Bioactive Compounds in Baby Spinach (*Spinacia oleracea* L.)

Effects of Pre- and Postharvest Factors

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Abstract

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A high intake of fruit and vegetables is well known to have positive effects on human health, and has been correlated to a decreased risk of most degenerative diseases of ageing, such as cardiovascular disease, cataracts and several forms of cancer. These protective effects have been attributed to high concentrations of bioactive compounds (ascorbic acid, flavonoids, carotenoids) in fruit and vegetables, partly due to the antioxidative action of some of these compounds. Maintaining a high level of these compounds in fruit and vegetables is therefore desirable. In addition, a high concentration of antioxidants in horticultural produce is believed to improve its storability and reduce the rate of deterioration.

This thesis investigated the effects of pre- and postharvest factors on the concentrations of bioactive compounds in baby spinach (*Spinacia oleracea* L.). The factors studied included sowing time, growth stage at harvest, use of shade nettings and postharvest storage temperature and duration. Bioactive compounds were analysed using reversed-phase HPLC and chlorophylls using a spectrophotometric method. Visual quality of the fresh and stored leaves was scored on a 1-9 scale, where 9 was the best.

The concentrations of ascorbic acid and flavonoids most often decreased during plant growth. Carotenoids showed relatively small increases or decreases, but nevertheless, plant growth had the largest impact on carotenoid concentration of the factors studied. Harvesting the leaves slightly earlier than is currently common practice may thus give increased concentrations of bioactive compounds. The variation in these compounds during the season was relatively small except for the flavonoids, which doubled in concentration in April-sown spinach compared to August-sown. Shading generally decreased ascorbic acid and total vitamin C concentrations, but most often increased carotenoid concentration. Flavonoid concentration showed different responses at different times of the season and at different growth stages. In some cases the concentration decreased due to shading, while in others it did not change significantly. There were considerable losses of ascorbic acid during storage, whereas carotenoids and flavonoids were more stable and sometimes increased. Ascorbic acid concentration at harvest was correlated with visual quality after storage, indicating that the antioxidative action of ascorbic acid may protect the plant tissue against oxidative stress and subsequent deterioration.

Keywords: ascorbic acid, vitamin C, carotenoids, flavonoids, antioxidants, growth stage, time of season, shade netting, postharvest storage.

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Appendix

Papers I-IV
The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Bergquist, S. Å. M., Gertsson, U. E. & Olsson, M. E. 2006. Influence of growth stage and postharvest storage in ascorbic acid and carotenoid content and visual quality of baby spinach (Spinacia oleracea L.). J. Sci. Food Agric. 86; 346-355.

II. Bergquist, S. Å. M., Gertsson, U. E., Knuthsen, P. & Olsson, M. E. 2005. Flavonoids in baby spinach (Spinacia oleracea L.): changes during plant growth and storage. J. Agric. Food Chem. 53; 9459-9464.

III. Bergquist, S. Å. M., Gertsson, U. E., Nordmark, L. Y. G. & Olsson, M. E. Bioactive compounds and visual quality of baby spinach as affected by shade netting and postharvest storage. Submitted.

IV. Bergquist, S. Å. M., Gertsson, U. E., Nordmark, L. Y. G. & Olsson, M. E. Effect of shade netting on baby spinach flavonoids. Submitted

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Paper I Society of Chemical Industry
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Objectives

The main objective of this doctoral thesis was to investigate the effects of some factors during production and postharvest handling on the concentration and composition of bioactive compounds in baby spinach (*Spinacia oleracea* L.). The bioactive compounds studied were ascorbic acid (vitamin C), carotenoids (total and individual) and flavonoids (total and individual).

The specific questions addressed were:

- How do the concentrations of the compounds vary during plant growth?
- How do the concentrations of the compounds vary during the season?
- How does shade netting affect the concentrations of the compounds?
- How do the concentrations of the compounds change during storage, and is the response different at different temperatures?
- Are visual quality and chlorophyll concentration affected by these factors, and is there a connection to the concentrations of bioactive compounds?

Introduction

Bioactive compounds, antioxidants and free radicals

There is strong evidence that a diet rich in fruit and vegetables has a positive effect on human health, offering protection against degenerative diseases of ageing, such as heart disease, cardiovascular disease, Alzheimer's disease, cataracts and several forms of cancer (Williamson, 1996; Liu *et al.,* 2000; Gandini *et al.,* 2000; Liu *et al.,* 2001; Joshipura *et al.,* 2001; Kang, Ascherio & Grodstein, 2005). Fruit and vegetables contain a wide range of substances that are suggested to be part of these health-enhancing effects. In addition to the major food constituents protein, fat and carbohydrate and micronutrients such as vitamins, minerals and trace elements, fruit and vegetables contain other compounds that may have a positive effect on human health. These phytochemicals (Box 1) include groups of compounds such as carotenoids, flavonoids and other polyphenols, phenolic acids, glucosinolates, allylic sulphides, isothiocyanates, dietary fibres, phytosterols and monoterpenes (Lister, 1999; Kris-Etherton *et al.,* 2002).

Antioxidants protect living cells against the harmful effects of free radicals and other reactive oxygen species. Some phytochemicals have an antioxidative action, for example, carotenoids, flavonoids and other phenolics. The vitamins C (ascorbic acid) and E (tocopherols and tocotrienols) and some enzymes are also part of the antioxidant defence system (Lurie, 2003). The mode of action of the phytochemical compounds in other words includes an antioxidative function, but they may also be antibacterial or have a hormonal action, stimulate the immune system or modulate enzyme activity (Lampe, 1999). Therefore, the compounds studied in this thesis are referred to as bioactive compounds, although they also have an antioxidative function (Box 1).

Box 1. Definitions

The definitions of these words are not unequivocal; on the contrary, they are subject to much discussion. The definitions used in this thesis are:

Bioactive compound – a constituent of foods or dietary supplements, other than those needed to meet basic human nutritional needs, that is responsible for positive changes in health status.

Antioxidant – a substance that offers protection against the harmful effect of free radicals and other reactive oxygen species. When present in low concentrations compared to those of an oxidisable substrate, they can delay or prevent its oxidation (Halliwell & Gutteridge, 1989).

Free radical – a species that possesses one or several unpaired electrons in the outer shell, and is thus very reactive (Halliwell & Gutteridge, 1989).

Phytochemical – a plant-derived substance that is nutritionally, medicinally or physiologically highly active.

Free radicals are mostly highly reactive and unstable, due to their unpaired valence electrons. Radicals are constantly generated by normal metabolism, *e.g.* when oxygen is reduced in mitochondria and ATP produced, or by a variety of enzymatic reactions (Finkel & Holbrook, 2000). There are many types of radicals, but those of most concern in biological systems are derived from oxygen. These radicals and other highly reactive oxygen-containing molecules are known collectively as reactive oxygen species, and include the superoxide anion ($O_2\cdot^-$), hydroxyl radical ($\cdot OH$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitric oxide ($NO\cdot$), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) (Halliwell & Gutteridge, 1989).

An overproduction of radicals may be triggered by radiation, pollutants, cigarette smoke, alcohol, exercise and stress. The immune system also produces radicals as a defence against pathogens. In the normal case there is a balance between the formation and elimination of free radicals. Oxidative stress occurs when the level of free radicals or reactive oxygen species is higher than the antioxidant defence system can cope with (Finkel & Holbrook, 2000). If not neutralised by antioxidants, the radicals may react with cellular components such as lipids, proteins and nucleic acids. Lipids may be especially prone to damage due to a process known as lipid peroxidation. When radicals react with polyunsaturated fatty acids in cellular membrane phospholipids, the fatty acids themselves become radicals that can react with other fatty acids, starting a chain reaction that may cause severe damage. This type of damage can cause cell death and eventually ageing or diseases in the organism. Free radicals have been found to be involved in ageing, several forms of cancer, heart disease, cardiovascular disease, Alzheimer's disease, inflammatory conditions, rheumatism, insulin resistance and cataracts (Halliwell & Gutteridge, 1989; Houstis, Rosen & Lander, 2006), and increasing antioxidant intake is therefore believed to decrease the risk of contracting these diseases (Berger, 2005).

Many antioxidants function by transiently becoming radicals themselves. These antioxidant radicals are not as reactive as the original free radicals and may in turn be regenerated by other antioxidants (Niki *et al.,* 1995).

Positive health effects of fruit and vegetables

As indicated by biochemical and in vitro studies, antioxidants in fruit and vegetables may provide protection against free radical damage. Since free radicals are involved in many degenerative diseases of ageing, the positive health effects of fruit and vegetables have been attributed to the relatively high antioxidant concentrations in fruit and vegetables (Ames, Shigenaga & Hagen, 1993; Rice-Evans & Miller, 1995). This is known as the antioxidant hypothesis (Stanner *et al.,* 2004). Epidemiological studies (Box 2) have also found associations between a high risk of diseases and a low intake of fruit and vegetables or bioactive compounds (Williamson, 1996; Gandini *et al.,* 2000; Liu *et al.,* 2000; Joshipura *et al.,* 2001; Liu *et al.,* 2001; Kris-Etherton *et al.,* 2002). Observational studies should not be used for drawing conclusions on the effects of a particular compound on a certain disease, but are valuable for providing hypotheses that may be tested by intervention studies (Hennekens *et al.,* 1995). Intervention trials are more expensive and time-consuming, but randomising supplementation across population groups diminishes the risk of confounding factors. In an observational epidemiological study comparing population groups, an association found between a high intake of a certain compound and a low incidence of a certain disease is not necessarily a causal relationship. The two groups may differ in ways other than in the compound under study. The bioactive compound measured may in fact be a marker of some other compound that also occurs in high concentrations in the same population group. A high intake of fruit and vegetables contributes to a high intake of several nutrients and phytochemicals, including vitamin C, carotenoids, flavonoids, folate, potassium, magnesium, dietary fibre, *etc*. (Lampe, 1999). Furthermore, a high intake of fruit and vegetables may also be associated with a healthy lifestyle in general, with more exercise and less smoking. These factors can be accounted for in the analyses, but other factors that may have an effect may be omitted.

Many epidemiological studies indicate that bioactive compounds may have a protective role against several diseases, although the results are seldom unambiguous. Observational studies have shown associations between a low intake of ascorbic acid, carotenoids and flavonoids and an increased risk of cardiovascular disease (Knekt *et al.,* 1996; Kritchevsky, 1999; Khaw *et al.,* 2001; Arts & Hollman, 2005). However, the eight intervention trials (mainly concerning β-carotene and α-tocopherol) reviewed by Asplund (2002) all failed to confirm this association. β-carotene has received much attention in this field, but it is possible that carotenoids other than β-carotene are more efficient (Krinsky & Johnson, 2005). Few intervention trials have studied the effects of vitamin C and flavonoids on cardiovascular disease (Christen, Gaziano & Hennekens, 2000; Manach, Mazur & Scalbert, 2005).

Box 2. Epidemiological studies

The definitions of these words are subject to discussion, but the definitions used in this thesis are:

Epidemiological studies – studies on human populations, which attempt to link human health effects (*e.g.* cancer) to a cause (*e.g.* low antioxidant intake). There are two types of epidemiological studies; observational and intervention studies.

Intervention studies – the investigator controls which treatment subjects receive, most often by random allocation. For example, one group receives a supplement (one or several compounds in the form of a pill), the other receives placebo, and the outcomes are compared.

Observational studies – the investigator measures but does not intervene. Observational studies include cohort studies (where a group of interest, *e.g.* smokers, and a comparison group are followed over time and the outcomes compared), and case-control studies (where patients with a certain condition is compared to people who do not and their histories are compared).

Observational studies have also found associations between a low intake of bioactive compounds, and an increased risk of various forms of cancer (Ziegler, 1989; Kris-Etherton *et al.,* 2002; Le Marchand, 2002; Neuhouser, 2004) although the association seems rather weak for flavonoids (Hollman, 2001). However, most intervention trials have not been able to confirm this relationship (Hennekens *et al.,* 1995). Some studies have even shown adverse effects, such as an increased risk of lung cancer from high β-carotene supplementation in smokers (ATBC, 1994; Omenn *et al.,* 1996). The reasons for this effect have been reviewed by Omaye *et al.* (1997). Again, few intervention trials have been performed for vitamin C and flavonoids. Vitamin C has been included in multi-supplement interventions, but only one small trial has used it as a single supplement (Forman & Altman, 2004). Observational studies also indicate positive effects of bioactive compounds on cataracts (Brown *et al.,* 1999) and Alzheimer's disease (Commenges *et al.,* 2000).

In addition to the aforementioned factors, the apparent ambiguities in these studies may be due to the fact that the bioactive compounds were given as synthetic supplements in the intervention trials, whereas in most observational trials the sources have been natural fruit and vegetables. Relatively high doses have been used in some of the intervention trials, and these compounds may have pro-oxidative effects at high doses (Rietjens *et al.,* 2002; Loo, 2003). Randomised intervention trials test the effect of supplements in addition to what is considered an adequate intake of these compounds, whereas observational studies generally compare a high intake group to a very low intake group (Moser, 2002). Furthermore, a mix of several compounds may be beneficial, rather than high doses of a certain compound (Böhm *et al.,* 1997), or different population groups, such as smokers, children, the elderly, pregnant women, *etc*. may have different needs and tolerance levels for these compounds. More intervention trials are clearly needed to ascertain whether the presumptive bioactive compounds have beneficial effects or not, and to determine the doses that should be used to obtain the best effect for different groups. In the meantime, the advice for public health remains to increase consumption of fruit and vegetables (Stanner *et al.,* 2004).

Bioactive compounds and product quality

In addition to the effects on human health when ingested in the diet, bioactive compounds are believed to affect storability of the fruits and vegetables that contain them. Reactive oxygen species are known to be involved in leaf senescence and the antioxidant defence system most likely plays an important role in determining the onset and rate of senescence (Philosoph-Hadas *et al.,* 1994; Meir *et al.,* 1995; Hodges & Forney, 2003). A probable theory is therefore that a product with a high concentration of antioxidants is well protected against oxidation, and may thereby retain its quality longer. Thus, increasing the concentrations of the presumed beneficial compounds (biotechnologically or by controlling and optimising preharvest factors or growing the right cultivars) in fruits and vegetables may not only have positive health effects for the people who eat them, but may also extend shelf life and increase stress tolerance, leading to lower postharvest losses of produce. Furthermore, since a high antioxidant concentration may be associated with a fresh appearance, freshness of a fruit or vegetable may to some extent be a marker for its food value as regards the content of bioactive compounds.

Variation in concentrations of bioactive compounds

The concentrations of bioactive compounds in fruit and vegetables are certainly not constant and are affected by many pre- and postharvest factors. In some cases, the effect of a certain factor on a certain compound is very clear, but in many cases, changes in one factor may lead to higher or lower concentrations of a certain compound, or no change in the concentration.

Genetic variation

Some bioactive compounds are ubiquitous among fruit and vegetables but may vary in concentration between species or cultivars, whereas other compounds are specific for a certain family or even species. Ascorbic acid (or its oxidised form dehydroascorbic acid) is apparently found in all fruits and vegetables in widely varying concentrations (Lee & Kader, 2000), whereas the glucosinolates, for example, are found mainly in the Brassicaceae family (Stoewsand, 1995). Genetic factors thus have a large influence on the content of bioactive compounds in fruit and vegetables. Variation between cultivars of the same species may also be large (Mercadante & Rodriguez-Amaya, 1991; DuPont *et al.,* 2000; Howard *et al.,* 2002).

Preharvest factors

Climatic conditions have a strong influence on the concentration of bioactive compounds (Weston & Barth, 1997). Climatic factors vary with growing site, during the season and from year to year. Temperature, both in terms of total or average temperature and the extremes during the growth period, may influence the chemical composition (Weston & Barth, 1997; Lefsrud *et al.,* 2005). Light is known to affect the concentrations of the types of compounds studied in this thesis. The light effect can be seen in lettuce heads, where the outer leaves contain considerably higher concentrations of vitamin C, carotenoids and flavonoids than the inner leaves, which receive less light (Poulsen, Johansen & Sørensen, 1995; Drews, Schonhof & Krumbein, 1997; Hohl *et al.,* 2001). Ascorbic acid is involved in photosynthesis as an electron donor and as a cofactor for violaxanthin de-epoxidase (Smirnoff, 2000). Some carotenoids are involved in photoprotection via the xanthophyll cycle (Demmig-Adams, Gilmore & Adams, 1996), and flavonoids are especially affected by the UV part of the spectrum (Liu, Gitz & McClure, 1995; Olsson *et al.,* 1998). Cultural practices including nutrient and water availability influence these compounds, as does soil type varying with growing site (Weston & Barth, 1997). The response to nitrogen availability may be difficult to predict; both increasing and decreasing concentrations due to fertilisation may occur, possibly depending on how plant growth responds to the fertilisation, so that a relative dilution or accumulation of the compounds may occur (Mozafar, 1993). Nitrogen fertilisation may also increase foliage, reducing light intensity reaching shaded parts of the plant, which, as stated above, may affect the concentration of these compounds (Mozafar, 1994; Lee & Kader, 2000).

Harvesting factors

There is considerable variation in concentration of bioactive compounds during growth and maturation of fruit and vegetables. This variation is probably especially evident in fruit ripening, where the carotenoid or flavonoid provide the colour of the ripe fruit (Kalt, 2005). Timing of harvest during the day is also of importance, as there may be large variation in the concentrations during the day, possibly related to water content (when concentrations are given on a fresh weight basis) or to light intensity (Mozafar, 1994; Veit *et al.,* 1996). Mechanical injuries may be inflicted by harvesting, which in turn may affect concentrations of bioactive compounds.

Postharvest factors

Storage conditions after harvest may also have a large impact on the bioactive compounds. Ascorbic acid quickly decreases during storage in many plant products, whereas carotenoids and flavonoids appear to be more stable (Kalt, 2005). Temperature management is crucial for maintaining the quality of harvested fruits and vegetables, both in terms of appearance and biochemical composition. A lower temperature decreases metabolic rates and thereby slows down deterioration. However, a temperature that is too low may cause chilling or freezing injury to the produce (Wills *et al.*, 1998). The duration of storage is of course also of major importance, since the concentrations change over time. Light conditions during storage generally affect concentrations of these compounds as well, although in different ways. Photosynthesis may be supported in light-stored leaves, and light storage may also increase ascorbic acid synthesis (Toledo *et al.,* 2003). Carotenoids, on the other hand, may decrease to a higher extent in vegetables stored in light (Kopas-Lane & Warthesen, 1995). Trimming, cutting and bruising, as well as processing, may also affect concentrations (Yadav & Sehgal, 1995; DuPont *et al.,* 2000). Furthermore, maintaining appropriate relative humidity and atmosphere during storage may increase shelf life and reduce losses of bioactive compounds. As stated above, storage not only affects the concentration of these compounds, but the concentration of these compounds may affect storability as well.

Ascorbic acid

Vitamin C is the collective name for ascorbic acid and dehydroascorbic acid (Fig. 1). The terms ascorbic acid and vitamin C are often used interchangeably. In many cases, this is of minor importance, since the dehydroascorbic acid concentration is often relatively low, less than 10% of the total vitamin C in most fruit and vegetables (Wills, Wimalasiri & Greenfield, 1984). In stored or processed fruit and vegetables, however, the concentration of dehydroascorbic acid is often considerably higher (Buescher, Howard & Dexter, 1999). In fresh vegetables a high dehydroascorbic acid concentration relative to ascorbic acid may also be found, then probably related to a high ascorbate oxidase activity, which immediately transforms ascorbic acid into dehydroascorbic acid (Gil, Ferreres & Tomás-Barberan, 1998). Vitamin C activity of the two redox forms is considered to be similar (Buescher, Howard & Dexter, 1999), but ascorbic acid has a much higher reducing capacity and antioxidative potential than dehydroascorbic acid (Davey *et al.,* 2000).

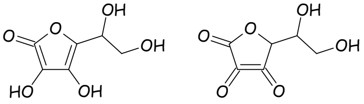

Figure 1. Ascorbic acid (left) and dehydroascorbic acid (right).

Ascorbic acid and dehydroascorbic acid are polar and thus hydrophilic. The radical monodehydroascorbate is the first oxidation product of ascorbic acid. This radical may be reduced back to ascorbic acid by monodehydroascorbate reductase, or two monodehydroascorbate molecules may spontaneously form one ascorbic acid and one dehydroascorbic acid molecule. Dehydroascorbic acid may be irreversibly oxidised to 2,3-diketogulonic acid if not reduced back to ascorbic acid by the glutathione-dependent enzyme dehydroascorbate reductase (Davey *et al.,* 2000).

As an antioxidant, ascorbic acid participates in the scavenging of reactive oxygen species (Smirnoff, 2000). Ascorbic acid also functions as a secondary antioxidant, regenerating tocopherols from their radical forms (Kunert & Ederer, 1985; Niki *et al.,* 1995; Smirnoff, 2000). Ascorbic acid is an important cofactor for some plant enzymes, including violaxanthin de-epoxidase, which is involved in photoprotection through the xanthophyll cycle, and is also involved in photosynthesis as an electron donor to photosystem II (Smirnoff, 2000). Furthermore, ascorbic acid has been implicated in control of cell expansion and cell division in plants (Smirnoff, 1996).

At higher concentrations, ascorbic acid and other antioxidant compounds may exert pro-oxidant activity as reviewed by Rietjens *et al.* (2002). Since it is a water-soluble compound, it does not accumulate in the human body (Hancock & Viola, 2005). The dose that can be considered safe has not yet been firmly established. The bioavailability of ascorbic acid depends on the dose, but ingestion of more than 0.5 g per day does not increase the uptake significantly (Davey *et al.,* 2000). Megadoses therefore appear to have no further advantage. The recommended daily intake of ascorbic acid ranges from 30 to 110 mg/day (Hancock & Viola, 2005). However, as that dose is required only to prevent deficiency, higher levels may be needed for further health benefits (Levine *et al.,* 1999). Requirements may also differ among population groups depending on stress, physical activity, use of certain medicines, smoking, or the presence of inflammatory conditions or diseases associated with increased oxidative stress. An estimate of the mean daily intake of vitamin C in 2000 was 102 mg in the USA and 72 mg in Canada (Institute of Medicine, 2000).

Fruit and vegetables are the major sources of ascorbic acid. Good dietary sources include citrus fruits, berries, peppers and broccoli (USDA, 2005).

Carotenoids

Carotenoids are yellow, orange or red pigments, responsible for the colour of many fruits, vegetables and flowers. They are also found in green vegetables, although their colour is disguised by chlorophyll. Carotenoids are tetraterpenoids, *i.e.* built upon a 5-carbon isoprenoid unit (Fig. 2), and are hydrophobic. There are two groups of carotenoids, the hydrocarbon carotenes and the oxygenated xanthophylls. Carotenes, such as α- and β-carotene and lycopene, are predominantly orange or red-orange pigments, whereas xanthophylls, such as lutein, violaxanthin, zeaxanthin, antheraxanthin and neoxanthin, are mainly yellow.

Carotenoids have important functions in photosynthesis. Some of them are accessory pigments that absorb light energy and transfer it to chlorophyll, which can use the energy for photosynthesis. β-carotene and lutein are prevalent in photosystems I and II, respectively (Demmig-Adams, Gilmore & Adams, 1996).

The xanthophylls violaxanthin, antheraxanthin and zeaxanthin are involved in photoprotection through the xanthophyll cycle (Demmig-Adams, Gilmore & Adams, 1996). Carotenoids are able to absorb energy that may otherwise lead to singlet oxygen formation from excited chlorophyll molecules. Carotenoids may also scavenge any singlet oxygen that forms during photosynthesis (Halliwell & Gutteridge, 1989).

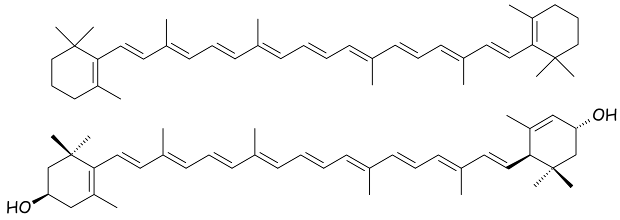

Figure 2. Carotenoid structure; β-carotene (top) and lutein (bottom).

The antioxidative activity varies between carotenoids, depending on their functional groups and chain length (Martin *et al.,* 1999). About 10% of all carotenoids found in fruit and vegetables have provitamin activity, *i.e.* are precursors of vitamin A. As antioxidants, carotenoids are more or less efficient quenchers of reactive oxygen species and excited chlorophyll (Martin *et al.,* 1999). When a carotenoid reacts with a radical, the unpaired electron is delocalised across the conjugated double bonds, forming a relatively stable radical (Krinsky & Johnson, 2005).

Toxicity of β-carotene has been found to occur at doses of 20 mg (ATBC, 1994). The level considered safe should thus be much smaller, less than 2 mg (Bast & Haenen, 2002). The average daily intake of carotenoids in 2000 in Denmark was estimated to be 4.8 mg (Leth, Jakobsen & Andersen, 2000), with a β-carotene intake alone of about 2 mg. Due to the conflicting results from observational and intervention studies for β-carotene other carotenoids, such as lutein, are suggested to be the compounds exerting the positive effects on human health (Krinsky & Johnson, 2005). Carotenoids may also act as pro-oxidants in some cases (Krinsky & Johnson, 2005).

Bioavailability of carotenoids depends on food matrix and processing, and varies among the individual carotenoids (Castenmiller *et al.,* 1999; van het Hof *et al.,* 2000). In spinach and other vegetables, bioavailability of lutein is five to nine times higher than that of β-carotene (van het Hof *et al.,* 1999; Castenmiller *et al.,* 1999).

A variety of vegetables contain large amounts of carotenoids, especially red, orange, yellow and dark green types (Müller, 1997). In the study by Leth *et al.* (2000), 9% of the carotenoid intake was from vegetables other than carrots (47%) and tomatoes (32%).

Flavonoids

Flavonoids are compounds with yellow to white or blue, purple to red colour. They are often referred to as polyphenols. The common feature of flavonoids is that they contain a C_6-C_3-C_6 skeleton (Fig. 3), and the major difference among the flavonoid subgroups is the oxidation state of the heterocyclic ring. Flavonoids include flavones, flavonols, anthocyanins, flavanones, flavans, proanthocyanidins, catechins, isoflavones and some other subgroups. Within each subgroup, compounds are distinguished by the substituents of the two benzene rings. Flavonoids occur mainly as glucosides, *i.e.* with one or several sugars bound to the flavonoid aglycone.

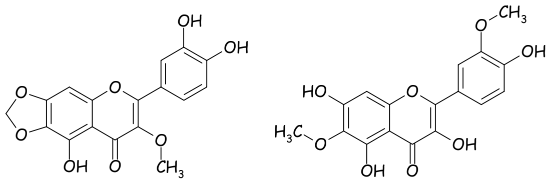

Figure 3.
Two spinach flavonoids; 5,3',4'-trihydroxy-3-methoxy-6:7-methylenedioxyflavone (left) and spinacetin (right).

Most flavonoids absorb strongly in the UV-B part of the spectrum and are believed to serve as UV-protection for underlying tissues (Greenberg *et al.,* 1996; Kolb *et al.,* 2001). Flavonoid concentration has in many cases been found to be increased by elevated UV-B radiation (Liu, Gitz & McClure, 1995; Greenberg *et al.,* 1996; Olsson *et al.,* 1998; Burchard, Bilger & Weissenböck, 2000). Flavonoids have an antioxidative action, and may scavenge reactive oxygen species or quench singlet oxygen. The antioxidative activity depends on the chemical structure, such as the number of hydroxyl groups substituted on the B ring (Rice-Evans, Miller & Paganga, 1996). Flavonoids are also involved in protection against herbivores and micororganisms, both as constitutive agents and as phytoalexins, *i.e.* produced in response to wounding (Harborne & Williams, 2000).

Like other antioxidants, flavonoids may act as pro-oxidants under certain conditions (Rietjens *et al.,* 2002). Toxic effects of flavonoids include carcinogenicity, interference with thyroid hormone biosynthesis, inhibition of iron uptake and alteration of bioavailability of certain medicines (Mennen *et al.,* 2005). The long-term effects of high-dose intake have not been established. However, an intake of quercetin as high as supplement manufacturers recommend is about 10 to 20 times what is consumed in a typical vegetarian diet, and may have a negative impact on human health (Skibola & Smith, 2000).

Bioavailability of flavonoids varies widely, and most likely depends on factors such as food matrix, human intestinal microflora and flavonoid structure (Manach *et al.,* 2005). The bioavailability of flavonoids is most probably lower than that of some other antioxidants, such as ascorbic acid.

In vegetables, the flavonoids most frequently studied are the flavones luteolin and apigenin, and the flavonols quercetin, kaempferol and myricetin. The mean intake of flavonols and flavanones in Denmark in 1995 was estimated to be 23 mg/day (Justesen *et al.,* 2000). Not all flavonoid subgroups were included in this analysis, however. Other estimates suggest a total polyphenol intake of 1 g/day (Scalbert & Williamson, 2000). Good dietary sources of flavonoids are kale, onions, broccoli, apples, oranges and blackcurrants (Hollman & Arts, 2000). Spinach is considered low in flavonoid content, but this is probably due to the fact that the specific flavonoids present in spinach are never included in these screening studies (see Paper II).

The crop

Spinach (*Spinacia oleracea* L.) is a leafy vegetable that belongs to the goosefoot family (Chenopodiaceae). The leaves are smooth, semi-savoy or savoy, depending on cultivar. Baby spinach is harvested after a shorter growth period and sown at closer density than regular spinach and thus the leaves are smaller, hence the name. In the present studies, we used the cultivar Emilia, which has round leaves that grow relatively upright, is slow bolting and is used both for baby leaf and regular production.

Spinach is a long-day plant that is prone to bolting during the summer. The bolting tendency varies between cultivars, and cultivars that are less prone to bolting could be used during the earlier part of the summer. For baby spinach, bolting is often not a problem because the leaves are harvested early.

Baby spinach is generally sold fresh in polypropylene bags and the maximum storage time is about 10 days. Leafy vegetables generally have relatively high respiration rates and a large surface-to-volume ratio, resulting in a relatively short storage time. Storability can be significantly improved by lowering temperatures, increasing humidity and by modifying the surrounding atmosphere (Wills *et al.*, 1998). Recommended storage temperature is close to 0 °C, but this recommendation is not always followed (Wills *et al.*, 1998).

Baby spinach was chosen for our studies for two main reasons. It is becoming an increasingly popular product in Sweden and elsewhere. Spinach is known to be a healthy product and contains relatively high concentrations of bioactive compounds (Gil, Ferreres & Tomás-Barberan, 1999; USDA, 2005). In addition, baby spinach has the advantage of a short culture time and shelf life, making it an excellent model crop.

Methodological aspects

This section gives an overview and brief discussion of the methods used in Papers I-IV. For specific details of the methods used, see the materials and methods section in the respective papers.

Field experiments

Baby spinach (*Spinacia oleracea* L.) cv. Emilia was used as a model crop in the studies reported in this thesis. For Papers I and II, the field experiment was established at a commercial production site (Öllöv) growing baby spinach and other baby leaves on beds. For Papers III and IV, the production system was the same, but at a different site (Hvilan). Both sites are located in southern Sweden, where the climate is warm temperate. The soil has a higher sand content at Öllöv than at Hvilan. The spinach was grown on field beds and harvested after 17-34 days, depending on weather conditions and growth stage at harvest (Table 1, Fig. 4). Time of harvest was determined by observation of emergence and growth, in particular leaf size, in order for the growth stages at different sowing dates to be as similar as possible (Fig. 5). The leaves were harvested manually using sharp knives, and any cotyledons that were harvested were removed.

The factors investigated in the field experiments were sowing time (Papers I-IV), growth stage at harvest (Papers I-IV) and the use of shade netting (Papers III-IV). After harvest, the effects of storage duration and temperature were studied (Papers I-IV).

Three types of shade netting with different characteristics were used for Papers III and IV. The colour and density of the weave were different, resulting in differences in transmittance and spectral influence between the netting types (Figs. 6, 7). An unshaded control was also included in the experiment.

Table 1. Growth conditions for spinach cultures sown and harvested at different times.

Site	Sowing	Growth stage	Culture time (days)	Mean temperature (°C)	Temperature sum (day-degrees, base 5°)	Leaf size
Öllöv	August	I	17	19.0	254	Small
		II	23	18.8	332	Normal
		III	29	17.9	394	Large
	June	I	18	15.4	200	Small
		II	24	15.7	269	Normal
		III	30	15.8	340	Large
	July	I	18	19.8	280	Small
		II	24	19.4	360	Normal
		III	30	19.2	434	Large
Hvilan	August	I	21	16.7	245	Small
		II	28	16.7	323	Normal
	April	I	27	11.5	176	Small
		II	34	11.3	214	Normal
		III	41	11.4	263	Large

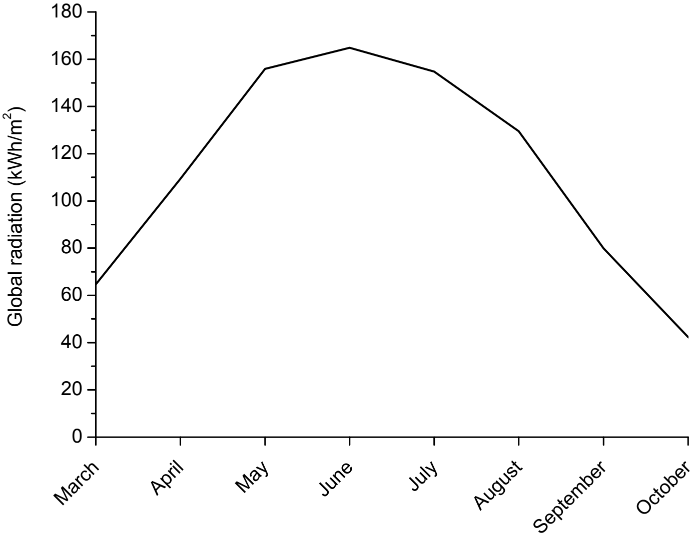

Figure 4. Normal global radiation for the area where field experiments were been performed, 1961-1990 (data for Lund from SMHI, Swedish Meteorological and Hydrological Institute).

Figure 5. Leaves of three sizes, representing the three growth stages, I, III and III.

Figure 6. Field experiment at Hvilan, using shade nettings.

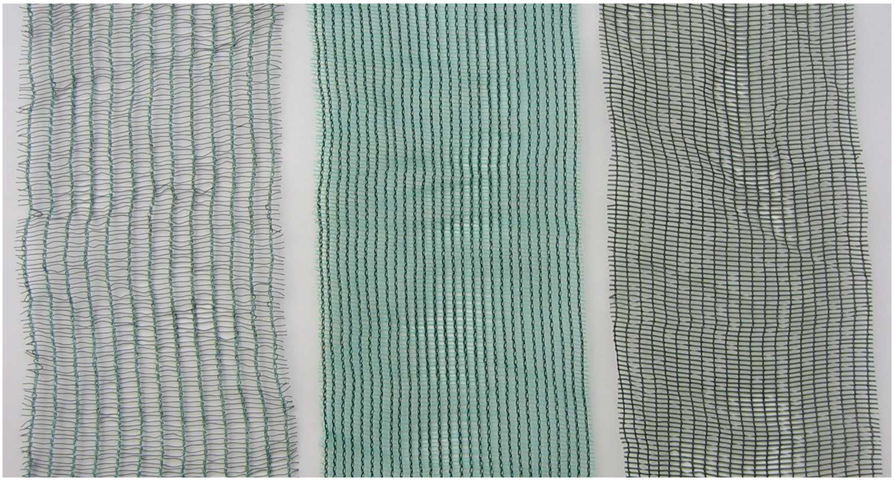

Figure 7. The three netting types used. From left: high transmittance, spectrum-altering, and low transmittance.

The studies were carried out in the field for a number of reasons. Field experiments have the advantage that the outcome is observed in a natural setting. Greenhouse or phytotron experiments, on the other hand, can control factors such as temperature and light conditions with much more precision. In a field experiment, several factors may vary at once, possibly causing problems in interpretation of the results. Seasonal changes in light and temperature occur concurrently with leaf development, for instance, making it difficult to distinguish the effects of leaf growth from those of season progression. However, repeating studies of changes during plant growth several times during the season, at different light and temperature conditions, allows different effects to be distinguished. When it comes to studies of plant pigments, a natural environment is important. Both artificial light sources and natural light filtered through the greenhouse material create un-natural light environments, for instance reducing UV radiation.

Chemical analyses of bioactive compounds

Analyses of the chosen bioactive compounds were performed using reversed-phase HPLC. Plant material was freeze-dried or used fresh, depending on analyte, and the compounds were extracted using suitable solvents. For ascorbic acid an isocratic HPLC method was used and for carotenoids and flavonoids gradient elution was used. The detector used for quantification purposes and identification of carotenoids was a diode array detector. Identification of carotenoids was done by comparing retention times and absorption spectra to those reported in the literature and by using standards when commercially available. Flavonoids were identified using LC-MS/MS. The compounds were quantified using external standards. Ascorbic acid was used for the ascorbic acid analyses, β-carotene for the carotenoids and spiraeoside (quercetin-4'-glucoside) for the flavonoids. All results are given on a dry weight basis unless otherwise stated.

Popular methods in antioxidant research include those measuring total antioxidative capacity, such as ORAC (oxygen radical absorbing capacity, Cao, Alession & Cutler, 1993), TEAC (trolox equivalent antioxidant capacity, Miller *et al.*, 1993), and FRAP (ferric reducing antioxidant power, Benzie & Strain, 1999). These methods attempt to give a measure of the total antioxidant capacity, in order to compare different foods or biological samples instead of quantifying the individual antioxidant compounds. However, the test system may not be relevant for the conditions in the plant or in the human body. This may be the case because different compounds have different effects in the organism, not to mention different degrees of bioavailability, and the total antioxidative capacity methods do not provide more than an indication of concentrations of specific compounds (Frankel & Meyer, 2000).

Visual quality assessment and chlorophyll analysis

The visual quality was scored on a scale from 9 to 1, where 9 was the best. The assessments were carried out by two people independently and their values were compared before a grade was agreed upon. On most occasions, the scores given by the two different judges was the same. The visual quality assessments included the "overall appearance", colour, turgour and occurrence of necrotic spots on the leaves.

Chlorophyll determinations were performed using a spectrophotometric method. We found no correlation between the chlorophyll concentration and the visual quality score, probably because the visual quality assessments took several other factors into consideration besides colour. For instance, yellow spots on the leaves have a large bearing on the visual quality score, but probably less on the chlorophyll concentration.

Results and discussion

Bioactive compounds in baby spinach – concentrations and composition

Unshaded baby spinach leaves had an ascorbic acid concentration of 2.5-8.3 mg/g dw and a total vitamin C concentration of 3.8-9.0 mg/g dw (Papers I, III). In food composition tables, concentrations of nutrients are often given per 100 g fresh weight. The ascorbic acid concentration of the spinach in this study per 100 g fresh weight was 14-100 mg, and vitamin C 19-109 mg, which is relatively high (USDA, 2005). Dehydroascorbic acid contributed between 2 and 34% of total vitamin C in the baby spinach at harvest. The higher proportion was often found in the leaves with lower vitamin C concentration.

Total carotenoid concentration at harvest ranged from 0.8 to 1.5 mg/g dw, corresponding to 6-13 mg/100 g fw (Papers I, III), which is relatively high compared to many other fruits and vegetables {Müller 1997 #6960}. The composition of carotenoids in baby spinach corresponded well with what has previously been reported for spinach (Eskling & Åkerlund, 1998; Kidmose *et al.,* 2001). The major carotenoid was lutein, making up 35-48% of the total carotenoids, measured as β-carotene equivalents. Violaxanthin contributed 16-35%, β-carotene 16-24% and neoxanthin 11-14%.

Total flavonoid concentration at harvest was 10-20 mg/g dw, corresponding to 76-250 mg/100 g fw (Papers II, IV). Spinach contains relatively rare flavonoid compounds, all flavonols. Compared to the 28 vegetables analysed by Hertog *et al.* (1992), the flavonoid concentration on a fresh weight basis was actually very high in baby spinach when these compounds were included. The major flavonoid compound found in this study was 5,3′,4′-trihydroxy-3-methoxy-6:7-methylenedioxyflavone-4′-glucuronide (Fig. 3), making up 33-47% of the total flavonoids, measured as spiraeoside equivalents. Spinatoside-4′-glucuronide contributed 9-15%, patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside 9-13%, patuletin-3-gentiobioside 7-12% and patuletin-3-(2′′-feroylglucosyl)-(1-6)[apiosyl(1-2)]-glucoside 6-9%.

Changes during plant growth

The normal harvest time for baby spinach in southern Sweden is about 3½ weeks. Optimum harvest time depends on leaf size, weather conditions, work load at the production site and demand for the product. Thus, there is some variation in leaf size at the time of harvest, and the optimum harvest time cannot be set to one particular day. Furthermore, leaf size at a certain time after sowing depends on growing conditions, such as temperature, irradiation and availability of water and nutrients. Hence, the time from sowing to normal harvest also varies with time of season. In this study, the leaf size at growth stage II corresponded to a leaf size when harvest is normally done. In fact, at the commercial production site (Papers I, II), the workers harvested the same cultivar at the same time as our harvest at growth stage II for the first sowing.

The bioactive compounds studied in this investigation changed in different ways during plant growth (Fig. 8). Ascorbic acid and vitamin C concentrations decreased significantly from growth stage I to growth stage II in both experiments (Papers I, III), except in the April-sown spinach. From stage II to stage III the concentrations increased significantly in the spinach sown in August 2002 and decreased in the spinach sown in April. These results indicate that the ascorbic acid and vitamin C concentrations in the product can generally be increased by harvesting earlier than the normal time. In some cases, the concentration could be increased by harvesting later, but this effect appears to be dependent on the time of sowing. Other studies of spinach report varying changes in ascorbic acid concentration during plant growth (Sugawara, 1959; Stino, Abdelfattah & Nassar, 1973; Luwe, Takahama & Heber, 1993; Watanabe, Uchiyama & Yoshida, 1994). These discrepancies may be due to the fact that leaves of different ages were studied by the different researchers. Had we compared only stages I and II in August, we would have reported a decreasing concentration with growth, whereas if only stages II and III had been compared, we would have reported an increasing concentration. In this study, three growth stages were studied, at five- or six-days intervals. However, a clearer pattern of change during growth might have been observed if samples had been taken at closer intervals.

The oxidised form of vitamin C, dehydroascorbic acid, generally constituted a larger proportion of the total vitamin C in the older leaves (Papers I, III). This may reflect a lower capacity of older plants to reduce dehydroascorbic acid to ascorbic acid (Toledo *et al.,* 2003; Hodges & Forney, 2003). Ascorbic acid has a higher reducing capacity and antioxidative potential than dehydroascorbic acid. The antioxidative capacity supplied by vitamin C thus decreased from growth stage I to stages II and III. This may have physiological implications, both for the plant and its defence against oxidative stress, and for human health when the spinach is ingested in the diet (Hancock & Viola, 2005).

Carotenoid concentrations changed in different directions during growth in the two studies (Fig. 8). In the first field experiment (Paper I), carotenoid concentration increased significantly from stages I and II to stage III in the June- and July-sown spinach, whereas differences were very small in the August-sown spinach. In the second experiment (Paper III), total carotenoid concentration decreased during growth. The individual carotenoid compounds showed rather similar changes during growth, except for violaxanthin in the August 2003 sowing, which decreased by 21%.

Flavonoid concentration changed during growth in a manner much like vitamin C and ascorbic acid, except in the April-sown spinach (Fig. 8). A decrease was most

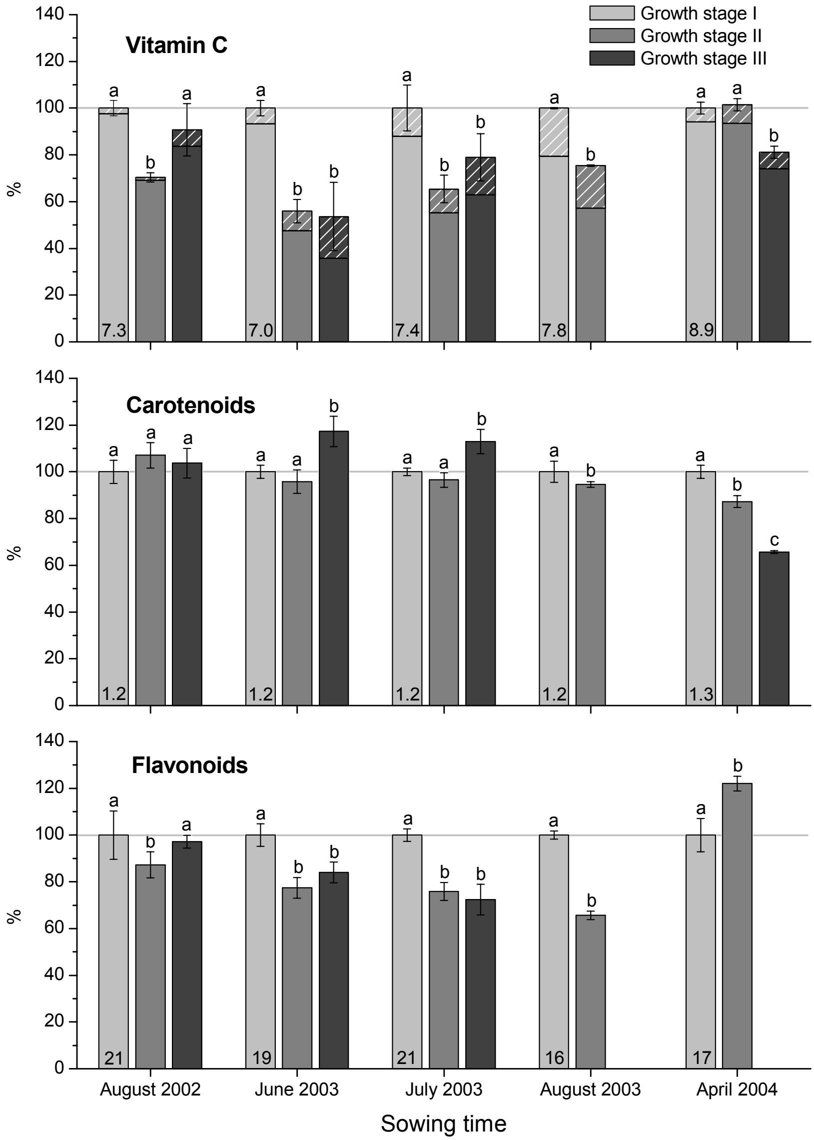

Figure 8. Relative concentrations of vitamin C, total carotenoids and total flavonoids during growth (unshaded) of baby spinach. The relative concentration at growth stage I is set to 100% for each sowing time. The accumulated bars for vitamin C indicate ascorbic acid (solid) and dehydroascorbic acid (striped) concentrations. Absolute concentrations (mg/g dw) are given in the bars representing growth stage I. Bars designated by the same letter within each sowing time and compound are not significantly different ($p<0.05$).

often observed from stage I to stage II. In the spinach sown in August 2002, total flavonoid concentration then increased again to stage III, whereas it remained stable in the spinach sown in June and July 2003 (Paper II). In April 2004, total flavonoid concentration increased significantly from stage I to stage II (Paper IV). As stated before, distinguishing between the effects of plant growth stage and season progression may be difficult. The end response is probably a combination of both, and naturally other factors as well. It is possible that total flavonoid concentration in baby spinach generally decreases as plant growth continues, but in the April-sown spinach the increasing radiation may have had a sufficiently large effect on flavonoid concentration to outweigh any decreases caused by plant growth alone. Studying plant growth under more controlled conditions would be of interest, but the concentrations and possibly also changes in concentrations may be altered by the use of an artificial light source.

Variation with sowing time

Swedish baby spinach is generally sown between April and September. Vitamin C and ascorbic acid concentrations varied between sowing dates, especially in the older leaves (growth stages II and III) in both field experiments (Papers I, III). The largest variation was found in ascorbic acid concentration at growth stage III, which was 2.4 times higher in the August-sown spinach than in the June-sown (Paper I). The concentrations were very similar at growth stage I, however. In the second field experiment, the ascorbic acid concentration was significantly higher in the April-sown spinach than in the August-sown. The higher concentration in the April-sown spinach may be due to the higher radiation during the growth period. The dehydroascorbic acid/vitamin C ratio was considerably higher in the June- and July-sown spinach than in the August-sown at all growth stages in the first field experiment (Paper I). However, the ratio was much higher in the August-sown spinach than in the April-sown in the second experiment (Paper III). This may indicate that the leaves were subjected to some sort of stress, which may also have had an effect on the ascorbic acid concentration. In other words, both the concentrations of ascorbic acid and vitamin C and the dehydroascorbic acid/vitamin C ratio showed large variations during the season. This has implications for the keeping quality, as will be pointed out later in this section.

Total carotenoid concentration varied somewhat between sowing dates, and different sowings gave the highest concentration at different growth stages. At growth stage II, the current normal harvest stage, the total carotenoid concentration was highest in the August-sown spinach and lowest in the July-sown (Paper I). The difference between the sowings was 12%. In the second field experiment (Paper III), total carotenoid concentration was very similar between the two sowings, varying by only 6%. Unexpectedly, violaxanthin concentration was lower in the April-sown spinach than in the August-sown. Violaxanthin is involved in photoprotection via the xanthophyll cycle (Demmig-Adams, Gilmore & Adams, 1996), and was therefore expected to be more abundant in April, when radiation is higher (Fig. 4) and when plants would thus require more protection against photodamage. The higher dry matter content in April than in August may explain this partly, but not fully, because then the decrease would have been equally evident in the other carotenoid compounds as well.

Total flavonoid concentration was higher in the August-sown spinach than in the July- and June-sown at all growth stages in the first experiment (Paper II). In the second experiment, however, the concentration was similar in the April-sown and in the August-sown spinach at growth stage I, whereas at growth stage II the concentration was 100 % higher in the April-sown spinach than in the August-sown (Paper IV). Since flavonoid concentration generally increases due to UV-B radiation (Liu, Gitz & McClure, 1995; Greenberg *et al.,* 1996; Olsson *et al.,* 1998; Burchard, Bilger & Weissenböck, 2000), and since radiation was higher for the April sowing than for the August sowing (Fig. 4) the results from the first experiment were somewhat surprising.

The effects of shade netting

The use of shade netting may entail changes to microclimate other than light quantity alone. In this study, three types of shade netting with different characteristics were used. These nets have some differences in absorption at different wavelengths, and consequently affect light quality in addition to light quantity. Temperature and relative humidity were measured, but no differences were found between the netting types (Papers III, IV).

Vitamin C and ascorbic acid concentrations were most often significantly lower in the plants grown under the nettings than in the unshaded plants. However, the response to the use of nettings varied with sowing time and growth stage (Paper III). The largest differences were observed in the April-sown spinach, possibly as a result of light intensity (Fig. 4). Ascorbic acid concentration is generally higher in plants receiving more light (Lee & Kader, 2000), as it is synthesised from sugars supplied through photosynthesis (Wheeler, Jones & Smirnoff, 1998) and is involved in photosynthesis as an electron donor and co-factor for violaxanthin de-epoxidase (Smirnoff, 2000). Higher global radiation brings about larger differences in radiation received by shaded and unshaded leaves, and hence the potential for a larger response. The ratio of dehydroascorbic acid to vitamin C was not affected as strongly as the concentrations of vitamin C and ascorbic acid by the use of shade nettings (Paper III).

The total carotenoid concentration was unchanged or higher under the shade nettings compared to the unshaded plants (Paper III). Total carotenoid concentration is generally said to be higher in sun leaves than in shade leaves (Thayer & Björkman, 1990; Demmig-Adams & Adams, 1992). However, this is when expressed per unit leaf area. As the dry matter content in sun leaves is usually higher per leaf area unit, carotenoid concentration per unit dry matter is generally lower in sun leaves than in shade leaves (Lichtenthaler *et al.,* 1981). In this study (Paper III), total carotenoid concentration per unit dry weight was inversely correlated to the radiation the plants received, when comparisons were made within each harvest (Fig. 9).

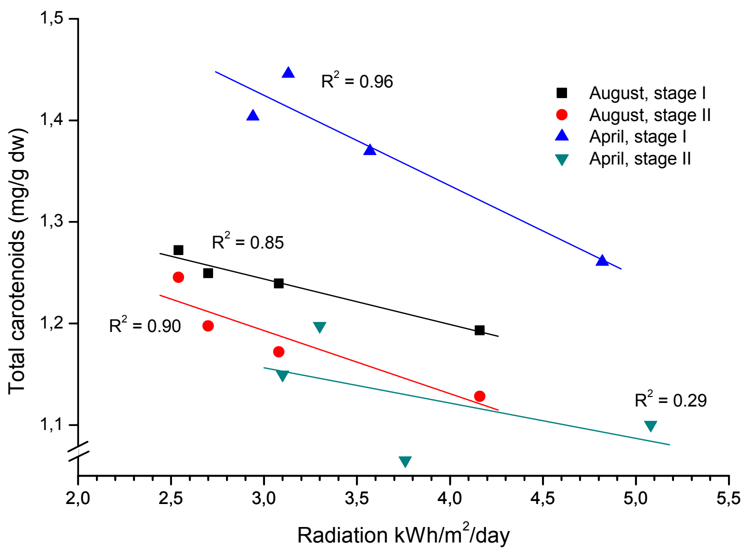

Figure 9. Total carotenoids as a function of radiation reaching the plants during the second field experiment.

Similarly to the carotenoids, total chlorophyll concentration was higher in the shaded leaves than in the unshaded, although the differences was even greater for chlorophyll (Paper III). The carotenoid/chlorophyll ratio was positively correlated to radiation received (Fig. 10). This is in agreement with the fact that sun leaves generally have a higher carotenoid/chlorophyll ratio than shade leaves, probably related to a greater need for photoprotection in full sun (Demmig-Adams, Gilmore & Adams, 1996).

The use of shade nettings affected total flavonoid concentration only in some cases (Paper IV). In the August-sown spinach harvested at stage I, the spectrum-altering netting, with the lowest UV-B transmittance, decreased flavonoid concentration significantly, whereas the August-sown spinach harvested at stage II and the April-sown spinach harvested at stage I showed no significant change in total flavonoid concentration due to shading. The largest effect by far was observed in the April-sown spinach harvested at stage II. This was also when global radiation was the highest (Fig. 4). This effect too may partly be explained by differences in dry matter content between the shaded and unshaded leaves (Paper IV). The individual flavonoid compounds responded in different ways to shading. The concentration of the major flavonoid compound, 5,3´,4´-trihydroxy-3-methoxy-6:7-methylene-dioxyflavone-4´-glucuronide responded differently at different harvests. Patuletin-3-gentiobioside concentration was most often significantly lower in shaded leaves than in unshaded (Paper IV).

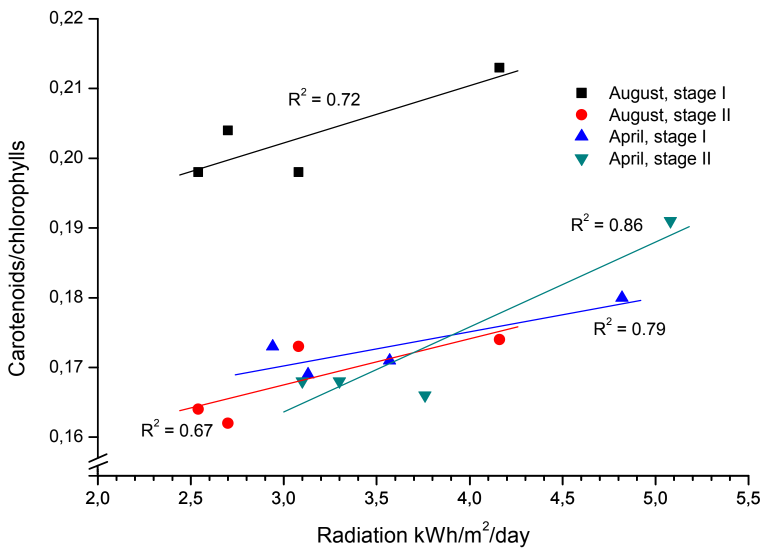

Figure 10. Total carotenoids / total chlorophylls as a function of radiation reaching the plants during the second field experiment.

Postharvest changes

Most fruits and vegetables are ideally harvested shortly before eating, since many processes that may lead to deterioration of the produce start immediately after harvest. Today, long transport distances and distribution chains with several steps may nevertheless render the time from harvest until the produce is available in to the consumer quite long. Leafy vegetables with a high surface/volume ratio and relatively high respiration are quite perishable and storage life may be as short as a week or even less (Wills *et al.*, 1998). Postharvest changes may include deterioration in visual and microbial quality, as well as in biochemical composition.

We found that vitamin C and ascorbic acid concentrations generally decreased during storage at 2°C and 10°C, with the most pronounced changes at 10°C (Papers I, III). The decrease was most evident in the first experiment and in the August-sown spinach in the second experiment. In some cases all ascorbic acid was lost during the nine-day storage period. In the April-sown spinach, however, the concentrations were more stable during storage. The largest change was a 37% decrease in ascorbic acid content in produce grown under the spectrum-altering netting and harvested at stage II. The observed decreases are in agreement with previous reports on spinach (Favell, 1998; Gil, Ferreres & Tomás-Barberan, 1999; Hodges & Forney, 2003). In the second experiment, however, ascorbic acid and vitamin C concentrations actually increased during storage in some cases (only at 2°C). The changes in dry matter content of the leaves during storage were much too small to explain the increase. It therefore appears that ascorbic acid was being synthesised during storage. Postharvest synthesis of ascorbic acid has previously been observed in barley exposed to light during the storage period (Smirnoff, 2000). To our knowledge, the present study is the first to report an increase in ascorbic acid concentration in dark-stored leaves.

The dehydroascorbic acid/vitamin C ratio generally increased during storage (Papers I, III), as is common in fruit and vegetables during storage (Wills, Wimalasiri & Greenfield, 1984). The initial dehydroascorbic acid/vitamin C ratio and the concentration of ascorbic acid were considerably higher in the August-sown spinach than in the April-sown in the second experiment.

Visual quality also decreased during storage in most cases (Papers I, III). The quality deterioration was considerably greater in the August-sown spinach than in the April-sown in the second experiment. This is possibly related to the higher initial ascorbic acid concentration, providing a better defence against oxidative stress (Hodges & Forney, 2003). We found correlations between visual quality retention and initial ascorbic acid concentration, and initial dry matter content (Paper I, Fig. 11, 12). The visual quality decreased more in the older leaves in the first experiment and in the April-sown spinach in the second experiment, probably also related to the difference in initial ascorbic acid concentration. However, even though there was a correlation between initial ascorbic acid concentration and visual quality after storage, this does not constitute proof of a cause-effect relationship. It is possible that ascorbic acid is simply a marker for another factor that may affect visual quality. One such factor could be dry matter, but that correlation for the April-sown spinach was very weak (Fig. 12), making this explanation less plausible.

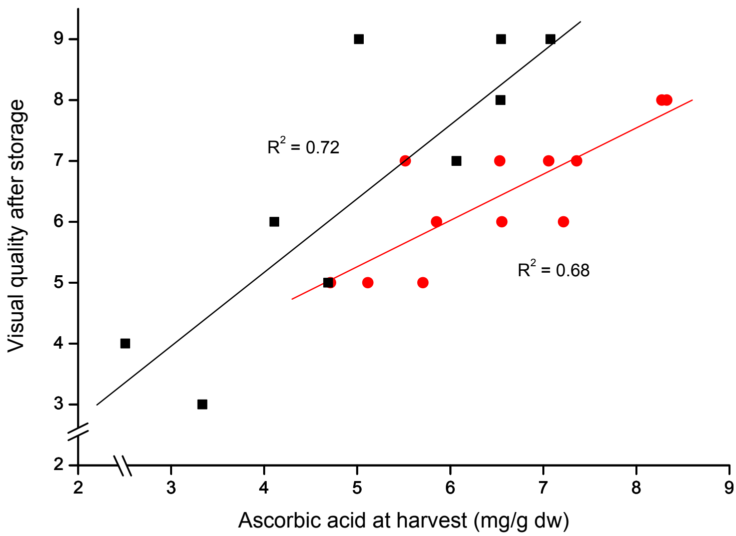

Figure 11. Visual quality after storage as a function of ascorbic acid concentration at harvest. Black squares represent field experiment 1 (Öllöv), data from all sowings. Red dots represent field experiment 2 (Hvilan), data only from the sowing in April, including shaded and unshaded plants.

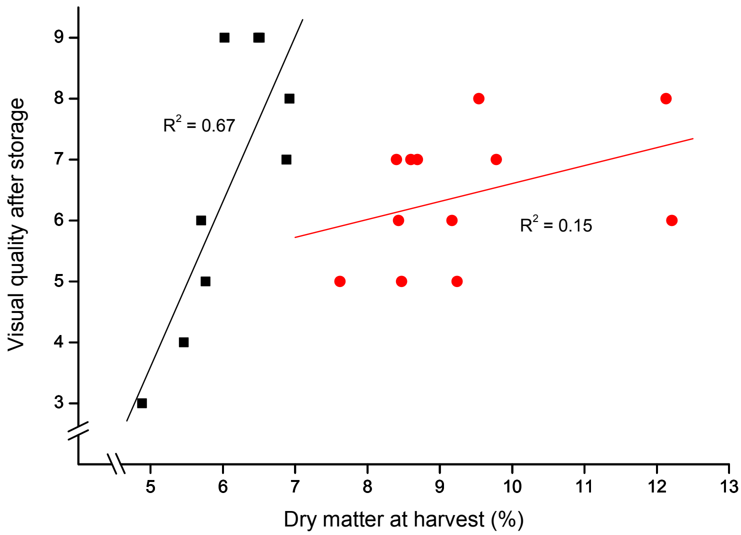

Figure 12. Visual quality after harvest as a function of dry matter content at harvest. Symbols and data sources as in Fig. 11.

The changes in carotenoid concentration during storage were not as large or as consistent as the changes in ascorbic acid concentration. Total carotenoid concentration increased in most cases from harvest to nine days of storage in the first experiment, although significantly so only in the June- and July-sown spinach stored at 10°C (Paper I). In the second experiment, an increase was most often observed at six days of storage, at 2°C and 10°C, but between six and nine days total carotenoid concentration often decreased again, especially at 10°C (Paper III). The baby spinach in this study clearly retained carotenoids to a higher extent than has previously been reported for spinach (Pandrangi & Laborde, 2004).

Total flavonoid concentration also showed relatively small and inconsistent changes during storage (Papers II, IV). The individual flavonoid compounds changed in different ways during storage, with patuletin-3-glucosyl-(1-6)[apiosyl(1-2)]-glucoside and patuletin-3-gentiobioside generally decreasing, and patuletin-3-(2´´-feroylglucosyl)-(1-6)[apiosyl(1-2)]-glucoside increasing during storage.

Concluding remarks and future perspectives

Consumer awareness of the health benefits of fruits and vegetables are increasing, possibly thanks to campaigns like "five-a-day" initiative encouraging increased consumption. Nevertheless, the consumption of fruits and vegetables is well below the recommended daily intake in many populations (Naska *et al.,* 2000). Even though the results of epidemiological studies are ambiguous, some health benefits from bioactive compounds have been confirmed. Therefore, it is of great importance that the levels of bioactive compounds in fruit and vegetables can be maintained from harvest to consumption, and possibly also increased in the produce at harvest by varying or controlling preharvest factors or choosing the right cultivars. A high concentration of bioactive compounds may be positive not only for human health, but also for produce storability, as can be seen by the correlation between ascorbic acid and visual quality in this study. The antioxidant activity and health effects vary among the individual compounds in each of the groups of bioactive compounds studied. Therefore, it is not only the total concentration, but also the composition of carotenoids, flavonoids and vitamin C that are important.

This study investigated some factors that affect the concentration and composition of some bioactive compounds. However, the effects of a certain factor are seldom clear-cut. Instead, an increase in ascorbic acid concentration, for example, often goes hand in hand with a decrease in carotenoid or flavonoid concentration, and *vice versa*. However, the following relatively straightforward results were obtained:

- Harvesting baby spinach leaves early, while they are slightly smaller than at conventional harvest may give increased concentrations of bioactive compounds. However, there are economic considerations to this as well, as an earlier harvest gives a lower yield. On the other hand, when the culture time is shorter there is scope for more sowings, and thus also more harvests, during the season. The storability of younger leaves appears to be higher, which should give lower postharvest losses. Harvesting later than the current conventional time may give a higher yield, but the concentrations of bioactive compounds may not be improved. Dehydroascorbic acid/vitamin C ratio is likely to be higher at a later harvest.

- There is generally relatively little variation in bioactive compounds in baby spinach during the season. Flavonoids, however, can vary considerably during the season, especially in unshaded plants, and ascorbic acid can vary greatly with time of sowing in later growth stages.

- The use of shade netting to protect baby spinach cultures from severe weather conditions including excess radiation appears to have relatively small effects on the concentrations of bioactive compounds and could therefore be considered acceptable as regards the concentration of the bioactive compounds investigated.

- There are considerable decreases in vitamin C and, above all, ascorbic acid concentration during storage, especially at higher temperature (10°C compared to 2°C). Postharvest stability is much greater for carotenoids and flavonoids. After a nine-day storage period at 10°C, all ascorbic acid may be lost and the visual quality may be lower than the limit of saleability. At 2°C, visual quality is likely to be better, and ascorbic acid retention higher, although only a small proportion of the initial ascorbic acid concentration may be retained. This demonstrates the importance of maintaining an appropriate storage temperature for baby spinach.

- Storability, measured as visual quality after storage, appears to be related to ascorbic acid concentration at harvest. A higher initial concentration probably offers better protection against oxidative stress, thus reducing deterioration of plant membranes and other tissues.

The work reported in this thesis provides some important pieces of information, adding to the increasing knowledge on how pre- and postharvest factors affect the concentrations of bioactive compounds in fruits and vegetables. The mechanisms behind some of the findings are currently not fully understood, and would be interesting targets for future research. The correlation between ascorbic acid concentration and visual quality retention seems promising, but even though there is a probable explanation for this correlation, it is possible that ascorbic acid concentration is simply a marker for something else, for example dry matter content, that is also correlated to visual quality retention. Since storage life of baby spinach and other leafy vegetables is short, improving storability with retention of visual quality and maintenance of bioactive compound concentrations is an important task for the future.

Other factors may also affect bioactive compounds in baby spinach, for instance fertilization regime and cultivar differences, and it would be interesting to investigate these too. Use of biotechnological methods to increase the concentrations of bioactive compounds may be another option, but legislation and consumer attitudes may make this less applicable. More frequent sampling during storage would also be of interest, in order to monitor postharvest changes more closely.

References

Ames, B.N., Shigenaga, M.K. & Hagen, T.M. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the national academy of sciences of the United States of America* 90, 7915-7922.

Arts, I.C.W. & Hollman, P.C.H. 2005. Polyphenols and disease risk in epidemiologic studies. *American journal of clinical nutrition* 81, 317S-325S.

Asplund, K. 2002. Antioxidant vitamins in the prevention of cardiovascular disease: a systematic review. *Journal of internal medicine* 251, 372-392.

ATBC 1994. The alpha tocopherol beta carotene cancer prevention study group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England journal of medicine* 330, 1029-1035.

Bast, A. & Haenen, G.R.M.M. 2002. The toxicity of antioxidants and their metabolites. *Environmental toxicology and pharmacology* 11, 251-258.

Benzie, I.F.F. & Strain, J.J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity in biological fuilds and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology* 299, 15-27.

Berger, M.M. 2005. Can oxidative damage be treated nutritionally? *Clinical nutrition* 24, 172-183.

Brown, L., Rimm, E.B., Seddon, J.M., Giovannucci, E.L., Chasan-Taber, L., Spiegelman, D., willett, W.C. & Hankinson, S.E. 1999. A prospective study of carotenoid intake and risk of cataract extraction in US men. *American journal of clinical nutrition* 70, 517-524.

Buescher, R., Howard, L. & Dexter, P. 1999. Postharvest Enhancement of Fruits and Vegetables for Improved Human Health. *Hortscience* 34, 1167-1170.

Burchard, P., Bilger, W. & Weissenböck, G. 2000. Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. *Plant cell and environment* 23, 1373-1380.

Böhm, F., Edge, R., Land, E.J., McGarvey, D.J. & Truscott, T.G. 1997. Carotenoids enhance vitamin E antioxidant efficiency. *Journal of the american chemical society* 119, 621-622.

Cao, G., Alessio, H.M. & Cutler, R.G. 1993. Oxygen-radical absorbance assay for antioxidants. *Free radical biology and medicine* 14, 303-311.

Castenmiller, J.J.M., West, C.E., Linssen, J.P.H., van het Hof, K.H. & Voragen, A.G.J. 1999. The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. *Journal of nutrition* 129, 349-355.

Christen, W.G., Gaziano, J.M. & Hennekens, C.H. 2000. Design of Physicians' Health Study II--a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. *Annals of epidemiology* 10, 125-134.

Commenges, D., Scotet, V., Renaud, S., Jacqmin Gadda, H., Barberger Gateau, P. & Dartigues, J.-F. 2000. Intake of flavonoids and risk of dementia. *European journal of epidemiology* 16, 357-363.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D. & Fletcher, J. 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the science of food and agriculture* 80, 825-860.

Demmig-Adams, B. & Adams, W.W.I. 1992. Carotenoid composition in sun and shade leaves of plants with different life forms. *Plant, cell and environment* 15, 411-419.

Demmig-Adams, B., Gilmore, A.M. & Adams, W.W.I. 1996. *In vivo* functions of carotenoids in higher plants. *FASEB journal* 10, 403-412.

Drews, M., Schonhof, I. & Krumbein, A. 1997. Content of minerals, vitamins, and sugars in iceberg lettuce (*Lactuca sativa* var. *capitata* L.) grown in the greenhouse dependent on cultivar and development stage. *Gartenbauwissenschaft* 62, 65-72.

DuPont, M.S., Mondin, Z., Williamson, G. & Price, K.R. 2000. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *Journal of agricultural and food chemistry* 48, 3957-3964.

Eskling, M. & Åkerlund, H.E. 1998. Changes in the quantities of violaxanthin de-epoxidase, xanthophylls and ascorbate in spinach upon shift from low to high light. *Photosynthesis research* 57, 41-50.

Favell, D.J. 1998. A comparison of the vitamin C content of fresh and frozen vegetables. *Food chemistry* 62, 59-64.

Finkel, T. & Holbrook, N.J. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239-247.

Forman, D. & Altman, D. 2004. Vitamins to prevent cancer: supplementary problems. *The lancet* 364, 1193-1194.

Frankel, E.N. & Meyer, A.S. 2000. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the science of food and agriculture* 80, 1925-1941.

Gandini, S., Merzenich, H., Robertson, C. & Boyle, P. 2000. Meta-Analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. *European journal of cancer* 36, 636-646.

Gil, M.I., Ferreres, F. & Tomás-Barberan, F.A. 1998. Effect of modified atmosphere packaging on the flavonoids and vitamin C content of minimally processed Swiss chard (*Beta vulgaris* subspecies *cycla*). *Journal of agricultural and food chemistry* 46, 2007-2012.

Gil, M.I., Ferreres, F. & Tomás-Barberan, F.A. 1999. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of agricultural and food chemistry* 47, 2213-2217.

Greenberg, B.M., Wilson, M.I., Gerhardt, K.E. & Wilson, K.E. 1996. Morphological and physiological responses of *Brassica napus* to ultraviolet-B radiation: photomodification of ribulose-1,5-bisphosphate carboxylase/oxygenase and potential acclimation processes. *Journal of plant physiology* 148, 78-85.

Halliwell, B. & Gutteridge, J.M.C. 1989. *Free radicals in biology and medicine*. 2nd edn. Oxford University Press. New York.

Hancock, R.D. & Viola, R. 2005. Improving the nutritional value of crops through enhancement of L-ascorbic acid (vitamin C) content: rationale and biotechnological opportunities. *Journal of agricultural and food chemistry* 53, 5249-5257.

Harborne, J.B. & Williams, C.A. 2000. Advances in flavonoid research since 1992. *Phytochemistry* 55, 481-504.

Hennekens, C.H., Gaziano, J.M., Manson, J.E. & Buring, J.E. 1995. Antioxidant vitamin - cardiovascular disease hypothesis is still promising, but still unproven: the need for randomized trials. *American journal of clinical nutrition* 62, 1377S-1380S.

Hertog, M.G.L., Hollman, P.C.H. & Katan, M.B. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of agricultural and food chemistry* 40, 2379-2383.

Hodges, D.M. & Forney, C.F. 2003. Postharvest ascorbate metabolism in two cultivars of spinach differing in their senescence rates. *Journal of the american society for horticultural science* 128, 930-935.

Hohl, U., Neubert, B., Pforte, H., Schonhof, I. & Böhm, H. 2001. Flavonoid concentrations in the inner leaves of head lettuce genotypes. *European food research and technology* 213, 205-211.

Hollman, P.C.H. 2001. Evidence for health benefits of plant phenols: local or systemic effects? *Journal of the science of food and agriculture* 81, 842-852.

Hollman, P.C.H. & Arts, I.C.W. 2000. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. *Journal of the science of food and agriculture* 80, 1081-1093.

Houstis, N., Rosen, E.D. & Lander, E.S. 2006. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 440, 944-948.
Howard, L.R., Pandjaitan, N., Morelock, T. & Gil, M.I. 2002. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *Journal of agricultural and food chemistry* 50, 5891-5896.
Institute of Medicine 2000. *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. National Academy Press. Washington, D. C.
Joshipura, K.J., Hu, F.B., Manson, J.E., Stampfer, M.J., Rimm, E.B., Speizer, F.E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D. & Willett, W.C. 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. *Annals of i nternal medicine* 134, 1106-1114.
Justesen, U., Knuthsen, P., Andersen, N.L. & Leth, T. 2000. Estimation of daily intake distribution of flavonols and flavanones in Denmark. *Scandinavian journal of nutrition* 44, 158-160.
Kalt, W. 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of food science* 70, 11-19.
Kang, J.H., Ascherio, A. & Grodstein, F. 2005. Fruit and vegetable consumption and cognitive decline in aging women. *Annals of neurology* 57, 713-720.
Khaw, K.-T., Bingham, S., Welch, A., Luben, R., Wareham, N., Oakes, S. & Day, N. 2001. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. *The lancet* 357, 657-663.
Kidmose, U., Knuthsen, P., Edelenbos, M., Justesen, U. & Hegelund, E. 2001. Carotenoids and flavonoids in organically grown spinach (*Spinacia oleracea* L) genotypes after deep frozen storage. *Journal of the science of food and agriculture* 81, 918-923.
Knekt, P., Jarvinen, R., Reunanen, A. & Maatela, J. 1996. Flavonoid intake and coronary mortality in Finland: a cohort study. *British medical journal* 312, 478-481.
Kolb, C.A., Käser, M.A., Kopecký, J., Zotz, G., Riederer, M. & Pfündel, E.E. 2001. Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. *Plant physiology* 127, 863-875.
Kopas-Lane, L.M. & Warthesen, J.J. 1995. Carotenoid photostability in raw spinach and carrots during cold storage. *Journal of food science* 60, 773-776.
Krinsky, N.I. & Johnson, E.J. 2005. Carotenoid actions and their relation to health and disease. *Molecular aspects of medicine* 26, 459-516.
Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E. & Etherton, T.D. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American journal of medicine* 113, 71S-88S.
Kritchevsky, S.B. 1999. Beta-carotene, carotenoids and the prevention of coronary heart disease. *Journal of nutrition* 129, 5-8.
Kunert, K.J. & Ederer, M. 1985. Leaf aging and lipid peroxidation: the role of the antioxidants vitamin C and E. *Physiologia plantarum* 65, 85-88.
Lampe, J.W. 1999. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *American journal of clinical nutrition* 70, 475S-490S.
Le Marchand, L. 2002. Cancer preventive effects of flavonoids - a review. *Biomedicine & pharmacotherapy* 56, 296-301.
Lee, S.K. & Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest biology and technology* 20, 207-220.
Lefsrud, M.G., Kopsell, D.A., Kopsell, D.E. & Curran-Celentano, J. 2005. Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. *Hortscience* 40, 2026-2030.
Leth, T., Jakobsen, J. & Andersen, N.L. 2000. The intake of carotenoids in Denmark. *European journal of lipid science and technology* 102, 128-132.
Levine, M., Rumsey, S.C., Daruwala, R., Park, J.B. & Wang, Y. 1999. Criteria and recommendations for vitamin C intake. *JAMA: Journal of the Amreican medical association* 281, 1415-1423.
Lichtenthaler, H.K., Buschmann, C., Döll, M., Fietz, H.-J., Bach, T., Kozel, U., Meier, D. & Rahmsdorf, U. 1981. Photosyntehtis activity, chloroplast ultrastructure, and leaf

characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis research* 2, 115-141.
Lister, C.E. 1999. The medicine of life. Vitamins and other bioactive food components. *Food technology* 34, 18-20, 35.
Liu, L., Gitz, D.C.I. & McClure, J.W. 1995. Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Physiologia plantarum* 93, 725-733.
Liu, S., Manson, J.E., Lee, I.M., Cole, S.R., Hennekens, C.H., Willett, W.C. & Buring, J.E. 2000. Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. *American journal of clinical nutrition* 72, 922-928.
Liu, S., Lee, I.-M., Ajani, U., Cole, S.R., Buring, J.E. & Manson, J.E. 2001. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: the physicians' health study. *International journal of epidemiology* 30, 130-135.
Loo, G. 2003. Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation (Review). *Journal of nutritional biochemistry* 14, 64-73.
Lurie, S. 2003. Antioxidants. In: Hodges, D.M. (Ed.) *Postharvest oxidative stress in horticultural crops*, pp. 131-150. New York: Food Products Press.
Luwe, M.W.F., Takahama, U. & Heber, U. 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia Oleracea* L.) leaves. *Plant physiology* 101, 969-976.
Manach, C., Mazur, A. & Scalbert, A. 2005. Polyphenols and prevention of cardiovascular diseases. *Current opinion in lipidology* 16, 77-84.
Manach, C., Williamson, G., Morand, C., Scalbert, A. & Rémésy, C. 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American journal of clinical nutrition* 81, 230S-242S.
Martin, H.D., Ruck, C., Schmidt, M., Sell, S., Beutner, S., Mayer, B. & Walsh, R. 1999. Chemistry of carotenoid oxidation and free radical reactions. *Pure and applied chemistry* 71, 2253-2262.
Meir, S., Kanner, J., Akiri, B. & Philosoph-Hadas, S. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal of agricultural and food chemistry* 43, 1813-1819.
Mennen, L.I., Walker, R., Bennetau-Pelissero, C. & Scalbert, A. 2005. Risks and safety of polyphenol consumption. *American journal of clinical nutrition* 81, 326S-329S.
Mercadante, A.Z. & Rodriguez-Amaya, D.B. 1991. Carotenoid composition of a leafy vegetable in relation to some agricultural variables. *Journal of agricultural and food chemistry* 39, 1094-1097.
Miller, N.J., Rice-Evans, C.A., Davies, M.J., Gopinathan, V. & Milner, A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical science* 84, 407-412.
Moser, U. 2002. Are clinical trials suitable to prove efficacy of nutrients? *Free radical research* 36, 27-30.
Mozafar, A. 1993. Nitrogen fertilizers and the amount of vitamins in plants: a review. *Journal of plant nutrition* 16, 2479-2506.
Mozafar, A. 1994. *Plant vitamins: agronomic, physiological and nutritional aspects*. CRC Press. Boca Raton, Florida.
Müller, H. 1997. Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A* 204, 88-94.
Naska, A., Vasdekis, V.G.S., Trichopoulou, A., Friel, S., Leonhauser, I.U., Moreiras, O., Nelson, M., Remaut, A.M., Schmitt, A., Sekula, W., Trygg, K.U. & Zajkas, G. 2000. Fruit and vegetable availability among ten European countries: how does it compare with the 'five-a-day' recommendation? *British journal of nutrition* 84, 549-556.
Neuhouser, M.L. 2004. Dietary flavonoids and cancer risk: Evidence from human population studies. *Nutrition and cancer-an international journal* 50, 1-7.
Niki, E., Noguchi, N., Tsuchihashi, H. & Gotoh, N. 1995. Interaction among vitamin-C, vitamin-E, and β-carotene. *American journal of clinical nutrition* 62, S1322-S1326
Olsson, L.C., Veit, M., Weissenböck, G. & Bornman, J.F. 1998. Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. *Phytochemistry* 49, 1021-1028.

Omaye, S.T., Krinsky, N.I., Kagan, V.E., Mayne, S.T., Liebler, D.C. & Bidlack, W.R. 1997. β-carotene: friend or foe? *Fundamental and applied toxicology* 40, 163-174.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Valanis, B., Williams, J.H., Barnhart, S. & Hammar, S. 1996. Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. *New England journal of medicine* 334, 1150-1155.

Pandrangi, S. & Laborde, L.F. 2004. Retention of folate, carotenoids, and other quality characteristics in commercially packaged fresh spinach. *Journal of food science* 69, C702-C707

Philosoph-Hadas, S., Meir, S., Akiri, B. & Kanner, J. 1994. Oxidative defense systems in leaves of three edible herb species in relation to their senescence rate. *Journal of agricultural and food chemistry* 42, 2376-2381.

Poulsen, N., Johansen, A.S. & Sørensen, J.N. 1995. Influence of growth conditions on the value of crisphead lettuce: 4. Quality changes during storage. *Plant foods for human nutrition* 47, 157-162.

Rice-Evans, C. & Miller, N.J. 1995. Antioxidants - the case for fruit and vegetables in the diet. *British food journal* 97, 35-40.

Rice-Evans, C.A., Miller, N.J. & Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine* 20, 933-956.

Rietjens, I.M.C.M., Boersma, M.G., de Haan, L., Spenkelink, B., Awad, H.M., Cnubben, N.H.P., van Zanden, J.J., van der Woude, H., Alink, G.M. & Koeman, J.H. 2002. The pro-oxidant chemistry and toxicity of the natural antioxidants vitamin C, vitamin E, beta-carotene and flavonoids. *Environmental toxicology and pharmacology* 11, 321-333.

Scalbert, A. & Williamson, G. 2000. Dietary intake and bioavailability of polyphenols. *Journal of nutrition* 130, 2073S-2085S.

Skibola, C.F. & Smith, M.T. 2000. Potential health impacts of excessive flavonoid intake. *Free radical biology and medicine* 29, 375-383.

Smirnoff, N. 1996. The function and metabolism of ascorbic acid in plants. *Annals of botany* 78, 661-669.

Smirnoff, N. 2000. Ascorbate biosynthesis and function in photoprotection. *Philosophical transactions of the Royal society of London series B - biological sciences* 355, 1455-1464.

Stanner, S.A., Hughes, J., Kelly, C.N.M. & Buttriss, J. 2004. A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public health nutrition* 7, 407-422.

Stino, K.R., Abdelfattah, M.A. & Nassar, H. 1973. Studies on vitamin C and oxalic acid concentration in spinach. *Agricultural research review* 51, 109-114.

Stoewsand, G.S. 1995. Bioactive organosulfur phytochemicals in Brassica oleracea vegetables - a review. *Food and chemical toxicology* 33, 537-543.

Sugawara, T. 1959. Studies on vitamin C in field and garden crops. *Horticultural abstracts* 35, 1390

Thayer, S.S. & Björkman, O. 1990. Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosynthesis research* 23, 331-343.

Toledo, M.E.A., Ueda, Y., Imahori, Y. & Ayaki, M. 2003. L-ascorbic acid metabolism in spinach (*Spinacia oleracea* L.) during postharvest storage in light and dark. *Postharvest biology and technology* 28, 47-57.

USDA. 2005. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 18.

van het Hof, K., West, C.E., Weststrate, J.A. & Hautvast, J.G.A.J. 2000. Dietary factors that affect the bioavailability of carotenoids. *Journal of nutrition* 130, 503-506.

van het Hof, K.H., Brouwer, I.A., West, C.E., Haddeman, E., Steegers-Theunissen, R.P.M., van Dusseldorp, M., Weststrate, J.A., Eskes, T.K.A.B. & Hautvast, G.A.J. 1999. Bioavailability of lutein from vegetables is 5 times higher than that of ß-carotene. *American journal of clinical nutrition* 70, 261-268.

Veit, M., Bilger, T., Muhlbauer, T., Brummet, W. & Winter, K. 1996. Diurnal Changes in Flavonoids. *Journal of plant physiology* 148, 478-482.

Watanabe, Y., Uchiyama, F. & Yoshida, K. 1994. Compositional changes in spinach (*Spinacia oleracea* L.) grown in the summer and in the fall (English abstract). *Journal of the Japanese society for horticultural science* 62, 889-895.
Weston, L.A. & Barth, M.M. 1997. Preharvest factors affecting postharvest quality of vegetables. *Hortscience* 32, 812-816.
Wheeler, G.L., Jones, M.A. & Smirnoff, N. 1998. The biosynthetic pathway of vitamin C in higher plants. *Nature* 393, 365-369.
Williamson, G. 1996. Protective effects of fruit and vegetables in the diet. *Nutrition & food science* 96, 6-10.
Wills, R.B.H., Wimalasiri, P. & Greenfield, H. 1984. Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. *Journal of agricultural and food chemistry* 32, 836-838.
Wills, R., McGlasson, B., Graham, D. & Joyce, D. 1998. *Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals*. 4th edn. CAB International. Wallingford Oxon, UK.
Yadav, S.K. & Sehgal, S. 1995. Effect of home processing on ascorbic acid and beta-carotene content of spinach (*Spinacia oleracea*) and amaranth (*Amaranthus tricolor*) leaves. *Plant foods for human nutrition* 47, 125-131.
Ziegler, R.G. 1989. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *Journal of nutrition* 119, 116-122.

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