic content by day 3 followed by an increase at day 9. 3 out of 4 cultivars had higher phenolic content levels in the samples stored at 25 °C compared to 6 °C over the storage period. The trends in the total phenolic content of this study are similar to the results in Vinhas study, except that there were found no significant differences in phenolic content over the storage period between RT and ST samples in this study, due to high variations in the samples.

Chlorogenic acid has been reported to be one of the main phenolic acids in tomato (Minnogio et al., 2003). The chlorogenic acid content measured in the fresh samples on the day of harvest in this study (23.30 ± 7.80 mg/100 g DW) is similar to concentrations reported in other studies (Gómez-Romero et al., 2010; Mattila and Hellström, 2007). The chlorogenic acid decreased to near 0 over the first 7 days of the storage period in the RT samples. The ST samples decreased on day 3 before stabilising over the rest of the storage period, but there was no significant difference found between the RT and ST samples. It is odd that no similar trends in total phenolic content and chlorogenic acid content were found in the present data, other than a decrease from harvest day to day 3. This difference might be contributed to the effect of other phenolic compounds on total phenolic content.

The antioxidant activity in fresh tomato samples in this study ranged from 3.34-4.84 mmol/100 g DW. Carlsen et al. (2010) listed antioxidant content for many different cultivars of fresh tomatoes from different countries between 2.7-6.29 mmol/100 g DW, showing that there is a large variation between cultivars in antioxidant activity of tomato. The antioxidant activity was found to decrease over the storage period in both RT and ST samples, with a more rapid decline in ST samples by day 3, but the two treatments evened out by day 10. Soluble phenolics have been found to be a major contributor, together with ascorbic acid, to soluble antioxidant activity in tomatoes (Toor and Savage, 2005). Using a simple correlation test in Excel the antioxidant activity was found to correlate well with the total phenolic content in the ST samples ($r>0.9$) but not in the RT samples($r<0.3$). The low correlation in the RT samples might be due to an increased accumulation of lycopene when tomatoes are stored in higher temperatures, affecting the antioxidant activity. Since the total phenolic content remained stable, and ascorbic acid content has been reported to decrease upon storage (Rai et al., 2012), the antioxidant levels in this study could be expected. However, other studies have found an increase in AOX activity upon storage: One study reported no significant changes in samples stored in room temperature for 7 days and significantly higher concentrations in samples stored in 5 °C for 14 days (Javanmardi and Kubota, 2006). Another study found an increase in antioxidant activity in pink fruits when

stored 15 days at both 6 °C, 12 °C and 25 °C (Vinha et al., 2013). These increases might arise from ripening, biotic and abiotic stresses and genetic, and environmental conditions.

4-aminobutyric acid decreased in both RT and ST samples over the storage period with no significant difference between the treatments. This was to be expected as GABA levels have been found to be highest in the mature-green stage of ripening, and decline throughout further ripening (Akihiro et al., 2008).

In tomato 50 % of dry matter have been reported to be soluble sugars, including 25 % fructose, 22 % glucose and 1 % sucrose. Organic acids were found to make up 13 % of the dry matter, including 9 % citrate and 4 % malate (Davies and Hobson, 1981). These results do not resemble data found in this study with glucose being higher than fructose in the fresh samples on harvest day, and sucrose although smaller, accounts for around 11 % of the three sugars. Other studies have had samples at 8 days past anthesis and in the breaker stage having higher glucose levels than fructose levels suggesting that the tomatoes in this study, although red, might not have been fully ripened (Mounet et al., 2007; Davies and Kempton, 1975). Davies also reported that when ripe the total reducing sugars decreased at further ripening. There was found no significant differences in glucose and fructose concentration between the RT and ST samples, or over the storage period in RT or ST samples. It should be noted that many of the samples showed high variation, i.e. use of a larger sample size (n>3) might have resulted in significantly different results. Sucrose concentrations in RT samples were found to be different than in ST samples with sucrose in RT samples decreasing over the storage period. No significant differences in ST samples were found over the storage period, but there seemed to be an increase from the day of harvest to day 3 before stabilising. A decrease in sucrose upon ripening has also been reported in other studies (Davies and Kempton, 1975).

The citric and malic acid contents in this study on the day of harvest were 3827.01 mg/100 g DW and 1517.03 mg/100 g DW, respectively. This is in the same range as previous studies have reported which have found malic acid concentrations in ripe tomatoes between 822-1333 mg/100 g DW and concentrations of citric acid between 3741-5645 mg/100 g DW (Belitz et al., 2009; Vinha et al., 2013; Morvai and Molnár-Perl, 1992). The citric acid remained stable throughout the storage period with no significant differences between the RT and ST samples. Malic acid concentrations decreased over the storage period in RT samples, and seemed to decrease to a lesser extent in ST samples, but there were found no significant differences in the ST samples over the storage period. The decrease in malic acid is expected due to an increase in malic acid catabolism during the ripening stage (Davies and Hobson, 1981). Previous studies have also found that citric and malic acid decrease occurs slower at lower temperatures (Thorne and Efiuvwevwere, 1988; Gómez et al., 2009).

α-Tocopherol concentrations were very small in the fresh samples of this study, and did not significantly change over the storage period, or differed in RT and ST samples. Other studies have reported higher concentrations of 8.5 mg/100 g DW in fresh tomatoes (Chun et al., 2006) and 12.9 mg/100 g DW (Belitz et al., 2009). The different results might arise from the use of different cultivars at different ripening stages (Abushita et al., 1997; Raffo et al., 2002). α-Tocopherol levels have also been reported to be higher in processed tomato products compared to fresh tomato Chun et al. (2006). This is also true for the heat treated tomatoes of this study, as discussed in section 4.2.2.

The PCA and Hierarchical clustering gave no distinct results relating to the storage period in the samples, instead the samples seemed to cluster related to the different treatments. The clustering according to treatments in the PCA and hierarchical clustering indicates that the treatments had a larger impact on overall metabolite composition of the tomatoes than the storage period. The PCA of fresh samples did however show a slight separation of RT and ST samples with RT samples mostly located on the top right of the score plot in Figure 3.14. The loading plot indicated that alcohols, some of the sugars, phosphates and acids are the metabolites contributing to the variation towards the top right. The metabolites contributing towards the lower left include chlorogenic acid, malic acid, sucrose and 4-aminobutyric acid. This is in accordance with the results discussed above. With the first two components accounting for only 37.7 % of the variance of the fresh samples in PCA, there are other components that also contribute largely to the sample pattern. The plot with the first two components has therefore to be considered a simplified perspective of the sample grouping.

4.2.2 Effects of heat treatment

The dry matter estimate of 6.2 % is around the level expected in Norwegian raw tomatoes according to the Norwegian Food Composition Table (Norwegian Food Safety Authority, Directorate of Health and the University of Oslo, 2014). The tomatoes dried at 60 °C over night retained no water and resembled the dry matter content, whereas the samples dried at 40 °C over night were heavier indicating that they still retained water.

Total phenolic concentrations decreased significantly in the dried samples compared to the fresh samples. The phenolic content remained stable over the storage period in both D40 and D60 samples. Other studies have reported an increase in total phenolic content upon heat processing (Sahlin et al., 2004; Lavelli et al., 1999; Gahler et al., 2003), but these studies have used higher temperatures than in this study, and over shorter time periods. The increase at higher temperatures have been reported to be a result of hydrolysis of flavonoid glucosides and the release of cell wall phenolics (Lavelli et al., 1999). Toor and Savage (2006) reported a large decrease in total phenolic content when drying samples at 42 °C over 18 h, which is a similar method used and results found in this study. The decrease in phenolic content at lower temperatures arise from oxidative enzymes such as polyphenoloxidases and peroxidases (Shahidi and Naczk, 1995). There has also been reported no change in phenolics while drying on high heat for a short time interval (30 min) (Dewanto et al., 2002). This suggests that total phenolic content only increase at higher temperatures, over shorter time periods, and variably in different cultivars. The chlorogenic acid concentrations followed the pattern of total phenolic content. It decreased significantly in the dried samples and remained stable throughout the storage period. This was to be expected as chlorogenic acid is a large contributor to the total phenolic content of tomatoes.

The antioxidant activity decreased significantly in the dried samples compared to the fresh samples, with dried samples showing less than half the concentration of the fresh samples. The antioxidant activity remained stable over the storage period with no significant differences between the D40 and D60 samples. Studies have reported differing results in how heat treatment affects antioxidant activity. Many studies of high temperature over shorter time intervals have reported an increase in antioxidant activity (Dewanto

et al., 2002; Halvorsen et al., 2006; Nicoli et al., 1997). The reported increases of antioxidant activity at high temperatures might arise from the increase in total phenolic content at higher temperatures as well as products formed under Maillard reactions (Nicoli et al., 1997). Another study found a decrease at high temperatures (200 °C) over a short time interval (18 min) (Sahlin et al., 2004). The concentration has also been reported to decrease at lower temperatures 42 °C over a longer time interval (18 h) (Toor and Savage, 2006). The loss of antioxidant activity is likely due to a decrease in phenolic content, as well as possibly in ascorbic acid, which are the two main contributors to antioxidant activity in tomato. A simple correlation test found high correlation between total phenolic content and antioxidant activity of D40 and D60 samples (r>0.8). Lycopene and other carotenoids which contribute to antioxidant activity has also been found to decrease upon heat treatment (Chang and Liu, 2007; Zanoni et al., 1999). When drying tomato samples in air there will be antioxidant reduction due to oxidation conditions. To prevent this the samples could be dried in a vacuum or by nitrogen purging (Chang and Liu, 2007).

The amino acid 4-aminobutyric acid and the organic acids malic and citric acid decreased to a small concentration upon heat treatment. The citric acid content in the samples seemed to increase slightly over the storage period, but there was found no significant difference in the samples over the storage period. The decrease might be a result of degradation of amino acids and organic acids at higher temperatures.

Regarding the reducing sugars, glucose and fructose, a large increase in the D60 samples compared to the fresh samples was found, which seemed to increase over the storage period, but the samples were not found to be significantly different over the storage period. The D40 samples on the other hand remained stable around the same as the fresh samples in fructose concentrations, and seemed to decrease in glucose concentrations, but there was found no significant difference in the samples over the storage period. The increase in reducing sugars might be due to starch hydrolysis. Kolusheva and Marinova (2007) reported a large increase in starch hydrolysis in samples heated to 60 °C compared to samples heated to 30 °C, which explains the large difference in the D40 and D60 samples. Sucrose on the other hand decreased in the dried samples and were slightly higher in the D40 samples than the D60 samples, both remaining stable throughout the storage period.

α-Tocopherol concentration increased significantly in the dried samples compared to the fresh samples and was much higher in D60 samples than in D40 samples. D40 samples remained stable over the storage period, whereas the D60 samples seemed to increase, but there was found no significant differences in the D60 samples over the storage period. Seybold et al. (2004) found an increase in α-tocopherol concentrations of tomatoes baked for 45 minutes at 180 °C, 200 °C and 220 °C where the increase was higher in the samples baked at the higher temperatures. This is the same indication that was found in this study. Seybold et al. suggested that the increase might result from α-tocopherol being released from its binding sites when heat treated at higher temperatures.

The PCA and cluster analysis clearly clustered the dried and fresh samples separately. The first two components of the PCA accounted for 69.3 % of the variance in the data set. Much of the variance is therefore described trough the sample pattern depicted in Figure 3.12. The metabolites contributing to the variation towards the dried samples included some of the sugars (glucose, fructose), some organic acids, myo-inositol, monoglyceride and the tocopherols/tocopheryls. The other metabolites grouped distinctly towards the

fresh samples. 10 of the metabolites that grouped towards the dried samples in PCA clustered together in the hierarchical clustering as well. These metabolites were mainly metabolites involved in fatty acid synthesis. The remaining 4 metabolites from PCA that grouped together towards the dried samples (fructose, glucose, myo-inositol and galacturonic acid), clustered next to them but were more closely clustered together with the other metabolites. This could be due to the small differences between fresh and dried samples of these metabolites. The similar results from PCA and cluster analysis gives confidence to these results in the study.

Chapter 5

Conclusion

Short term storage of potato at room temperature was found to have low impact on metabolite composition, however at 4 °C, low temperature sweetening occurred causing a significant increase in reducing sugars and chlorogenic acid synthesis, which correlated well with the increase found in total phenolic content. A lower storage temperature in tomatoes slowed the metabolism and further ripening of the fruit, with significantly smaller changes in some metabolites such as malic acid and sucrose in samples stored at 4 °C. Short term storage of tomatoes at low temperatures can therefore extend the shelf life of tomatoes. Potatoes that will be heat-treated should however not be stored at low temperatures due to potentially increased acrylamide formation with reducing sugars.

When the potatoes were heat-treated there was found a decrease in total phenolic content, antioxidant activity and levels of most of the metabolites identified by GC-MS. There was however found an increase in levels of fatty acids, chlorogenic acid, fructose and glucose in the dried samples. The changes over the storage period generally followed the trend from the samples stored at 4 °C. Similar patterns were observed in tomatoes with a decrease in most metabolites except fructose, glucose and vitamin E. Baking at 40-60 °C over 18 hours were therefore found to lead to overall losses in nutritional quality.

To obtain the desired quality of the final product when producing a vegetable or fruit product it is important to choose the cultivar carefully and further adjust storage times, conditions, and processing methods to optimise the quality of the vegetable product. Further studies might include a longer storage period in both tomato and potato, analysis using methods such as LC-MS to include an assessment of non-volatile metabolites such as ascorbic acid and lycopene, heat treatment at higher temperatures over shorter storage periods, assessing specific cultivars for specific purposes, assessing environmental factors, storage treatments and other processing methods. A large number of studies assessing quality in both potatoes and tomatoes have been published, but all with different cultivars that have been grown, stored and processed under different conditions. Large-scale studies that monitor all aspects of growth, storage and processing could therefore be of importance, as well as thorough literature reviews that can provide a collected source of information for researchers in the food industry.

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Appendices

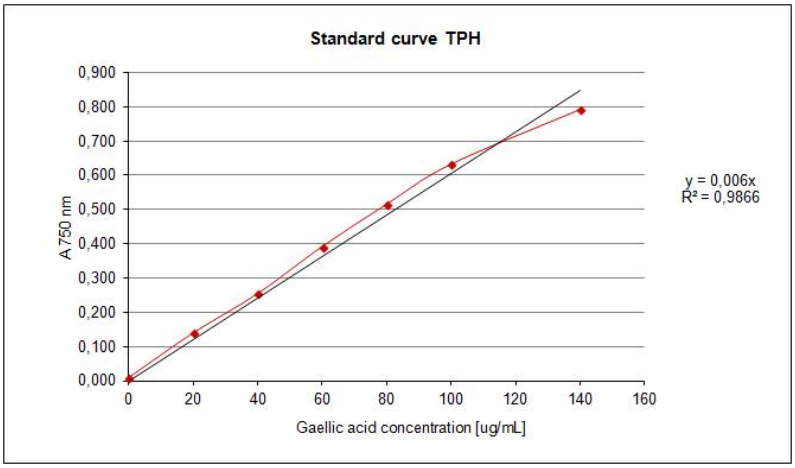

Figure A.1: Standard curve of gallic acid used to calculate of total phenolic content concentration.

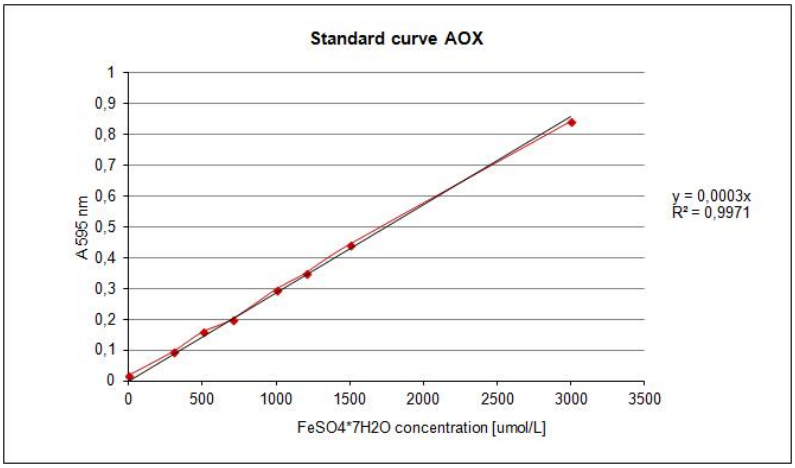

Figure A.2: Standard curve of iron (III) sulfate heptahydrate used to calculate antioxidant activity concentration.

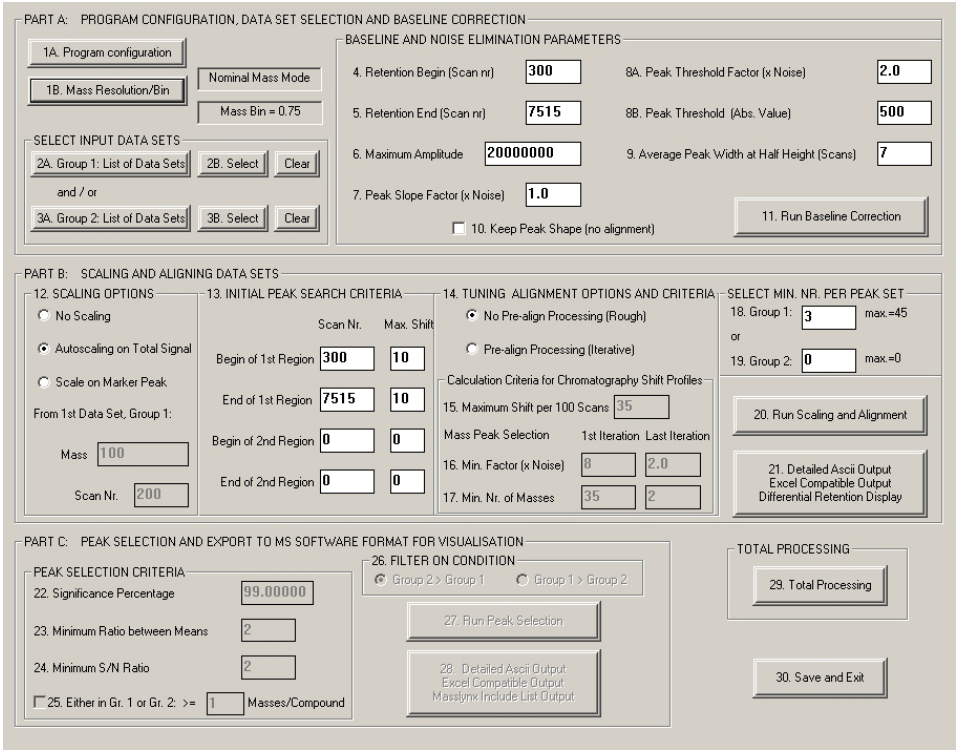

Figure A.3: Screenshot of MetAlign setup used.

Table A.1: Total phenolic content of potato. Day is the time of measurement after harvest, RT were stored in room temperature, ST were stored at storage temperature.

Day	RT	*Stdev*	ST	*Stdev*	40	*Stdev*	60	*Stdev*
				mg GAE / 100 g DW				
0	102.04	*4.86*	102.04	*4.86*	19.04	*2.14*	20.28	*2.84*
3	106.49	*3.89*	113.92	*6.70*	19.16	*1.69*	19.21	*2.75*
7	99.49	*7.15*	116.67	*3.20*	21.65	*1.34*	19.21	*0.83*
14	112.64	*19.01*	109.67	*8.21*	18.33	*1.28*	16.33	*2.21*

Table A.2: Antioxidant activity of potato. Day is the time of measurement after harvest, RT were stored in room temperature, ST were stored at storage temperature.

Day	RT	*Stdev*	ST	*Stdev*	40	*Stdev*	60	*Stdev*
				mmol / 100 g DW				
0	0.44	*0.03*	0.44	*0.03*	0.03	*0.01*	0.05	*0.01*
3	0.35	*0.02*	0.37	*0.08*	0.03	*0.01*	0.04	*0.00*
7	0.38	*0.08*	0.52	*0.11*	0.04	*0.01*	0.05	*0.01*
14	0.39	*0.04*	0.42	*0.02*	0.02	*0.00*	0.03	*0.01*

Table A.3: Total phenolic content of tomato. Day is the time of measurement after harvest, RT were stored in room temperature, ST were stored at storage temperature.

Day	RT	*Stdev*	ST	*Stdev*	40	*Stdev*	60	*Stdev*
				mg GAE / 100 g DW				
0	565.32	*121.77*	565.32	*121.77*	246.46	*25.55*	309.00	*38.01*
3	525.00	*50.69*	479.84	*55.40*	250.37	*21.24*	297.89	*23.49*
7	494.35	*103.84*	479.84	*43.57*	229.89	*32.89*	260.45	*13.21*
10	559.68	*13.32*	505.65	*23.08*	221.54	*10.17*	288.78	*5.73*

Table A.4: Antioxidant activity of tomato. Day is the time of measurement after harvest, RT were stored in room temperature, ST were stored at storage temperature.

Day	RT	*Stdev*	ST	*Stdev*	40	*Stdev*	60	*Stdev*
				mmol / 100 g DW				
0	4.84	*0.79*	4.84	*0.79*	1.78	*0.33*	1.64	*0.28*
3	3.94	*0.41*	2.97	*0.60*	1.80	*0.11*	1.36	*0.11*
7	3.82	*0.08*	3.63	*0.08*	1.26	*0.20*	1.24	*0.16*
10	3.34	*0.25*	3.34	*0.08*	1.45	*0.01*	1.41	*0.02*

Table A.5: Metabolite composition of potato tubers extracted the day of harvest (P0). Fresh tubers were extracted directly after harvest, 40 were dried at 40 °C over night before extraction and 60 were dried at 60 °C over night before extraction.

			PO				
Compound	Fresh control	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
			mg/ 100 g DW				
Acids							
2,4-Dihydroxybutanoic acid	0.10	*0.02*	0.27	*0.09*	1.33	*0.18*	0.000
2-Hethyl-maleic acid	4.61	*0.78*	1.93	*0.35*	1.89	*0.30*	0.001
3-Hydroxypropanoic acid	0.10	*0.02*	1.63	*0.07*	1.18	*0.21*	0.000
Aconitic acid	10.07	*1.96*	11.14	*4.50*	16.70	*2.72*	0.093
Ascorbic acid	10.20	*17.28*	6.57	*2.10*	9.84	*8.87*	0.911
Citric acid	655.07	*40.87*	253.60	*7.09*	235.34	*0.99*	0.000
Dehydroascorbic acid dimer	13.24	*11.40*	7.73	*1.22*	14.21	*3.01*	0.499
Fumaric acid	17.78	*10.96*	27.87	*6.44*	28.28	*1.45*	0.224
Galactaric acid	8.07	*2.70*	2.87	*0.24*	3.35	*0.17*	0.012
Glyceric acid	5.56	*0.30*	8.97	*1.26*	8.53	*1.30*	0.015
Itaconic acid	4.16	*3.96*	4.52	*0.91*	4.82	*0.24*	0.942
Lactic acid	0.13	*0.04*	0.06	*0.02*	31.92	*27.81*	0.081
Lyxonic acid	9.59	*0.08*	8.07	*0.89*	12.22	*1.35*	0.005
Malic acid	264.62	*109.67*	158.53	*10.86*	127.86	*10.25*	0.087
Malonic acid	2.77	*1.09*	1.89	*0.75*	1.91	*0.09*	0.342
Oxalic acid	3.87	*4.81*	0.06	*0.02*	0.08	*0.02*	0.233
Pyruvic acid	0.17	*0.10*	1.01	*0.89*	1.28	*1.18*	0.321
Quinic acid	48.82	*7.72*	61.79	*10.70*	73.56	*20.97*	0.187
Succinic acid	27.95	*17.58*	115.76	*15.42*	96.31	*9.67*	0.001
Threonic acid	16.29	*1.39*	14.87	*2.15*	15.23	*3.05*	0.744
Alcohols and esters							
Glycerol	3.96	*4.86*	1.14	*1.85*	0.08	*0.02*	0.329
Mannitol	15.15	*2.97*	16.48	*3.80*	16.67	*2.68*	0.822
Myo-inositol	124.47	*25.92*	115.98	*5.69*	113.82	*8.68*	0.708
Amines, amides and N-compounds							
Adenosine	2.98	*1.01*	22.67	*2.66*	34.40	*2.96*	0.000
Adenine	1.59	*1.70*	5.21	*2.00*	7.52	*1.60*	0.018
Allantoin	20.77	*5.90*	12.42	*3.64*	10.17	*1.92*	0.045
Butyro-1,4-lactam	0.72	*0.21*	1.37	*0.18*	1.66	*0.09*	0.001
Calystegine A3	33.74	*10.50*	27.49	*4.42*	30.90	*8.63*	0.669
Calystegine B2	40.87	*39.44*	35.27	*5.09*	40.59	*15.50*	0.952
Calystegine B4	19.09	*21.30*	7.33	*2.28*	5.88	*6.71*	0.442
Ethanolamine	37.27	*8.81*	31.93	*4.63*	30.36	*5.99*	0.464
Guanine	0.13	*0.04*	0.95	*0.28*	0.49	*0.25*	0.011
Guanosine	0.55	*0.04*	6.40	*1.91*	14.00	*0.78*	0.000
Nicotinic acid	2.59	*1.27*	3.68	*0.72*	3.95	*0.75*	0.255
p-Tyramine	18.54	*8.77*	11.21	*3.52*	19.09	*4.45*	0.278
Pantothenic acid	1.49	*0.39*	2.61	*0.31*	3.01	*0.08*	0.002
Pipecolinic acid	4.64	*3.28*	7.27	*6.43*	5.34	*2.27*	0.756
Putrescine	32.26	*12.37*	21.38	*3.88*	24.76	*4.24*	0.296
Spermidine	2.48	*1.35*	0.89	*0.23*	0.98	*0.19*	0.088
Uracil	0.88	*0.54*	0.91	*0.18*	1.88	*0.12*	0.018
Urea	0.48	*0.63*	0.49	*0.39*	3.32	*1.09*	0.006
Amino acids							
3-Cyano-alanine	5.35	*3.48*	2.32	*0.87*	2.61	*0.84*	0.237
4-Aminobutyric acid	793.99	*36.94*	364.23	*8.11*	336.54	*2.40*	0.000
Alanine	341.53	*54.58*	224.97	*51.20*	237.28	*16.01*	0.034
Asparagine	458.01	*143.22*	184.70	*31.53*	176.37	*15.70*	0.011
Aspartic acid	724.06	*175.34*	253.28	*15.57*	195.99	*26.76*	0.001

Table A.5: Continued.

Compound	Fresh control	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
			mg/ 100 g DW				
Butyric acid	6.05	*1.41*	5.22	*2.77*	5.05	*1.40*	0.807
Glutamic acid	650.26	*60.29*	120.19	*16.59*	151.62	*28.68*	0.000
Glutamine	688.77	*109.67*	271.89	*11.32*	257.18	*4.36*	0.000
Glycine	140.80	*11.98*	63.48	*11.60*	73.67	*3.63*	0.000
Histidine	52.38	*31.18*	36.21	*10.37*	39.09	*8.63*	0.589
Homoserine	1.89	*0.88*	1.80	*0.49*	2.94	*0.49*	0.132
Isoleucine	215.51	*42.34*	229.17	*51.22*	211.74	*61.28*	0.913
Leucine	92.24	*39.53*	103.04	*23.66*	82.75	*17.84*	0.699
Lysine	126.37	*14.34*	49.50	*21.55*	51.11	*19.37*	0.004
Methionine	82.50	*9.00*	58.51	*12.39*	52.41	*11.87*	0.036
Ornithine	15.32	*7.16*	3.30	*1.46*	4.54	*1.92*	0.028
Phenylalanine	202.72	*26.64*	220.11	*11.89*	230.47	*29.64*	0.415
Proline	5.55	*4.42*	4.51	*2.53*	4.24	*0.98*	0.855
Pyroglutamic acid	837.93	*213.51*	397.90	*60.41*	275.03	*238.12*	0.024
Serine	277.79	*41.81*	137.08	*14.49*	134.36	*9.02*	0.001
Threonine	146.41	*14.74*	123.73	*24.96*	117.90	*22.23*	0.290
Tryptophan	148.06	*46.26*	200.47	*90.39*	209.41	*145.69*	0.742
Tyrosine	469.80	*204.28*	329.31	*70.41*	372.97	*116.66*	0.503
Valine	394.76	*59.37*	325.88	*25.38*	290.48	*45.53*	0.077
β-Alanine	45.93	*15.61*	21.97	*2.65*	28.29	*2.31*	0.045
Aromatics							
Chlorogenic acid	1.36	*0.29*	3.52	*2.33*	2.76	*1.16*	0.284
Fatty acids							
Eicosanoic acid	0.19	*0.07*	2.02	*0.55*	2.21	*0.69*	0.005
Hexadecanoic acid	121.97	*83.48*	127.38	*7.78*	118.65	*11.17*	0.976
Octadecadienoic acid	1.76	*1.53*	22.24	*6.91*	26.38	*4.00*	0.001
Octadecadienoic acid methyl ester	0.10	*0.02*	3.89	*2.11*	0.98	*0.11*	0.021
Octadecanoic acid	49.59	*40.37*	57.97	*2.00*	53.88	*4.67*	0.002
Octadecatrienoic acid	0.19	*0.13*	3.10	*0.99*	3.45	*0.71*	0.910
Octadecenoic acid, 9 -(Z)-	0.16	*0.09*	1.63	*0.54*	1.90	*0.35*	0.003
Octadecenoic acid, 9 -(E)-	0.13	*0.04*	0.67	*0.24*	0.70	*0.13*	0.008
Phosphates							
Fructose-6-phosphate	2.89	*1.12*	1.14	*0.20*	2.07	*0.45*	0.061
Glucose-6-phosphate	6.37	*3.06*	1.89	*0.51*	2.34	*0.52*	0.043
Glycerol-3-phosphate	13.14	*1.22*	17.06	*1.71*	17.67	*2.85*	0.068
Monomethylphosphate	20.46	*10.60*	0.90	*0.30*	0.70	*0.16*	0.011
Myo-inositol-1-phosphate	1.17	*0.39*	0.52	*0.14*	0.51	*0.03*	0.024
Phosphoric acid	1248.39	*171.37*	496.73	*60.93*	479.70	*7.25*	0.000
Sugars							
Fructose	22.46	*17.19*	32.19	*8.75*	50.69	*6.69*	0.066
Galactose	8.63	*10.34*	4.52	*1.16*	3.40	*0.38*	0.565
Glucose	69.72	*74.81*	156.05	*35.83*	129.46	*18.58*	0.168
Mannose	5.93	*5.08*	5.84	*1.60*	5.64	*0.46*	0.993
Ribose	2.54	*2.51*	1.42	*1.03*	1.38	*0.37*	0.623
Sucrose	1043.41	*78.61*	401.43	*11.69*	370.68	*2.84*	0.000
Unidentified compounds							
A144004	5.39	*0.53*	2.14	*0.07*	2.09	*0.08*	0.000
A148003	6.10	*5.12*	3.80	*2.92*	3.34	*1.36*	0.610
A160005	35.79	*50.93*	19.63	*30.21*	19.18	*11.86*	0.808
A171003	28.37	*11.79*	14.74	*2.40*	15.41	*1.22*	0.093
A207006 (sugar)	3.71	*3.12*	3.79	*1.36*	3.07	*2.41*	0.923
NA133011	2.28	*0.52*	0.85	*0.15*	0.94	*0.04*	0.002

Table A.6: Metabolite composition of potato tubers extracted 3 days after harvest (P3). RT were stored in room temperature, ST were stored at storage temperature (≈ 4 °C), 40 were stored at storage temperature and dried at 40 °C over night before extraction and 60 were stored at storage temperature and dried at 60 °C over night before extraction.

	P3								
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
	mg/ 100 g DW								
Acids									
2,4-Dihydroxybutanoic acid	0.11	*0.04*	0.12	*0.03*	0.30	*0.05*	1.26	*0.13*	0.000
2-Hethyl-maleic acid	4.01	*0.22*	3.52	*0.22*	2.08	*0.42*	2.01	*0.32*	0.000
3-Hydroxypropanoic acid	0.11	*0.03*	0.12	*0.03*	1.83	*0.25*	1.42	*0.19*	0.000
Aconitic acid	2.30	*3.77*	5.53	*4.71*	15.01	*5.26*	16.83	*4.13*	0.011
Ascorbic acid	18.40	*7.61*	7.54	*5.05*	5.20	*5.39*	5.91	*4.68*	0.074
Citric acid	666.17	*11.28*	646.82	*21.07*	249.65	*5.57*	233.53	*3.45*	0.000
Dehydroascorbic acid dimer	22.42	*3.15*	17.03	*16.63*	7.53	*1.98*	5.70	*1.16*	0.126
Fumaric acid	18.88	*2.31*	12.96	*3.68*	28.30	*3.65*	35.52	*8.33*	0.003
Galactaric acid	8.70	*0.57*	9.50	*0.90*	3.87	*0.31*	3.53	*0.24*	0.000
Glyceric acid	4.89	*0.28*	3.46	*0.31*	8.12	*0.47*	8.01	*0.39*	0.000
Itaconic acid	8.02	*1.55*	4.47	*4.20*	4.97	*1.29*	5.73	*1.94*	0.383
Lactic acid	0.11	*0.03*	0.12	*0.03*	52.53	*45.44*	69.20	*10.56*	0.012
Lyxonic acid	11.08	*0.41*	10.26	*0.77*	9.76	*0.20*	12.65	*1.20*	0.007
Malic acid	352.66	*32.96*	338.53	*13.25*	177.23	*8.36*	161.68	*16.35*	0.000
Malonic acid	2.32	*0.36*	2.12	*0.20*	2.23	*0.54*	2.34	*0.62*	0.930
Oxalic acid	0.57	*0.79*	0.11	*0.03*	0.07	*0.02*	0.08	*0.02*	0.391
Pyruvic acid	0.47	*0.10*	0.55	*0.75*	0.97	*0.96*	0.34	*0.42*	0.668
Quinic acid	94.02	*14.69*	47.59	*13.84*	30.68	*10.28*	33.07	*8.86*	0.001
Succinic acid	25.15	*9.32*	64.07	*13.76*	114.93	*18.60*	100.56	*7.23*	0.000
Threonic acid	13.78	*1.02*	13.91	*1.22*	13.26	*1.33*	15.36	*1.65*	0.316
Alcohols and esters									
Glycerol	8.87	*0.20*	9.01	*0.97*	0.07	*0.02*	0.08	*0.02*	0.000
Mannitol	18.35	*2.11*	16.84	*0.19*	21.83	*4.52*	21.40	*2.63*	0.167
Myo-inositol	175.70	*44.82*	202.99	*33.78*	107.93	*18.32*	114.67	*16.29*	0.013
Amines, amides and N-compounds									
Adenosine	1.84	*0.21*	3.77	*1.01*	30.77	*1.91*	44.74	*7.01*	0.000
Adenine	1.08	*0.12*	1.24	*0.70*	6.44	*2.30*	11.90	*4.48*	0.002
Allantoin	28.80	*10.22*	17.83	*7.20*	15.37	*1.73*	11.73	*1.72*	0.052
Butyro-1,4-lactam	0.84	*0.10*	0.71	*0.05*	1.15	*0.19*	1.36	*0.19*	0.002
Calystegine A3	48.63	*12.49*	31.10	*8.96*	28.11	*5.50*	24.62	*3.15*	0.031
Calystegine B2	55.32	*6.64*	41.68	*5.91*	36.96	*10.27*	36.58	*3.74*	0.037
Calystegine B4	12.17	*1.74*	7.63	*4.63*	5.12	*4.30*	7.39	*4.44*	0.249
Ethanolamine	34.88	*3.94*	35.96	*6.96*	34.29	*3.90*	27.46	*3.18*	0.193
Guanine	0.13	*0.03*	0.11	*0.03*	2.08	*0.36*	1.15	*0.42*	0.000
Guanosine	0.46	*0.14*	0.56	*0.11*	8.93	*0.88*	17.27	*1.79*	0.000
Nicotinic acid	2.69	*0.79*	2.15	*0.32*	3.64	*0.87*	3.14	*0.52*	0.109
p-Tyramine	22.74	*3.75*	23.33	*7.27*	13.16	*3.19*	15.18	*4.54*	0.078
Pantothenic acid	1.88	*0.35*	1.76	*0.20*	2.69	*0.53*	2.92	*0.30*	0.010
Pipecolinic acid	4.87	*2.51*	1.70	*0.53*	1.34	*0.19*	2.70	*1.00*	0.053
Putrescine	43.19	*11.36*	54.63	*15.26*	20.71	*2.69*	18.77	*4.44*	0.005
Spermidine	4.01	*0.42*	3.60	*0.55*	1.10	*0.04*	1.00	*0.16*	0.000
Uracil	0.99	*0.09*	0.75	*0.49*	0.77	*0.08*	1.47	*0.27*	0.048
Urea	0.13	*0.03*	0.11	*0.03*	0.08	*0.02*	4.65	*1.09*	0.000
Amino acids									
3-Cyano-alanine	6.60	*0.41*	7.05	*3.03*	2.75	*0.56*	2.78	*0.41*	0.014
4-Aminobutyric acid	840.19	*55.10*	780.80	*7.50*	350.87	*5.36*	334.54	*3.31*	0.000
Alanine	241.31	*30.51*	244.43	*45.51*	236.85	*42.94*	222.72	*31.11*	0.900
Asparagine	523.07	*29.52*	504.21	*54.66*	193.77	*9.40*	186.91	*8.37*	0.000

Table A.6: Continued.

Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Aspartic acid	859.60	*47.64*	1407.65	*39.37*	375.96	*27.11*	319.68	*18.94*	0.000
Butyric acid	4.53	*0.63*	5.25	*1.43*	5.60	*2.05*	5.16	*1.13*	0.822
Glutamic acid	659.18	*13.08*	425.60	*28.51*	84.91	*22.02*	66.63	*13.87*	0.000
Glutamine	705.81	*29.92*	697.79	*40.29*	265.42	*11.97*	249.71	*8.85*	0.000
Glycine	125.00	*7.73*	118.11	*15.07*	62.21	*8.28*	69.25	*14.06*	0.000
Histidine	38.73	*6.37*	43.42	*7.94*	40.53	*9.47*	39.11	*5.79*	0.867
Homoserine	1.79	*0.51*	2.18	*0.46*	2.14	*0.03*	3.35	*0.34*	0.005
Isoleucine	166.19	*16.73*	173.49	*44.53*	166.96	*33.28*	201.59	*34.31*	0.560
Leucine	47.84	*13.11*	54.45	*20.84*	57.80	*6.46*	79.04	*8.64*	0.094
Lysine	94.54	*25.64*	116.58	*24.62*	46.84	*19.75*	55.71	*24.09*	0.021
Methionine	67.27	*2.73*	69.55	*7.75*	51.73	*8.68*	53.84	*8.92*	0.040
Ornithine	11.13	*2.90*	9.48	*1.21*	2.91	*0.96*	3.40	*0.89*	0.001
Phenylalanine	203.24	*13.06*	216.84	*41.28*	178.03	*13.28*	194.84	*14.95*	0.314
Proline	2.89	*0.37*	3.55	*1.60*	6.09	*1.38*	4.34	*1.01*	0.050
Pyroglutamic acid	783.39	*92.56*	841.12	*80.56*	426.49	*17.98*	415.62	*13.62*	0.000
Serine	226.78	*12.68*	213.05	*5.24*	107.82	*14.58*	127.26	*23.61*	0.000
Threonine	120.17	*4.79*	137.83	*12.56*	99.22	*14.46*	121.49	*18.54*	0.049
Tryptophan	86.51	*19.88*	225.78	*41.82*	236.66	*68.52*	262.98	*66.75*	0.014
Tyrosine	428.35	*29.30*	499.15	*118.40*	313.88	*83.44*	356.51	*75.62*	0.102
Valine	348.49	*46.86*	397.38	*56.77*	262.66	*40.52*	272.66	*23.34*	0.016
β-Alanine	39.01	*9.90*	33.40	*2.44*	21.14	*4.75*	20.24	*5.17*	0.014
Aromatics									
Chlorogenic acid	1.40	*0.36*	4.12	*2.27*	16.24	*4.38*	10.60	*0.81*	0.000
Fatty acids									
Eicosanoic acid	0.27	*0.07*	0.18	*0.10*	2.28	*0.67*	2.53	*0.54*	0.000
Hexadecanoic acid	169.52	*54.82*	127.95	*52.16*	127.55	*11.19*	121.75	*7.98*	0.446
Octadecadienoic acid	2.54	*1.43*	1.98	*1.13*	24.89	*6.55*	29.46	*6.10*	0.000
Octadecadienoic acid methyl ester	0.17	*0.10*	0.12	*0.03*	5.08	*1.20*	1.49	*0.26*	0.000
Octadecanoic acid	71.24	*33.87*	48.78	*30.23*	55.96	*3.95*	51.23	*2.55*	0.642
Octadecatrienoic acid	0.18	*0.12*	0.19	*0.14*	3.67	*1.14*	3.81	*0.88*	0.000
Octadecenoic acid, 9 -(Z)-	0.11	*0.03*	0.16	*0.07*	1.82	*0.50*	2.12	*0.43*	0.000
Octadecenoic acid, 9 -(E)-	0.13	*0.03*	0.11	*0.03*	0.54	*0.20*	0.60	*0.10*	0.001
Phosphates									
Fructose-6-phosphate	3.06	*0.88*	5.66	*2.42*	1.50	*0.22*	1.66	*0.28*	0.015
Glucose-6-phosphate	6.83	*2.34*	13.65	*5.61*	2.56	*0.49*	1.93	*0.51*	0.005
Glycerol-3-phosphate	15.16	*2.28*	13.76	*3.34*	19.64	*2.88*	18.90	*0.74*	0.056
Monomethylphosphate	12.78	*2.91*	17.30	*4.25*	1.00	*0.22*	0.73	*0.13*	0.000
Myo-inositol-1-phosphate	1.59	*0.21*	1.63	*0.39*	0.53	*0.13*	0.73	*0.18*	0.001
Phosphoric acid	1148.50	*37.74*	1158.23	*129.27*	547.73	*17.25*	528.90	*21.86*	0.000
Sugars									
Fructose	8.87	*2.16*	25.50	*7.63*	51.33	*3.65*	55.01	*4.36*	0.000
Galactose	4.42	*0.86*	10.61	*7.63*	8.44	*0.95*	4.30	*1.27*	0.210
Glucose	33.05	*17.80*	102.28	*67.99*	293.66	*11.46*	190.86	*29.67*	0.000
Mannose	2.60	*0.36*	5.33	*1.98*	10.44	*0.56*	7.52	*1.07*	0.000
Ribose	2.40	*0.67*	2.19	*1.71*	1.84	*0.97*	2.17	*0.92*	0.943
Sucrose	1054.70	*17.46*	1021.90	*32.77*	397.54	*5.83*	373.55	*2.08*	0.000
Unidentified compounds									
A144004	5.67	*0.22*	5.60	*0.05*	2.10	*0.09*	2.02	*0.06*	0.000
A148003	4.42	*2.00*	5.62	*1.32*	3.45	*2.34*	2.85	*0.88*	0.295
A160005	12.82	*7.31*	8.36	*8.44*	35.67	*27.72*	42.79	*12.07*	0.079
A171003	23.28	*5.93*	29.21	*7.55*	12.87	*2.55*	13.15	*2.27*	0.011
A207006 (sugar)	3.32	*0.63*	2.48	*1.13*	3.21	*1.87*	4.78	*1.65*	0.308
NA133011	2.48	*0.34*	2.62	*0.03*	0.95	*0.13*	0.90	*0.02*	0.000

Table A.7: Metabolite composition of potato tubers extracted 7 days after harvest (P7). RT were stored in room temperature, ST were stored at storage temperature (≈ 4 °C), 40 were stored at storage temperature and dried at 40 °C over night before extraction and 60 were stored at storage temperature and dried at 60 °C over night before extraction.

					P7				
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Acids									
2,4-Dihydroxybutanoic acid	0.10	*0.03*	0.12	*0.03*	0.21	*0.01*	0.97	*0.08*	0.000
2-Hethyl-maleic acid	3.65	*0.06*	3.70	*0.38*	1.91	*0.12*	1.95	*0.23*	0.000
3-Hydroxypropanoic acid	0.10	*0.03*	0.12	*0.03*	1.61	*0.06*	1.37	*0.11*	0.000
Aconitic acid	8.33	*3.74*	5.40	*4.73*	12.41	*3.58*	17.00	*6.59*	0.079
Ascorbic acid	24.41	*19.70*	9.06	*8.62*	8.04	*2.81*	11.36	*4.89*	0.313
Citric acid	651.92	*5.91*	650.91	*16.12*	248.48	*6.88*	228.38	*5.64*	0.000
Dehydroascorbic acid dimer	23.29	*6.96*	12.67	*1.99*	8.05	*1.02*	7.00	*1.97*	0.003
Fumaric acid	22.03	*3.68*	9.28	*1.82*	20.65	*4.09*	24.32	*1.30*	0.001
Galactaric acid	9.82	*2.22*	9.18	*0.36*	2.65	*0.26*	2.58	*0.36*	0.000
Glyceric acid	4.44	*0.97*	3.81	*0.76*	6.95	*1.06*	7.70	*0.44*	0.001
Itaconic acid	6.61	*1.95*	4.02	*3.43*	4.73	*0.68*	5.91	*0.76*	0.450
Lactic acid	0.12	*0.03*	0.11	*0.03*	16.27	*28.06*	38.96	*33.66*	0.177
Lyxonic acid	8.50	*2.25*	9.29	*0.81*	6.99	*0.97*	9.47	*0.24*	0.157
Malic acid	286.64	*73.86*	341.43	*27.94*	172.60	*9.88*	157.78	*10.21*	0.001
Malonic acid	2.00	*0.43*	1.95	*0.41*	2.05	*0.68*	2.36	*0.54*	0.785
Oxalic acid	0.10	*0.03*	0.12	*0.03*	0.08	*0.02*	0.07	*0.02*	0.119
Pyruvic acid	0.34	*0.23*	0.31	*0.38*	0.41	*0.31*	0.21	*0.23*	0.870
Quinic acid	58.78	*5.80*	17.49	*8.07*	17.38	*4.28*	19.52	*9.48*	0.000
Succinic acid	13.22	*2.59*	18.42	*1.31*	86.81	*10.52*	76.98	*7.31*	0.000
Threonic acid	12.50	*0.66*	12.32	*0.16*	11.83	*1.37*	14.12	*2.71*	0.364
Alcohols and esters									
Glycerol	8.09	*0.72*	7.73	*0.22*	0.07	*0.02*	0.08	*0.02*	0.000
Mannitol	13.53	*3.21*	14.14	*0.94*	17.88	*2.61*	18.66	*2.29*	0.070
Myo-inositol	176.18	*12.19*	136.20	*2.89*	84.00	*5.33*	95.15	*12.93*	0.000
Amines, amides and N-compounds									
Adenosine	2.86	*0.65*	5.61	*2.10*	24.69	*3.73*	37.49	*3.32*	0.000
Adenine	1.00	*0.28*	1.56	*0.29*	4.26	*0.30*	8.25	*1.36*	0.000
Allantoin	26.21	*9.85*	15.76	*0.70*	11.53	*2.42*	9.75	*0.37*	0.017
Butyro-1,4-lactam	0.90	*0.18*	0.71	*0.15*	1.33	*0.18*	1.56	*0.15*	0.001
Calystegine A3	29.95	*7.01*	27.44	*6.67*	28.40	*3.48*	24.04	*3.16*	0.606
Calystegine B2	37.69	*10.92*	22.76	*18.14*	33.02	*6.07*	34.00	*4.44*	0.452
Calystegine B4	11.32	*9.48*	6.65	*5.24*	7.97	*3.13*	6.80	*1.58*	0.733
Ethanolamine	33.53	*1.76*	34.63	*4.04*	29.90	*1.95*	28.40	*3.33*	0.093
Guanine	0.12	*0.03*	0.11	*0.03*	1.06	*0.18*	0.84	*0.15*	0.000
Guanosine	0.54	*0.14*	0.90	*0.26*	6.05	*1.12*	12.38	*0.76*	0.000
Nicotinic acid	2.86	*0.58*	2.76	*0.65*	3.45	*0.16*	3.47	*0.55*	0.273
p-Tyramine	26.74	*3.72*	25.28	*4.35*	15.66	*4.20*	20.25	*2.29*	0.024
Pantothenic acid	1.56	*0.19*	1.70	*0.13*	3.17	*0.53*	2.90	*0.20*	0.000
Pipecolinic acid	4.95	*3.28*	2.73	*2.26*	3.15	*1.25*	1.88	*0.87*	0.401
Putrescine	33.97	*1.86*	60.25	*3.89*	17.81	*2.08*	17.30	*3.88*	0.000
Spermidine	4.11	*0.97*	3.32	*0.01*	0.79	*0.23*	0.76	*0.18*	0.000
Uracil	0.83	*0.33*	0.72	*0.05*	0.66	*0.12*	1.30	*0.25*	0.029
Urea	0.69	*0.53*	0.12	*0.03*	0.84	*0.81*	4.71	*2.46*	0.010
Amino acids									
3-Cyano-alanine	8.04	*0.77*	7.85	*0.55*	2.22	*0.71*	2.64	*0.68*	0.000
4-Aminobutyric acid	810.04	*58.63*	772.62	*103.20*	350.57	*8.28*	323.46	*3.13*	0.000
Alanine	176.59	*42.72*	175.45	*89.94*	220.05	*35.97*	254.20	*43.69*	0.335
Asparagine	556.59	*87.82*	557.98	*24.47*	187.77	*23.37*	173.27	*18.96*	0.000

Table A.7: Continued.

Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
				mg/ 100 g DW					
Aspartic acid	843.25	*41.43*	1335.49	*20.37*	406.04	*66.94*	318.41	*25.98*	0.000
Butyric acid	3.76	*1.27*	4.39	*1.36*	4.80	*1.83*	6.34	*2.85*	0.455
Glutamic acid	690.14	*15.47*	559.41	*26.93*	84.52	*11.71*	85.68	*14.85*	0.000
Glutamine	658.35	*49.75*	681.78	*46.72*	258.57	*13.40*	237.04	*6.38*	0.000
Glycine	87.64	*13.77*	88.84	*35.10*	62.51	*11.68*	68.71	*15.37*	0.374
Histidine	35.04	*15.75*	43.15	*13.25*	37.06	*11.30*	38.74	*14.15*	0.900
Homoserine	1.08	*0.28*	1.71	*0.35*	1.65	*0.40*	3.39	*0.54*	0.001
Isoleucine	183.60	*22.47*	168.01	*58.37*	197.01	*58.07*	192.41	*62.48*	0.910
Leucine	60.44	*12.64*	63.41	*44.17*	81.45	*34.34*	86.67	*36.08*	0.729
Lysine	104.22	*22.27*	122.00	*39.34*	42.62	*14.18*	43.49	*16.41*	0.008
Methionine	68.60	*6.46*	68.10	*14.08*	55.70	*14.38*	54.09	*12.43*	0.366
Ornithine	10.06	*4.18*	10.39	*2.07*	2.56	*0.44*	2.90	*1.23*	0.005
Phenylalanine	191.75	*24.16*	160.04	*42.15*	183.12	*24.20*	190.16	*25.20*	0.568
Proline	4.04	*1.17*	3.21	*0.46*	4.80	*1.46*	6.03	*1.18*	0.075
Pyroglutamic acid	699.20	*104.72*	764.26	*145.96*	417.96	*22.02*	270.39	*234.14*	0.011
Serine	177.60	*27.77*	220.39	*58.45*	129.99	*21.33*	121.24	*22.02*	0.031
Threonine	126.85	*18.00*	131.49	*32.52*	111.16	*22.52*	103.96	*22.22*	0.506
Tryptophan	218.48	*55.25*	231.17	*35.04*	195.65	*35.75*	222.34	*102.14*	0.912
Tyrosine	622.58	*65.35*	506.91	*115.10*	345.04	*71.35*	362.88	*65.30*	0.010
Valine	362.09	*89.76*	343.99	*104.97*	275.83	*46.66*	271.17	*46.97*	0.401
β-Alanine	27.78	*5.12*	27.15	*8.23*	20.81	*2.43*	23.50	*3.97*	0.401
Aromatics									
Chlorogenic acid	1.92	*0.82*	8.23	*2.58*	21.50	*3.77*	24.09	*2.92*	0.000
Fatty acids									
Eicosanoic acid	0.20	*0.06*	0.20	*0.10*	2.16	*0.40*	2.72	*0.77*	0.000
Hexadecanoic acid	110.62	*11.77*	103.52	*45.02*	121.21	*8.94*	121.68	*7.03*	0.754
Octadecadienoic acid	1.85	*0.49*	1.45	*0.84*	25.86	*5.31*	34.01	*6.64*	0.000
Octadecadienoic acid methyl ester	0.10	*0.03*	0.12	*0.03*	5.65	*0.98*	1.82	*0.28*	0.000
Octadecanoic acid	43.24	*7.94*	37.84	*23.07*	52.06	*1.71*	49.58	*2.02*	0.519
Octadecatrienoic acid	0.14	*0.05*	0.17	*0.11*	4.20	*0.89*	5.54	*1.41*	0.000
Octadecenoic acid, 9 -(Z)-	0.12	*0.07*	0.12	*0.03*	1.81	*0.35*	2.41	*0.50*	0.000
Octadecenoic acid, 9 -(E)-	0.12	*0.03*	0.11	*0.03*	0.43	*0.15*	0.51	*0.15*	0.003
Phosphates									
Fructose-6-phosphate	2.20	*0.06*	6.37	*0.78*	0.99	*0.21*	1.52	*0.34*	0.000
Glucose-6-phosphate	4.88	*0.60*	12.46	*2.37*	1.61	*0.27*	1.55	*0.35*	0.000
Glycerol-3-phosphate	14.06	*3.79*	15.67	*0.68*	21.70	*3.82*	23.63	*2.82*	0.013
Monomethylphosphate	15.55	*1.29*	18.71	*1.69*	0.97	*0.11*	0.83	*0.12*	0.000
Myo-inositol-1-phosphate	0.90	*0.05*	1.52	*0.06*	0.50	*0.07*	0.71	*0.04*	0.000
Phosphoric acid	1029.62	*58.70*	1008.40	*26.61*	496.70	*42.27*	490.39	*9.50*	0.000
Sugars									
Fructose	16.17	*13.92*	100.85	*21.87*	52.99	*5.78*	93.52	*16.19*	0.001
Galactose	5.81	*2.28*	7.19	*1.43*	4.23	*0.74*	3.46	*1.00*	0.059
Glucose	26.11	*16.80*	183.56	*42.89*	217.59	*25.17*	211.06	*62.62*	0.001
Mannose	2.75	*0.77*	5.46	*0.94*	6.96	*0.32*	7.55	*0.68*	0.000
Ribose	1.42	*0.92*	2.26	*0.74*	1.27	*1.06*	2.16	*1.62*	0.635
Sucrose	1026.61	*5.66*	1023.98	*23.89*	391.22	*11.71*	365.67	*6.32*	0.000
Unidentified compounds									
A144004	5.51	*0.27*	5.41	*0.13*	2.13	*0.19*	1.91	*0.01*	0.000
A148003	3.84	*2.42*	1.73	*0.64*	1.71	*0.87*	4.49	*0.41*	0.072
A160005	8.01	*12.04*	5.63	*6.04*	18.49	*20.79*	51.31	*38.57*	0.131
A171003	17.85	*3.31*	25.25	*5.09*	12.00	*1.05*	12.04	*1.89*	0.003
A207006 (sugar)	4.10	*3.12*	2.89	*2.23*	4.16	*1.07*	6.34	*3.05*	0.450
NA133011	2.29	*0.10*	2.30	*0.34*	0.89	*0.13*	0.77	*0.09*	0.000

Table A.8: Metabolite composition of potato tubers extracted 14 days after harvest (P14). RT were stored in room temperature, ST were stored at storage temperature (≈ 4 °C), 40 were stored at storage temperature and dried at 40 °C over night before extraction and 60 were stored at storage temperature and dried at 60 °C over night before extraction.

					P14				
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Acids									
2,4-Dihydroxybutanoic acid	0.11	*0.03*	0.17	*0.02*	0.22	*0.04*	0.91	*0.10*	0.000
2-Hethyl-maleic acid	3.64	*0.37*	3.46	*0.28*	1.61	*0.34*	1.90	*0.12*	0.000
3-Hydroxypropanoic acid	0.11	*0.03*	1.05	*0.01*	1.72	*0.15*	1.34	*0.08*	0.000
Aconitic acid	4.83	*4.05*	3.25	*2.74*	10.10	*1.41*	16.70	*1.37*	0.001
Ascorbic acid	15.57	*13.73*	2.76	*3.39*	4.60	*3.49*	10.94	*1.36*	0.201
Citric acid	638.32	*4.94*	623.69	*16.82*	230.45	*3.21*	225.81	*2.60*	0.000
Dehydroascorbic acid dimer	19.39	*2.16*	20.32	*9.29*	5.64	*4.00*	9.13	*1.22*	0.019
Fumaric acid	50.26	*21.00*	13.84	*3.17*	14.39	*6.31*	21.99	*3.55*	0.012
Galactaric acid	11.36	*1.79*	9.89	*1.63*	2.53	*0.71*	2.57	*0.45*	0.000
Glyceric acid	4.21	*0.78*	3.93	*0.25*	4.96	*1.50*	6.22	*0.71*	0.063
Itaconic acid	2.30	*3.78*	4.32	*3.67*	3.05	*2.61*	5.43	*0.61*	0.602
Lactic acid	0.11	*0.03*	0.13	*0.03*	9.67	*16.63*	27.87	*24.41*	0.148
Lyxonic acid	10.64	*1.23*	10.75	*1.12*	6.37	*0.93*	8.70	*0.60*	0.002
Malic acid	403.95	*34.67*	421.44	*2.42*	154.22	*29.53*	161.19	*5.02*	0.000
Malonic acid	3.57	*0.12*	2.07	*0.21*	1.66	*0.45*	2.50	*0.15*	0.000
Oxalic acid	0.11	*0.03*	0.67	*0.49*	0.13	*0.08*	0.07	*0.02*	0.050
Pyruvic acid	0.13	*0.04*	0.40	*0.48*	0.28	*0.36*	0.08	*0.02*	0.582
Quinic acid	69.89	*21.99*	33.97	*18.85*	20.96	*8.73*	29.01	*10.51*	0.024
Succinic acid	9.59	*2.42*	10.97	*1.36*	58.67	*22.55*	64.14	*5.41*	0.001
Threonic acid	15.03	*2.58*	16.76	*1.16*	10.31	*2.16*	12.70	*2.14*	0.024
Alcohols and esters									
Glycerol	4.62	*3.90*	9.11	*0.67*	0.06	*0.02*	0.08	*0.02*	0.001
Mannitol	21.40	*2.45*	20.20	*1.68*	16.55	*3.96*	18.35	*1.48*	0.188
Myo-inositol	184.71	*51.39*	156.73	*8.21*	94.82	*13.81*	99.68	*4.73*	0.009
Amines, amides and N-compounds									
Adenosine	1.95	*0.92*	3.94	*1.01*	21.35	*2.88*	36.95	*2.57*	0.000
Adenine	0.84	*0.31*	1.80	*0.63*	3.25	*0.61*	6.81	*1.18*	0.000
Allantoin	35.43	*9.08*	23.23	*2.39*	6.33	*4.97*	9.61	*2.15*	0.001
Butyro-1,4-lactam	0.94	*0.19*	0.79	*0.04*	1.69	*1.02*	1.66	*0.14*	0.138
Calystegine A3	32.13	*3.60*	33.07	*5.65*	17.59	*1.80*	21.63	*2.26*	0.002
Calystegine B2	49.76	*9.97*	27.49	*22.64*	24.84	*2.17*	25.36	*2.29*	0.110
Calystegine B4	10.78	*6.73*	1.10	*0.97*	3.07	*2.46*	5.66	*2.56*	0.066
Ethanolamine	35.85	*2.13*	34.38	*4.13*	28.13	*5.44*	28.92	*0.40*	0.070
Guanine	0.13	*0.04*	0.11	*0.04*	0.80	*0.19*	0.48	*0.03*	0.000
Guanosine	0.56	*0.27*	0.61	*0.19*	4.09	*0.65*	11.95	*1.33*	0.000
Nicotinic acid	3.23	*0.13*	2.60	*0.64*	2.59	*0.74*	3.58	*0.37*	0.126
p-Tyramine	19.66	*3.90*	22.02	*0.80*	12.46	*1.23*	19.79	*1.69*	0.004
Pantothenic acid	1.91	*0.31*	1.79	*0.31*	2.61	*0.20*	3.25	*0.11*	0.000
Pipecolinic acid	3.80	*2.54*	5.06	*0.87*	5.81	*5.55*	2.93	*0.81*	0.685
Putrescine	24.60	*1.39*	52.44	*3.14*	14.52	*1.22*	15.55	*1.38*	0.000
Spermidine	4.16	*0.43*	3.40	*0.21*	0.51	*0.25*	0.76	*0.03*	0.000
Uracil	0.66	*0.16*	0.85	*0.17*	0.56	*0.12*	1.03	*0.16*	0.022
Urea	0.13	*0.04*	0.11	*0.04*	0.07	*0.02*	4.57	*0.38*	0.000
Amino acids									
3-Cyano-alanine	6.62	*1.43*	5.28	*1.11*	1.86	*0.47*	2.43	*0.15*	0.001
4-Aminobutyric acid	818.67	*33.72*	742.57	*31.74*	330.21	*3.04*	321.15	*3.15*	0.000
Alanine	213.58	*66.51*	110.83	*17.77*	127.37	*106.96*	236.44	*16.81*	0.107
Asparagine	513.19	*36.88*	425.34	*69.47*	140.36	*17.06*	162.55	*3.25*	0.000

Table A.8: Continued.

Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Aspartic acid	903.15	*42.69*	1385.95	*31.97*	354.64	*95.31*	384.06	*8.09*	0.000
Butyric acid	4.42	*0.08*	3.47	*0.93*	4.06	*1.59*	5.07	*0.45*	0.290
Glutamic acid	706.93	*14.13*	620.14	*14.77*	89.74	*16.87*	113.67	*6.28*	0.000
Glutamine	648.84	*12.91*	217.20	*355.86*	154.81	*133.94*	240.87	*8.53*	0.046
Glycine	110.16	*29.54*	79.59	*19.08*	44.83	*21.96*	67.38	*1.52*	0.028
Histidine	52.24	*10.72*	32.07	*7.83*	19.43	*12.76*	37.86	*3.94*	0.017
Homoserine	1.19	*0.15*	1.35	*0.46*	1.15	*0.38*	3.40	*0.20*	0.000
Isoleucine	197.48	*81.63*	145.81	*25.95*	133.56	*23.73*	185.22	*18.37*	0.320
Leucine	85.34	*34.76*	59.57	*19.47*	59.17	*12.22*	86.51	*13.71*	0.295
Lysine	141.95	*65.08*	93.86	*15.27*	27.39	*7.33*	36.91	*5.62*	0.010
Methionine	81.47	*17.58*	51.96	*1.43*	31.78	*12.29*	52.90	*4.92*	0.004
Ornithine	8.60	*4.45*	5.31	*2.49*	1.30	*0.44*	2.81	*0.84*	0.039
Phenylalanine	185.20	*30.66*	145.68	*12.58*	130.32	*49.02*	196.23	*16.39*	0.087
Proline	8.46	*2.56*	3.97	*0.35*	4.25	*3.42*	7.01	*0.91*	0.097
Pyroglutamic acid	807.49	*53.06*	546.57	*149.77*	357.09	*49.86*	272.71	*236.24*	0.008
Serine	207.72	*71.32*	209.93	*48.97*	99.35	*24.04*	131.56	*16.73*	0.041
Threonine	145.73	*33.56*	103.73	*18.82*	79.15	*12.26*	107.83	*14.11*	0.031
Tryptophan	194.48	*120.37*	146.70	*42.26*	145.04	*50.02*	216.63	*39.98*	0.553
Tyrosine	583.54	*293.77*	525.69	*62.90*	276.54	*55.20*	366.16	*26.73*	0.128
Valine	424.56	*69.98*	310.73	*36.01*	221.84	*37.53*	260.19	*13.92*	0.003
β-Alanine	25.76	*2.33*	20.72	*7.12*	15.21	*7.14*	24.51	*0.42*	0.132
Aromatics									
Chlorogenic acid	1.57	*0.87*	9.39	*2.93*	15.39	*6.18*	18.66	*4.98*	0.005
Fatty acids									
Eicosanoic acid	0.19	*0.08*	0.12	*0.05*	1.71	*0.21*	2.55	*0.31*	0.000
Hexadecanoic acid	117.76	*50.78*	115.16	*46.01*	106.78	*5.08*	118.64	*4.07*	0.972
Octadecadienoic acid	1.73	*1.17*	1.77	*1.33*	18.34	*4.07*	32.80	*3.27*	0.000
Octadecadienoic acid methyl ester	0.11	*0.03*	0.13	*0.03*	4.24	*1.21*	1.63	*0.20*	0.000
Octadecanoic acid	48.60	*27.45*	44.25	*25.59*	45.77	*1.87*	48.95	*2.24*	0.987
Octadecatrienoic acid	0.13	*0.04*	0.25	*0.24*	3.05	*0.55*	5.13	*0.52*	0.000
Octadecenoic acid, 9 -(Z)-	0.13	*0.04*	0.16	*0.08*	1.25	*0.28*	2.22	*0.18*	0.000
Octadecenoic acid, 9 -(E)-	0.13	*0.04*	0.11	*0.04*	0.35	*0.05*	0.46	*0.06*	0.000
Phosphates									
Fructose-6-phosphate	2.67	*0.62*	6.63	*1.72*	1.26	*0.25*	1.34	*0.28*	0.000
Glucose-6-phosphate	5.77	*1.21*	13.46	*1.41*	1.90	*0.54*	1.45	*0.30*	0.000
Glycerol-3-phosphate	16.41	*1.47*	15.83	*2.14*	19.41	*2.19*	21.06	*0.76*	0.018
Monomethylphosphate	15.15	*1.36*	18.64	*1.20*	0.86	*0.16*	0.82	*0.05*	0.000
Myo-inositol-1-phosphate	0.82	*0.16*	1.97	*0.36*	0.55	*0.16*	0.70	*0.04*	0.000
Phosphoric acid	1255.13	*124.96*	1187.05	*72.95*	421.56	*142.37*	493.58	*10.66*	0.000
Sugars									
Fructose	21.33	*10.99*	240.39	*84.52*	151.33	*37.24*	168.03	*13.33*	0.003
Galactose	9.85	*5.09*	16.93	*0.97*	9.13	*3.37*	4.45	*1.38*	0.009
Glucose	51.03	*14.81*	415.91	*119.07*	295.99	*48.42*	227.36	*9.40*	0.001
Mannose	2.89	*1.53*	9.05	*0.61*	10.14	*1.83*	8.84	*1.53*	0.001
Ribose	1.40	*0.05*	1.13	*0.54*	0.66	*0.54*	1.87	*0.59*	0.082
Sucrose	1014.92	*2.86*	989.99	*29.14*	368.38	*6.63*	361.42	*4.28*	0.000
Unidentified compounds									
A144004	5.42	*0.16*	5.06	*0.21*	1.70	*0.46*	2.04	*0.06*	0.000
A148003	4.20	*2.93*	2.71	*0.66*	3.30	*2.80*	4.95	*3.22*	0.740
A160005	3.99	*4.05*	5.48	*7.27*	19.49	*17.20*	38.32	*10.06*	0.015
A171003	17.70	*0.35*	13.21	*5.84*	7.85	*2.31*	11.39	*1.37*	0.033
A207006 (sugar)	2.82	*1.87*	0.59	*0.59*	2.87	*2.10*	4.68	*0.32*	0.051
NA133011	2.23	*0.05*	2.23	*0.33*	0.67	*0.23*	0.81	*0.04*	0.000

Table A.9: Metabolite composition of tomatoes extracted the day of harvest (T0). Fresh tubers were extracted directly after harvest, 40 were dried at 40 °C over night before extraction and 60 were dried at 60 °C over night before extraction.

			TO				
Compound	Fresh control	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
			mg/ 100 g DW				
Acids							
1,4-Lactonethreonic acid	0.63	*0.24*	16.93	*15.36*	1.31	*0.37*	0.111
2-Methyl-maleic acid	39.24	*5.42*	0.63	*0.22*	1.70	*0.98*	0.000
2-Oxoglutaric acid	18.70	*3.75*	0.77	*0.38*	1.31	*0.37*	0.000
Ascorbic acid	25.38	*34.42*	11.41	*18.45*	1.70	*0.98*	0.478
Citric acid	3827.01	*459.89*	56.00	*48.55*	87.90	*97.92*	0.000
Dehydroascorbic acid dimer	51.32	*18.53*	489.63	*309.51*	1596.43	*790.74*	0.021
Fumaric acid	35.61	*18.14*	83.57	*73.86*	3.54	*3.19*	0.161
Galactaric acid	128.44	*34.66*	0.63	*0.22*	1.70	*0.98*	0.000
Galacturonic acid	204.79	*43.39*	513.65	*192.98*	1270.09	*521.68*	0.017
Glyceric acid	8.97	*4.02*	5.01	*7.38*	1.70	*0.98*	0.267
Glycolic acid	0.73	*0.17*	28.82	*13.00*	39.41	*13.15*	0.011
Itaconic acid	75.64	*17.31*	328.87	*188.01*	1.31	*0.37*	0.024
Lactic acid	0.63	*0.24*	12.78	*3.61*	14.22	*11.20*	0.093
Malic acid	1517.03	*131.58*	31.52	*53.29*	1.70	*0.98*	0.000
Quinic acid	143.52	*19.56*	416.07	*373.26*	1.31	*0.37*	0.134
Succinic acid	32.97	*4.25*	0.63	*0.22*	1.70	*0.98*	0.000
Alcohols and esters							
Myo-inositol	750.14	*164.49*	805.85	*312.03*	1146.92	*542.86*	0.425
Threitol	10.87	*1.04*	8.41	*4.45*	2.87	*2.77*	0.047
α-Tocopherol	0.73	*0.17*	32.69	*4.81*	95.38	*36.00*	0.004
γ-Tocopherol	0.73	*0.17*	9.13	*3.67*	24.01	*7.21*	0.002
α-Tocopheryl acetate	0.73	*0.17*	14.55	*7.52*	69.52	*54.69*	0.082
Amines, amides and N-compounds							
5-Hydroxytryptamine	70.36	*6.80*	1.26	*0.43*	3.41	*1.96*	0.000
Adenosine	20.58	*2.82*	1.40	*0.46*	3.02	*1.22*	0.000
Ethanolamine	375.02	*21.27*	0.77	*0.38*	1.31	*0.37*	0.000
N-carboxymethylamine	89.85	*5.97*	0.63	*0.22*	1.70	*0.98*	0.000
Orotic acid	14.12	*4.74*	12.03	*19.53*	205.08	*353.24*	0.461
Putrescine	268.72	*65.37*	0.77	*0.38*	1.31	*0.37*	0.000
Uridine	15.23	*11.57*	2.40	*2.85*	1.70	*0.98*	0.092
Uracil	11.24	*4.91*	0.63	*0.22*	1.70	*0.98*	0.008
Amino acids							
4-Aminobutyric acid	3487.39	*673.52*	1.10	*0.95*	10.91	*9.28*	0.000
Alanine	77.77	*4.27*	1.40	*0.46*	3.02	*1.22*	0.000
Asparagine	32.05	*45.33*	0.72	*0.07*	1.70	*0.98*	0.319
Aspartic acid	545.58	*474.37*	1.54	*0.76*	3.08	*0.84*	0.081
Glutamic acid	3744.15	*733.69*	0.77	*0.38*	1.31	*0.37*	0.000
Glutamine	386.69	*455.92*	0.63	*0.22*	1.70	*0.98*	0.198
Glycine	63.74	*26.84*	0.63	*0.22*	1.70	*0.98*	0.004
Isoleucine	39.32	*11.57*	0.63	*0.22*	1.70	*0.98*	0.001
Leucine	34.99	*12.17*	0.63	*0.22*	1.70	*0.98*	0.002
Pyroglutamic acid	1182.82	*319.30*	0.63	*0.22*	1.70	*0.98*	0.000
Serine	180.31	*80.55*	1.54	*0.76*	2.63	*0.74*	0.005
Threonine	62.90	*17.20*	6.02	*1.22*	10.65	*5.53*	0.001
Valine	36.54	*13.90*	0.77	*0.38*	1.31	*0.37*	0.002
β-Alanine	46.84	*21.82*	0.77	*0.38*	1.31	*0.37*	0.006
Aromatics							
Chlorogenic acid	23.30	*7.80*	0.63	*0.22*	1.70	*0.98*	0.001

Table A.9: Continued.

			TO				
Compound	Fresh control	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
			mg/ 100 g DW				
Fatty acids							
Heptanoic acid	29.03	*4.26*	0.63	*0.22*	1.70	*0.98*	0.000
Hexadecanoic acid	191.68	*122.17*	24.28	*24.44*	100.77	*57.68*	0.105
Octadecanoic acid	56.86	*39.16*	3.44	*4.54*	2.11	*1.69*	0.042
Monoglyceride	1.89	*0.73*	21.00	*4.40*	43.50	*9.96*	0.001
Flavonoids							
Naringenin	41.12	*48.03*	0.63	*0.22*	1.70	*0.98*	0.206
Phosphates							
Fructose-6-phosphate	21.06	*6.70*	0.77	*0.38*	1.31	*0.37*	0.001
Glucose-6-phosphate	40.21	*14.12*	0.77	*0.38*	1.31	*0.37*	0.002
Inositol-1-phosphate	10.79	*1.03*	0.77	*0.38*	1.31	*0.37*	0.000
Phosphoric acid	4811.99	*247.36*	4100.82	*964.94*	8893.98	*2164.26*	0.011
Sugars							
Arabinose	13.65	*3.41*	11.85	*9.84*	1.31	*0.37*	0.090
Cellobiose	16.54	*3.17*	12.08	*2.80*	1.31	*0.37*	0.001
Fructose	6445.15	*2318.00*	5873.72	*2382.79*	15137.89	*8254.31*	0.121
Galactose	1749.91	*43.78*	916.55	*815.59*	768.45	*1327.90*	0.410
Glucose	10066.66	*2818.59*	4777.86	*2620.04*	13296.26	*5698.01*	0.097
Laminaribose	23.17	*1.73*	31.54	*5.70*	32.02	*13.11*	0.399
Ribose	76.06	*44.18*	12.93	*10.21*	1.31	*0.37*	0.026
Sucrose	1966.74	*286.58*	1201.39	*482.06*	626.37	*234.86*	0.010
Sugar (O-methyl)	83.78	*43.13*	1416.17	*369.70*	3537.22	*1728.93*	0.017
Xylose	10.82	*1.14*	9.70	*8.49*	1.31	*0.37*	0.108
Unidentified compounds							
A207006 (sugar)	4.09	*5.65*	33.80	*13.16*	33.25	*31.24*	0.192
A212004	58.70	*15.57*	0.63	*0.22*	1.70	*0.98*	0.000
A213001	48.87	*13.32*	8.80	*2.65*	11.49	*3.18*	0.001

Table A.10: Metabolite composition of tomatoes extracted 3 days after harvest (T3). RT were stored in room temperature, ST were stored at storage temperature ($\approx$ 4 °C), 40 were stored at storage temperature and dried at 40 °C over night before extraction and 60 were stored at storage temperature and dried at 60 °C over night before extraction.

			T3						
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Acids									
1,4-Lactonethreonic acid	1.51	*1.47*	0.73	*0.21*	15.82	*4.43*	1.08	*0.09*	0.000
2-Methyl-maleic acid	25.08	*10.29*	24.70	*4.08*	0.47	*0.15*	1.38	*0.59*	0.001
2-Oxoglutaric acid	11.37	*10.76*	3.01	*4.08*	0.56	*0.17*	1.08	*0.09*	0.156
Ascorbic acid	9.84	*7.57*	5.02	*2.63*	12.23	*15.07*	1.99	*0.47*	0.491
Citric acid	3771.88	*325.91*	3906.18	*66.69*	51.09	*21.47*	129.34	*19.98*	0.000
Dehydroascorbic acid dimer	51.25	*11.66*	50.34	*3.60*	355.28	*57.95*	1244.73	*216.97*	0.000
Fumaric acid	31.60	*32.99*	39.51	*5.21*	25.38	*33.12*	14.64	*13.58*	0.661
Galactaric acid	113.64	*9.19*	112.00	*50.27*	0.47	*0.15*	1.38	*0.59*	0.001
Galacturonic acid	619.39	*364.44*	391.91	*249.95*	315.10	*49.29*	900.26	*299.77*	0.103
Glyceric acid	8.38	*3.38*	9.59	*5.43*	1.14	*1.05*	1.38	*0.59*	0.022
Glycolic acid	0.66	*0.16*	0.73	*0.21*	7.07	*11.40*	18.98	*15.52*	0.143
Itaconic acid	54.09	*32.51*	62.30	*25.61*	142.34	*167.95*	128.98	*27.41*	0.528
Lactic acid	0.66	*0.16*	0.73	*0.21*	10.32	*8.97*	18.76	*3.04*	0.004
Malic acid	1178.15	*265.90*	1333.88	*277.54*	3.75	*5.55*	1.08	*0.09*	0.000
Quinic acid	140.55	*16.19*	95.13	*7.31*	488.95	*212.72*	1.38	*0.59*	0.003
Succinic acid	27.70	*5.28*	9.66	*2.03*	0.94	*0.71*	1.38	*0.59*	0.000
Alcohols and esters									
Myo-inositol	769.22	*175.92*	705.13	*197.07*	542.73	*20.20*	1198.90	*326.29*	0.026
Threitol	12.28	*3.09*	8.53	*1.54*	9.44	*1.10*	2.06	*1.78*	0.002
α-Tocopherol	0.80	*0.29*	0.62	*0.22*	35.22	*10.23*	96.99	*10.17*	0.000
α-Tocopheryl acetate	0.80	*0.29*	0.62	*0.22*	11.33	*5.71*	41.26	*14.85*	0.001
γ-Tocopherol	0.80	*0.29*	0.62	*0.22*	10.30	*2.93*	20.41	*5.14*	0.000
Amines, amides and N-compounds									
5-Hydroxytryptamine	63.71	*18.90*	51.12	*15.36*	0.94	*0.29*	2.77	*1.18*	0.000
Adenosine	39.07	*17.15*	22.24	*10.53*	1.93	*1.48*	2.46	*0.51*	0.005
Ethanolamine	381.69	*11.27*	404.45	*18.76*	0.56	*0.17*	1.08	*0.09*	0.000
N-carboxymethylamine	53.15	*50.73*	94.90	*18.59*	0.47	*0.15*	1.38	*0.59*	0.007
Orotic acid	11.56	*2.83*	3.38	*2.16*	0.56	*0.17*	1.08	*0.09*	0.000
Putrescine	334.78	*94.44*	326.86	*61.79*	0.56	*0.17*	1.08	*0.09*	0.000
Uridine	34.65	*18.88*	23.50	*16.57*	5.16	*7.98*	1.08	*0.09*	0.044
Uracil	10.73	*4.96*	6.80	*0.21*	0.47	*0.15*	1.38	*0.59*	0.003
Amino acids									
4-Aminobutyric acid	2635.34	*340.35*	3036.37	*444.72*	0.56	*0.17*	5.83	*2.01*	0.000
Alanine	104.24	*58.31*	65.51	*14.58*	1.03	*0.11*	2.46	*0.51*	0.007
Asparagine	55.33	*58.66*	12.90	*16.93*	0.69	*0.39*	1.38	*0.59*	0.173
Aspartic acid	548.90	*296.25*	433.40	*259.36*	1.12	*0.34*	2.16	*0.18*	0.016
Glutamic acid	4377.37	*468.65*	2216.18	*1555.08*	0.56	*0.17*	1.08	*0.09*	0.000
Glutamine	541.04	*259.72*	221.37	*277.94*	0.47	*0.15*	1.38	*0.59*	0.025
Glycine	70.72	*28.24*	61.88	*25.30*	0.47	*0.15*	1.38	*0.59*	0.002
Isoleucine	44.25	*19.00*	49.47	*20.09*	0.47	*0.15*	1.38	*0.59*	0.003
Leucine	39.39	*12.88*	39.24	*14.84*	0.47	*0.15*	1.38	*0.59*	0.001
Pyroglutamic acid	1470.60	*708.06*	1507.47	*644.74*	0.47	*0.15*	1.38	*0.59*	0.005
Serine	215.40	*139.45*	202.79	*75.07*	1.12	*0.34*	2.16	*0.18*	0.013
Threonine	84.38	*36.23*	76.34	*31.06*	2.22	*1.82*	6.65	*7.63*	0.005
Valine	40.50	*16.87*	33.28	*11.34*	0.56	*0.17*	1.08	*0.09*	0.002
β-Alanine	37.58	*19.74*	44.90	*10.73*	0.56	*0.17*	1.08	*0.09*	0.002
Aromatics									
Chlorogenic acid	9.45	*8.62*	4.85	*2.89*	0.47	*0.15*	1.38	*0.59*	0.144

Table A.10: Continued.

					T3				
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Fatty acids									
Heptanoic acid	21.51	*4.80*	23.89	*5.30*	0.47	*0.15*	1.38	*0.59*	0.000
Hexadecanoic acid	408.85	*180.60*	235.12	*107.91*	69.53	*77.42*	131.64	*20.29*	0.028
Octadecanoic acid	126.78	*67.11*	59.20	*42.12*	15.39	*25.71*	1.08	*0.09*	0.024
Monoglyceride	2.90	*0.37*	1.79	*0.93*	23.44	*6.32*	47.03	*3.98*	0.000
Flavonoids									
Naringenin	14.29	*1.44*	10.51	*8.36*	0.47	*0.15*	1.38	*0.59*	0.010
Phosphates									
Fructose-6-phosphate	23.79	*3.20*	18.91	*4.70*	0.56	*0.17*	1.08	*0.09*	0.000
Glucose-6-phosphate	38.52	*3.92*	36.18	*13.84*	0.56	*0.17*	1.08	*0.09*	0.000
Inositol-1-phosphate	20.53	*9.76*	10.71	*5.57*	0.56	*0.17*	1.08	*0.09*	0.007
Phosphoric acid	4468.43	*3983.18*	4736.49	*1643.70*	3309.31	*245.23*	7299.99	*1283.18*	0.249
Sugars									
Arabinose	27.85	*8.22*	14.05	*5.33*	14.50	*2.78*	1.38	*0.59*	0.002
Cellobiose	23.64	*5.12*	19.02	*8.16*	22.45	*12.97*	1.38	*0.59*	0.032
Fructose	6369.50	*2369.78*	5296.89	*604.05*	4482.24	*331.97*	11901.93	*2703.32*	0.004
Galactose	1871.58	*85.41*	1836.81	*135.22*	236.17	*408.14*	825.10	*1426.96*	0.071
Glucose	10824.60	*2355.46*	10634.10	*2831.82*	4801.76	*506.42*	9615.89	*2013.02*	0.026
Laminaribose	28.82	*3.99*	22.13	*3.48*	23.45	*2.50*	36.19	*8.44*	0.037
Ribose	117.30	*66.87*	64.07	*17.14*	22.36	*5.14*	1.38	*0.59*	0.015
Sucrose	1551.54	*224.18*	2900.20	*885.01*	1091.82	*288.81*	929.75	*222.03*	0.005
Sugar (O-methyl)	36.05	*35.40*	61.63	*45.19*	837.70	*308.61*	2591.24	*471.26*	0.000
Xylose	13.22	*3.20*	11.09	*3.29*	11.24	*1.47*	1.38	*0.59*	0.001
Unidentified compounds									
A207006 (sugar)	4.60	*5.08*	3.22	*2.31*	29.26	*3.33*	38.10	*8.60*	0.000
A212004	78.87	*17.20*	63.88	*24.69*	0.47	*0.15*	1.38	*0.59*	0.000
A213001	149.80	*59.46*	105.83	*26.39*	29.46	*10.30*	13.29	*0.72*	0.003

Table A.11: Metabolite composition of tomatoes extracted 7 days after harvest (T7). RT were stored in room temperature, ST were stored at storage temperature ($\approx$ 4 °C), 40 were stored at storage temperature and dried at 40 °C over night before extraction and 60 were stored at storage temperature and dried at 60 °C over night before extraction.

			T7						
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Acids									
1,4-Lactonethreonic acid	1.36	*1.35*	0.77	*0.24*	18.43	*15.97*	1.54	*0.54*	0.071
2-Methyl-maleic acid	27.27	*6.05*	19.12	*1.52*	0.94	*0.52*	1.54	*0.54*	0.000
2-Oxoglutaric acid	0.71	*0.22*	3.39	*4.62*	0.74	*0.20*	1.92	*1.08*	0.503
Ascorbic acid	12.86	*5.56*	20.01	*20.10*	6.95	*8.62*	1.92	*1.08*	0.305
Citric acid	3431.14	*526.81*	3652.46	*305.33*	84.85	*41.23*	132.31	*82.00*	0.000
Dehydroascorbic acid dimer	35.39	*16.20*	60.19	*5.31*	697.23	*364.90*	1914.86	*974.74*	0.007
Fumaric acid	82.70	*39.86*	37.49	*26.01*	20.23	*20.02*	1.54	*0.54*	0.025
Galactaric acid	166.80	*24.28*	138.33	*43.18*	0.74	*0.20*	1.92	*1.08*	0.000
Galacturonic acid	761.66	*91.15*	404.10	*131.60*	727.19	*287.79*	1457.33	*557.10*	0.023
Glyceric acid	8.27	*3.88*	9.29	*3.01*	0.94	*0.52*	1.54	*0.54*	0.005
Glycolic acid	0.84	*0.23*	0.64	*0.16*	34.16	*8.00*	23.91	*22.91*	0.021
Itaconic acid	76.04	*3.66*	53.09	*16.39*	119.86	*128.84*	7.17	*8.81*	0.273
Lactic acid	0.71	*0.22*	0.77	*0.24*	6.78	*9.72*	16.70	*13.18*	0.130
Malic acid	889.48	*208.57*	1327.87	*203.84*	6.38	*9.33*	4.39	*4.39*	0.000
Quinic acid	82.14	*71.51*	121.72	*27.44*	393.73	*680.81*	460.74	*794.50*	0.760
Succinic acid	21.27	*4.95*	9.97	*1.72*	0.94	*0.52*	1.54	*0.54*	0.000
Alcohols and esters									
Myo-inositol	935.78	*294.43*	575.36	*112.66*	707.55	*143.19*	1353.31	*291.08*	0.014
Threitol	13.41	*0.88*	9.77	*1.08*	6.36	*2.00*	3.45	*2.78*	0.001
α-Tocopherol	0.84	*0.23*	0.64	*0.16*	36.42	*6.92*	108.56	*35.16*	0.000
α-Tocopheryl acetate	0.84	*0.23*	0.64	*0.16*	20.48	*13.52*	89.37	*49.09*	0.008
γ-Tocopherol	0.84	*0.23*	0.64	*0.16*	9.98	*4.60*	28.00	*5.08*	0.000
Amines, amides and N-compounds									
5-Hydroxytryptamine	45.10	*3.59*	54.56	*21.01*	1.47	*0.40*	3.84	*2.16*	0.000
Adenosine	37.10	*4.16*	25.72	*5.23*	1.68	*0.63*	3.46	*1.37*	0.000
Ethanolamine	382.63	*11.64*	400.88	*14.52*	0.94	*0.52*	1.54	*0.54*	0.000
N-carboxymethylamine	114.35	*18.62*	98.62	*22.14*	6.55	*9.88*	1.92	*1.08*	0.000
Orotic acid	7.95	*4.14*	6.31	*2.73*	0.74	*0.20*	238.42	*410.68*	0.453
Putrescine	298.15	*88.52*	344.78	*20.73*	0.94	*0.52*	1.54	*0.54*	0.000
Uridine	51.24	*20.81*	20.73	*10.84*	0.74	*0.20*	1.92	*1.08*	0.002
Uracil	15.39	*8.29*	8.27	*3.91*	0.74	*0.20*	1.92	*1.08*	0.016
Amino acids									
4-Aminobutyric acid	2972.03	*362.22*	2950.11	*480.21*	1.62	*1.67*	13.79	*12.21*	0.000
Alanine	94.52	*10.92*	81.26	*21.98*	1.68	*0.63*	3.46	*1.37*	0.000
Asparagine	28.51	*30.10*	37.04	*54.09*	0.74	*0.20*	1.92	*1.08*	0.414
Aspartic acid	371.44	*12.75*	521.43	*525.22*	1.88	*1.03*	3.42	*1.49*	0.094
Glutamic acid	3686.44	*1091.54*	2899.38	*1311.95*	0.94	*0.52*	1.54	*0.54*	0.001
Glutamine	273.50	*55.33*	478.49	*514.63*	0.74	*0.20*	1.92	*1.08*	0.142
Glycine	97.05	*7.50*	69.80	*2.65*	0.74	*0.20*	1.92	*1.08*	0.000
Isoleucine	45.06	*2.95*	57.54	*13.14*	0.74	*0.20*	1.92	*1.08*	0.000
Leucine	43.01	*2.26*	43.09	*6.34*	0.74	*0.20*	1.92	*1.08*	0.000
Pyroglutamic acid	2271.44	*117.35*	1449.94	*272.97*	0.74	*0.20*	1.92	*1.08*	0.000
Serine	291.95	*76.48*	281.45	*57.01*	1.88	*1.03*	3.08	*1.07*	0.000
Threonine	98.55	*15.44*	90.80	*7.68*	3.44	*2.43*	8.13	*8.28*	0.000
Valine	47.18	*4.11*	40.51	*6.38*	0.94	*0.52*	1.54	*0.54*	0.000
β-Alanine	57.81	*15.77*	54.21	*4.38*	0.74	*0.20*	1.92	*1.08*	0.000
Aromatics									
Chlorogenic acid	0.86	*0.24*	6.77	*6.79*	0.74	*0.20*	1.92	*1.08*	0.184

Table A.11: Continued.

					T7				
Compound	RT	*Stdev*	ST	*Stdev*	D40	*Stdev*	D60	*Stdev*	*p*-value
					mg/ 100 g DW				
Fatty acids									
Heptanoic acid	23.52	*6.81*	23.28	*3.84*	0.74	*0.20*	1.92	*1.08*	0.000
Hexadecanoic acid	172.20	*159.33*	188.90	*82.12*	79.65	*51.50*	125.02	*96.06*	0.598
Octadecanoic acid	40.11	*46.68*	44.43	*39.23*	0.74	*0.20*	10.33	*14.26*	0.303
Monoglyceride	2.68	*1.10*	2.32	*1.04*	20.15	*4.88*	42.95	*10.34*	0.000
Flavonoids									
Naringenin	1.70	*1.21*	48.64	*47.31*	0.74	*0.20*	1.92	*1.08*	0.096
Phosphates									
Fructose-6-phosphate	23.68	*6.00*	19.66	*2.10*	0.94	*0.52*	1.54	*0.54*	0.000
Glucose-6-phosphate	37.83	*7.49*	42.62	*7.23*	0.94	*0.52*	1.54	*0.54*	0.000
Inositol-1-phosphate	22.54	*6.45*	14.41	*2.34*	0.94	*0.52*	1.54	*0.54*	0.000
Phosphoric acid	6326.63	*441.84*	5837.42	*660.94*	4785.45	*870.57*	8542.22	*2299.81*	0.039
Sugars									
Arabinose	29.92	*8.80*	18.41	*2.92*	12.88	*10.45*	1.92	*1.08*	0.008
Cellobiose	20.63	*1.26*	17.23	*4.64*	4.14	*5.71*	1.54	*0.54*	0.001
Fructose	8136.03	*3257.98*	6129.49	*1147.89*	6210.15	*3859.51*	18446.13	*7814.53*	0.035
Galactose	1869.55	*164.73*	1795.42	*132.52*	441.95	*764.25*	1.92	*1.08*	0.001
Glucose	12742.04	*908.32*	11170.16	*2739.25*	5160.66	*3023.27*	15550.05	*7092.84*	0.073
Laminaribose	25.76	*3.08*	20.73	*2.08*	28.51	*2.96*	32.84	*2.15*	0.003
Ribose	95.32	*19.04*	92.83	*48.94*	0.74	*0.20*	1.92	*1.08*	0.002
Sucrose	1391.79	*253.18*	2390.05	*692.43*	1247.36	*409.82*	645.89	*269.65*	0.009
Sugar (O-methyl)	64.83	*45.82*	49.47	*44.43*	1938.87	*774.46*	3914.99	*2028.75*	0.007
Xylose	13.69	*1.98*	12.64	*0.67*	6.71	*5.49*	1.92	*1.08*	0.004
Unidentified compounds									
A207006 (sugar)	6.98	*3.22*	5.28	*3.89*	26.20	*11.72*	19.69	*14.41*	0.079
A212004	85.03	*7.10*	79.44	*1.80*	0.74	*0.20*	1.92	*1.08*	0.000
A213001	214.77	*78.07*	149.70	*46.57*	22.88	*8.03*	26.61	*6.63*	0.002

Table A.12: Metabolite composition of tomatoes extracted 10 days after harvest (T10). RT were stored in room temperature, ST were stored at storage temperature ($\approx$ 4 °C), 40 were stored at storage temperature and dried at 40 °C over night before extraction and 60 were stored at storage temperature and dried at 60 °C over night before extraction.

					T10				
Compound	RT	*Stdev*	ST	*Stdev*	D40[1]	*Stdev* [1]	D60[1]	*Stdev* [1]	*p*-value
					mg/ 100 g DW				
Acids									
1,4-Lactonethreonic acid	0.73	*0.23*	0.64	*0.18*	12.50	*15.58*	2.39	*0.83*	0.258
2-Methyl-maleic acid	30.08	*5.43*	34.36	*7.22*	1.89	*0.58*	2.39	*0.83*	0.001
2-Oxoglutaric acid	5.98	*9.18*	3.06	*4.15*	1.59	*1.25*	2.39	*0.86*	0.838
Ascorbic acid	9.19	*2.71*	15.80	*14.13*	6.55	*7.17*	2.39	*0.83*	0.448
Citric acid	3766.13	*413.65*	3664.85	*560.12*	112.50	*118.60*	254.71	*25.35*	0.000
Dehydroascorbic acid dimer	48.51	*5.93*	45.83	*27.98*	1049.60	*1483.77*	2592.84	*69.63*	0.012
Fumaric acid	68.37	*9.27*	30.12	*20.77*	3.15	*0.96*	2.39	*0.86*	0.004
Galactaric acid	127.21	*15.17*	128.16	*42.61*	0.95	*0.75*	2.39	*0.83*	0.002
Galacturonic acid	514.86	*152.85*	431.54	*185.47*	967.15	*676.98*	1840.57	*129.41*	0.010
Glyceric acid	9.97	*3.47*	14.90	*11.61*	0.95	*0.75*	2.39	*0.83*	0.195
Glycolic acid	0.73	*0.23*	0.64	*0.18*	21.23	*26.53*	19.35	*24.84*	0.331
Itaconic acid	92.52	*13.17*	58.35	*9.09*	22.21	*29.31*	2.39	*0.83*	0.002
Lactic acid	0.60	*0.16*	0.76	*0.24*	10.41	*12.62*	13.44	*14.81*	0.275
Malic acid	944.67	*235.40*	1189.54	*288.87*	1.59	*1.25*	2.39	*0.86*	0.002
Quinic acid	143.36	*11.72*	145.14	*20.44*	133.44	*185.22*	2.39	*0.86*	0.254
Succinic acid	16.47	*3.68*	11.15	*3.83*	0.95	*0.75*	2.39	*0.83*	0.004
Alcohols and esters									
Myo-inositol	715.75	*101.96*	595.30	*229.09*	1238.80	*835.33*	2174.77	*212.80*	0.015
Threitol	14.80	*1.22*	12.48	*1.91*	7.71	*7.41*	2.39	*0.86*	0.026
α-Tocopherol	0.73	*0.23*	0.64	*0.18*	35.13	*1.65*	134.68	*3.87*	0.000
α-Tocopheryl acetate	0.73	*0.23*	0.64	*0.18*	20.31	*19.15*	150.27	*14.86*	0.000
γ-Tocopherol	0.73	*0.23*	0.64	*0.18*	13.21	*9.59*	30.58	*5.38*	0.001
Amines, amides and N-compounds									
5-Hydroxytryptamine	55.02	*4.19*	64.51	*13.72*	1.91	*1.50*	4.78	*1.66*	0.000
Adenosine	45.13	*8.31*	28.28	*7.39*	2.83	*1.59*	4.78	*0.03*	0.001
Ethanolamine	372.94	*1.77*	368.70	*4.27*	1.59	*1.25*	2.39	*0.86*	0.000
N-carboxymethylamine	137.47	*17.32*	155.76	*33.14*	1.59	*1.25*	2.39	*0.86*	0.000
Orotic acid	6.87	*1.11*	10.58	*1.58*	1.59	*1.25*	403.83	*568.57*	0.294
Putrescine	314.47	*44.97*	321.08	*45.19*	1.09	*0.55*	2.39	*0.83*	0.000
Uridine	44.44	*5.62*	20.06	*13.28*	3.97	*2.12*	2.39	*0.86*	0.004
Uracil	11.70	*0.73*	10.37	*2.49*	0.95	*0.75*	2.39	*0.83*	0.001
Amino acids									
4-Aminobutyric acid	2377.17	*515.68*	2639.73	*728.75*	8.97	*11.28*	18.95	*11.90*	0.002
Alanine	99.99	*20.76*	94.79	*16.44*	2.54	*2.00*	4.78	*0.03*	0.001
Asparagine	4.81	*2.99*	47.43	*27.52*	0.95	*0.75*	2.39	*0.83*	0.038
Aspartic acid	338.47	*7.13*	750.88	*315.19*	3.18	*2.50*	4.92	*1.90*	0.010
Glutamic acid	3925.67	*828.97*	3703.83	*889.89*	1.59	*1.25*	2.39	*0.86*	0.001
Glutamine	49.89	*59.05*	473.85	*289.53*	0.95	*0.75*	2.39	*0.83*	0.046
Glycine	61.03	*22.26*	46.72	*12.83*	0.95	*0.75*	2.39	*0.83*	0.009
Isoleucine	34.71	*19.26*	36.56	*7.83*	0.95	*0.75*	2.39	*0.83*	0.027
Leucine	50.27	*21.78*	30.06	*5.97*	0.95	*0.75*	2.39	*0.83*	0.015
Pyroglutamic acid	1951.69	*36.59*	1337.98	*269.79*	0.95	*0.75*	2.39	*0.83*	0.000
Serine	141.66	*93.92*	150.49	*30.11*	3.18	*2.50*	4.79	*1.72*	0.045
Threonine	67.04	*30.62*	58.47	*6.31*	21.01	*26.24*	4.78	*0.03*	0.049
Valine	31.60	*15.75*	26.10	*2.04*	1.59	*1.25*	2.39	*0.86*	0.022
β-Alanine	27.69	*12.36*	36.84	*15.05*	1.59	*1.25*	2.39	*0.86*	0.030

[1] n=2, one replica was excluded due to unsatisfying GC-MS values.

Table A.12: Continued.

					T10				
Compound	RT	*Stdev*	ST	*Stdev*	D40[1]	*Stdev* [1]	D60[1]	*Stdev* [1]	*p*-value
					mg/ 100 g DW				
Aromatics									
Chlorogenic acid	0.73	*0.23*	6.10	*1.12*	0.95	*0.75*	2.39	*0.83*	0.001
Fatty acids									
Heptanoic acid	25.74	*3.36*	28.68	*0.12*	0.95	*0.75*	2.39	*0.83*	0.000
Hexadecanoic acid	246.69	*118.97*	193.65	*16.18*	34.32	*47.53*	66.19	*11.37*	0.050
Octadecanoic acid	71.13	*41.92*	51.94	*13.04*	9.44	*9.85*	2.39	*0.86*	0.068
Monoglyceride	3.07	*0.72*	1.39	*0.62*	28.36	*15.65*	62.20	*8.25*	0.000
Flavonoids									
Naringenin	0.73	*0.23*	29.18	*12.92*	0.95	*0.75*	2.39	*0.83*	0.010
Phosphates									
Fructose-6-phosphate	23.74	*3.89*	18.28	*5.58*	1.59	*1.25*	2.39	*0.86*	0.002
Glucose-6-phosphate	41.30	*7.36*	35.58	*9.92*	1.59	*1.25*	2.39	*0.86*	0.001
Inositol-1-phosphate	15.49	*1.88*	14.67	*5.36*	1.59	*1.25*	2.39	*0.86*	0.006
Phosphoric acid	4873.83	*835.33*	5265.48	*293.49*	8833.72	*6139.11*	4099.60	*5795.19*	0.561
Sugars									
Arabinose	17.43	*1.70*	27.45	*7.59*	14.01	*16.32*	2.39	*0.86*	0.071
Cellobiose	34.84	*11.35*	23.78	*8.71*	12.93	*14.79*	2.39	*0.86*	0.056
Fructose	6101.37	*1861.13*	7139.50	*3706.47*	12757.61	*12122.90*	27032.01	*325.54*	0.022
Galactose	1187.19	*1028.90*	1269.48	*1100.70*	0.95	*0.75*	2.39	*0.83*	0.286
Glucose	10353.42	*2273.46*	10613.36	*2719.43*	10735.32	*9970.62*	21148.13	*30.79*	0.124
Laminaribose	26.62	*5.53*	21.73	*6.95*	30.03	*4.48*	37.69	*10.27*	0.182
Ribose	60.03	*5.07*	100.06	*15.80*	9.34	*9.71*	2.39	*0.86*	0.000
Sucrose	1126.31	*149.32*	2582.16	*404.88*	1363.45	*179.38*	858.30	*45.66*	0.001
Sugar (O-methyl)	61.18	*3.86*	17.42	*28.95*	2788.40	*2316.54*	5978.88	*400.03*	0.002
Xylose	10.78	*0.61*	11.59	*3.23*	8.20	*8.10*	2.39	*0.86*	0.140
Unidentified compounds									
A207006 (sugar)	4.57	*4.47*	4.68	*5.36*	24.16	*8.83*	40.10	*11.83*	0.005
A212004	57.97	*1.72*	63.21	*10.03*	0.95	*0.75*	2.39	*0.83*	0.000
A213001	291.66	*140.93*	228.42	*138.45*	30.76	*21.35*	23.61	*2.38*	0.091

[1] n=2, one replica was excluded due to unsatisfying GC-MS values.