Quercetin Content in Yellow Onion (*Allium cepa* L.)

Effects of Cultivation Methods, Curing and Storage

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Abstract

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Flavonoids are polyphenolic substances that are common in many fruits and vegetables and may have positive protective effects against *e.g*. cardiovascular disease and some forms of cancer. These effects have mainly been attributed to their antioxidative properties. One of the most common flavonoids in food plants is quercetin, of which yellow onion (*Allium cepa* L.) is one of our main dietary sources.

The effects of cultivars, nitrogen fertiliser level, lifting time, curing, cultivation system and storage conditions on the quercetin content in yellow onion are investigated in this thesis. Analyses were performed using reversed-phase HPLC after extraction of raw onion samples in ethanol.

The main determinant of quercetin level was found to be the amount of global solar radiation during the end of the bulbing phase in August. Annual variations in quercetin content were higher than any treatment effect within each year. Cultivar differences in quercetin levels were significant but inconsistent. Early lifting, when 50% of the onions had fallen leaves, resulted in onions with better keeping quality and equal quercetin content compared with onions with later lifting times. Minimised split application of nitrogen fertilisers reduced the risk of mineral nutrient leaching without lowering the yield or quercetin content. Field curing generally resulted in significant increases in quercetin content compared with levels at lifting. The role of onion size for quercetin content was inconsistent but of minor importance. Quercetin levels were stable during cold storage at constant temperature. No significant differences were found between inorganically fertilised and organically fertilised onions of the same cultivar or between directly sown and transplanted onions. Quercetin content and soil water content within the field were not correlated.

Keywords: fertiliser level, flavonoids, polyphenols, antioxidants, lifting stage, global radiation, organic fertiliser, inorganic fertiliser, HPLC

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Populärvetenskaplig sammanfattning

Vanlig gul lök (*Allium cepa* L.) är rik på flavonoiden quercetin som ger löken dess gula färg. Flavonoider är fenolföreningar som anses ha positiv skyddande effekt mot välfärdssjukdomar som hjärt-kärl-sjukdomar och olika cancertyper. Dessa effekter anses främst bero på flavonoidernas antioxidativa egenskaper i kroppen. Man kan säga att de fungerar som rostskyddsmedel i kroppen och motverkar skador av så kallade reaktiva syreradikaler. Quercetin har visat sig ha hög antioxidantverkan och lök är en av de viktigaste källorna för quercetin i födan.

Forskningsprojektet som redovisas i denna doktorsavhandling hade som mål att fastställa om det går att påverka halterna av quercetin i gul lök genom att odla, skörda och lagra löken på olika sätt. Det som jämfördes var effekten av mängden kvävegödsel, samt olika kvävegödslingsmedel, löksorter, skördetidpunkter, fälttorkningsmetoder och lagringsförhållanden.

Ju mer solljus lökarna fick i slutet av mognadsfasen, i augusti, desto högre blev quercetinhalterna. Eftersom det var stora variationer i mängd solljus mellan åren, blev årsvariationen i mängden quercetin större än de flesta skillnaderna mellan olika behandlingar inom respektive år. Det fanns sortskillnader, men de var inte konsekventa över åren. Extra mycket kvävegödsel gav varken högre skörd eller högre quercetinhalter. Det gick inte heller att fastställa några skillnader i quercetinhalt beroende på om lökarna gödslats med handelsgödsel eller organisk kvävegödsel.

I Sverige är det vanligt att lökarna lossas från marken i mitten av augusti till mitten av september. Då är blasten fortfarande ganska grön. De lämnas sedan att fälttorka i strängar på fältet i knappt två veckor. Först därefter tas lökarna från fältet och skördas i egentlig mening. Fälttorkning av de lossade lökarna på hösten höjde halterna av quercetin, särskilt vid år med lite solljus. Tidig lossning har tidigare visat sig ge lökar med lägre benägenhet att gro när de tas ut i rumstemperatur efter långtidslagring vid låg temperatur. Att lossa tidigt påverkade inte halterna av quercetin i de lagrade lökarna. Dessutom var halterna av quercetin i lökarna mycket stabila under kyllagringen.

De viktigaste slutsatserna är att lökodling kan ske med minimerad mängd kvävegödsel, vilket minskar risken för skadlig utlakning till åar, sjöar och hav, utan att mängden nyttiga antioxidanter minskar. Det finns heller ingen anledning att inte lossa lökar avsedda för långtidslagring tidigt, vilket minskar skörden men ökar lagringsdugligheten, eftersom quercetinhalterna var lika höga som i senare lossade lökar efter långtidslagring vid konstant låg temperatur.

Contents

Appendix

Paper I-IV

The present thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Mogren, L.M., Olsson, M.E. & Gertsson, U.E. 2006. Effects of cultivar, lifting time and nitrogen fertiliser level on quercetin content in onion (*Allium cepa* L.) at lifting. *Journal of the Science of Food and Agriculture*. In press.

II. Mogren, L.M., Olsson, M.E. & Gertsson, U.E. 2006. Quercetin content in field-cured onions (*Allium cepa* L.): Effects of cultivar, lifting time, and nitrogen fertilizer level. *Journal of Agricultural and Food Chemistry* 54, 6185-6191.

III. Mogren, L.M., Olsson, M.E. & Gertsson, U.E. 2006. Quercetin content in stored onions (*Allium cepa* L.): Effects of storage conditions, cultivar, lifting time and nitrogen fertiliser level. Submitted.

IV. Mogren, L.M., Olsson, M.E., Caspersen, S. & Gertsson, U.E. 2006. Quercetin content in organically fertilized onions (*Allium cepa* L.): Effects of mycorrhizal inoculation and fertilizer placement. Manuscript.

Objectives

The main objective of this doctoral thesis was to investigate the effect of different factors during production and storage on the flavonol content of yellow onion (*Allium cepa* L.). The main flavonols in onion are quercetin glucosides, which today are regarded as positive for human health mainly due to their antioxidative abilities. Our objective was not to maximise the levels, but to investigate the factors altering the concentrations.

The specific questions addressed were:

How does the flavonol content in onion vary:

- due to the level of N fertiliser applied?
- in different cultivars?
- in fresh onions at lifting?
- after field curing?
- during and after storage?
- due to inorganic or organic fertilisers?

Introduction

Edible alliums have been cultivated since ancient times. Depictions of onion bulbs dating more than 5000 years back in time have been found in Egypt. The cultivated forms probably evolved from wild relatives in the mountainous regions of central Asia (Brewster, 1994). The genus *Allium* comprises more than 500 species and is found in the following botanical classification context:

Class: Monocotyledons
Superorder: Liliiflorae
Order: Asparagales
Family: Alliaceae
Genus: *Allium*

The common onion, *Allium cepa* L., does not exist as a wild species today. Within the species, there is great diversity in adaptation to photoperiod and temperature, in bulb storage life, dry matter content, flavour and skin colour. Modern cultivars are F1 hybrids, which have a narrow genetic base (Brewster, 1994). In northern Europe, long-day types derived from the old Dutch Rijnsburger type that require more than 16 h day length for bulbing are grown. They are sown in spring and bulb in the middle of the summer. As the bulb ripens, the outermost one to three sheaths develop into thin, dry protective skins. These dry skins enclose approximately four fleshy, swollen sheaths from bladed leaves. These in turn enclose approximately four swollen bladeless swollen bulb scales, and at the centre are found approximately five leaf initials with blades that will develop into sprout leaves during storage (Brewster, 1994).

The relative growth rate of onion seedlings is low compared with that of other crop species. The low vertically orientated leaf canopy is weakly competitive and onion crops are easily suppressed by overgrowing weeds. The onion root system is shallow and the root density in the soil profile low. Competition and stress accelerate bulbing, which indicates a stress-avoidance growth strategy, and after bulbing the onion crop is very stress-tolerant (Brewster, 1990).

Onions – a global benefit to health

World onion production (including all forms of edible alliums) is around 44 million tonnes, making it the second most important horticultural crop after tomatoes. Due to their storage characteristics and durability for shipping, onions have always been traded more widely than most vegetables and this holds true even today. Onions are rich in two chemical groups that are believed to have beneficial effects on human health, flavonoids and S-alk(en)yl cysteine sulphoxides (ACSOs) (Griffiths *et al.,* 2002).

Two main flavonoid subgroups are found in onion; flavonols such as quercetin, which are responsible for the yellow and brown skins of many varieties, and anthocyanins, which impart the red/purple colour to some varieties. The ACSOs are the flavour precursors, which when cleaved by the enzyme alliinase generate the characteristic odour and taste of onion. Onion compounds have been reported to have a range of health benefits, including anticarcinogenic properties, antiplatelet activity, antithrombotic activity, antiasthmatic and antibiotic effects (Griffiths *et al.,* 2002). Due to their importance in the diet globally, small changes in the content of health-promoting substances in onion could have significant effects on the world health situation.

Nitrate leaching

Nitrogen is absorbed by plants in the form of either ammonium (NH_4^+) or nitrate (NO_3^-) depending on species, cultivar, age and soil conditions. Ammonium is adsorbed on clay minerals and therefore is less mobile but nitrate is highly mobile in the soil. Excess nitrates leach down in the soil profile with irrigation or rain water, especially on sandy soils, or are carried away by runoff water. In developing countries, there is an alarming trend for groundwater pollution by nitrates (Prakasa Rao & Puttanna, 2000).

In Sweden, one of the major problems with nitrogen is the risk of leaching and thereby unwanted fertilisation of surface waters in the close vicinity of crop fields and subsequently of lakes and seas. Onions have shallow and stubby root systems and are often grown on sandy soils, which makes onion growing a potential risk for unwanted nitrogen leaching. Nitrate leaching can be minimised by substituting part of the inorganic fertilisers with organic fertilisers and matching the plant needs by split fertiliser applications (Prakasa Rao & Puttanna, 2000).

Free radicals, ROS and antioxidants

A free radical is any species capable of independent existence that contain one or more unpaired electrons, and thus is highly reactive (Halliwell & Gutteridge, 1989). The free radicals have been found to be involved in ageing, cardiovascular diseases and some forms of cancer. The radicals of most concern in biological systems are derived from oxygen, but recently the role of nitrogen reactive species has also attracted increasing attention (de Lima *et al.,* 2004). Mitochondria produce most of the energy in the cell, and correspondingly consume the bulk of intracellular oxygen. The higher the metabolic rate, the greater the production of reactive oxygen species (ROS). ROS encompass a variety of diverse chemical species including superoxide anions, hydroxyl radicals and hydrogen peroxide. The balance between ROS production and antioxidant defences determines the degree of oxidative stress. Environmental stimuli such as UV radiation can perturb the normal redox balance and shift cells into a state of oxidative stress. Consequences of this stress include modification to cellular proteins, lipids and DNA (Finkel, 2000).

An antioxidant is a substance that protects the cell from the harmful effects of free radicals and can be defined as 'a substance that, when present at low concentrations compared with those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell & Gutteridge, 1995). Onions (*A. cepa*) have higher radical scavenging activities than garlic (*A. sativum*), with red onion being more active than yellow onion (Nuutila *et al.,* 2003). In onions, both total phenolic and flavonoid content are strongly correlated with total antioxidant activity (Yang *et al.,* 2004). Antioxidant activity data for oxidation products of quercetin suggest that they also act as antioxidants and that in some cases they are even more active than quercetin (Ramos *et al.,* 2006).

Phenolics

Plant polyphenols are natural antioxidants and are candidates in exerting the protective effects of vegetables and fruit against some forms of cancer and cardiovascular diseases (Arts & Hollman, 2005). There is substantial genetic variation in the content of phenolics among fruit and vegetable cultivars. The levels of phenolic antioxidants appear to be sensitive to environmental conditions both before and after harvest (Kalt, 2005). Polyphenols are probably the most abundant antioxidants in the diet. Their total dietary intake could be as high as 1 g day^{-1}, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants (Scalbert, Johnson & Saltmarsh, 2005). Phenolic compounds are present in all plants (Kris-Etherton *et al.,* 2002). Their common feature is a hydroxyl-substituted benzene ring within their chemical structure. Phenolics that possess two ortho-positioned hydroxyl groups are very good antioxidants, though the disadvantage of this is their relative instability towards oxidation during storage or processing of plant foods. Phenolics are mostly synthesised from phenylalanine and the pathway is essentially chloroplastic and known in its entirety (Parr & Bolwell, 2000). A key step in the biosynthetic route is the introduction of one or more hydroxyl groups into the phenyl ring, thus

producing phenols. This means that phenols are derived from a common building block in their carbon skeleton, the phenylpropanoid unit C_6-C_3 that builds up the large variety of plant phenols, *e.g*. cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins (Hollman, 2001). These compounds may be synthesised in different cell types and during different stages of plant development and thus the regulation of phenolic accumulation in plants is very complex. The biological functions of phenolics in plants include structural polymers, UV-screens, antioxidants, attractants such as colour and smell, defence responses and signal compounds within the plant (Parr & Bolwell, 2000).

Flavonoids

One of the most abundant groups of polyphenolic compounds in plants is the flavonoids. Over 4000 different flavonoids occurring in plants have been described (Hollman, 2001). Among the more ubiquitous flavonoids, over 50 different glycosides have been identified (Crozier *et al.,* 1997) but the most common sugar is D-glucose (Meltzer & Malterud, 1997). Flavonoid formation depends on light and therefore the flavonoids are mainly concentrated in the outer tissues. According to Herrmann (1976), the concentration in free standing leaves exceeds that in other parts of the same plant considerably, except in onions. The flavonoids are known to control the level of auxins, one of the regulators of plant growth and differentiation. In food plants they add colour, texture and taste. Many of the biological roles played by the flavonoids are associated with their capacity to bind metals, *e.g.* iron and copper, which enhances the antioxidant and UV-screening actions of flavonoids (Formica & Regelson, 1995). The flavonoid content in plants is strongly influenced by extrinsic factors such as variations in plant type and growth, season, climate, degree of ripeness, food preparation and processing (Aherne & O´Brien, 2002).

Quercetin

There are seven major flavonoid compounds in onions. Quercetin aglycone, *i.e.* with no sugar molecule attached (Fig 1.), quercetin monoglucoside, quercetin diglucoside, isorhamnetin (a methyl ether of quercetin), isorhamnetin monoglucoside, rutin and kaempferol (Park & Lee, 1996). Quercetin diglucoside and monoglucoside account for up to 93% of the total flavonol content in onion (Lombard, Geoffriau & Peffley, 2002).

Onion is one of the most quercetin-rich crops (300 mg kg^{-1} fw) compared with *e.g.* kale (100 mg kg^{-1} fw), blackcurrants (40 mg kg^{-1} fw), and broccoli, black grapes and apple (30 mg kg^{-1} fw) (Hollman & Arts, 2000).

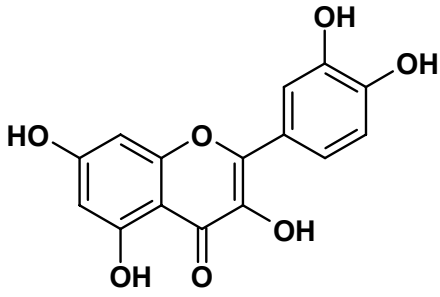

Figure 1. The molecular structure of quercetin.

The growing site of onions is a major environmental factor determining quercetin concentration, since soil and weather have a major influence on this parameter (Patil, Pike & Hamilton, 1995).

The role of UV-B light for the biosynthesis of quercetin has been studied in many crops. In leaves of barley (*Hordeum vulgare* L.), UV-B has been shown to markedly increase flavonoid accumulation in both the lower epidermis and underlying tissue (mesophyll). UV-A has a lesser effect, but is still significantly effective in increasing flavonoid accumulation (Liu, Gitz & McClure, 1995). In rape (*Brassica napus*), UV-B supplementation resulted in a marked, specific increase in the amount of quercetin glucosides by 70-150% (Olsson *et al.,* 1998). Quercetin content in onion can be doubled after harvest using UV light lamps (Higashio *et al.,* 2005). In onion, total quercetin content in the dry outer skins is significantly higher than that in the edible parts and a decrease is found from outer to inner parts on both a fresh and dry weight basis. Quercetin in the free form as aglycone is almost absent in the edible part but is bound with glucose molecules into quercetin glucosides and the distribution of quercetin and its glucosides is influenced by the accessibility of light (Patil & Pike, 1995). The level of quercetin is high in the dry outer skins. However the levels of quercetin glucosides in the dry outer skins are less than 10% of the levels in fleshy and partly dried scales. The probable mechanism is that quercetin is formed by deglucosidation of quercetin glucosides on the border between drying and dried brown areas on individual scales (Takahama & Hirota, 2000). Approximately 90% of the total quercetin of each scale is confined to the epidermal tissue, and the rest in the storage tissue. The total content of quercetin is higher in the upper part of an onion, compared with the lower part (root end) (Trammell & Peterson, 1976).

Colour genetics in onion

In onions belonging to *Allium cepa*, a variety of bulb colours exists, ranging from white, yellow, and brown to red with intermediate shades. The basic colour factor is likely to be a regulatory gene controlling chalcone synthase gene transcription. In white onions quercetin is under the detection level, suggesting that all or some enzymes involved in the early steps of the flavonoid biosynthesis pathway may be poorly functional in white onions (Kim, Yoo & Pike, 2005). Golden coloured onions contains significantly reduced amounts of quercetin compared with yellow

cultivars. This is probably an effect of inactivation of chalcone isomerase which results in a block in the flavonoid biosynthesis pathway and accumulation of chalcone derivatives, including a yellow pigment which might be responsible for the gold colour in onions. The kinds of specific pigments that are responsible for gold colours have not yet been identified, but they might be a good resource for health-promoting flavonoids in future breeding programmes (Kim *et al.,* 2004).

Health aspects of flavonoids, especially quercetin

For about one hundred years, flavonoids have been known as plant pigments. In the 1930s they were called vitamin P or bioflavonoids. Twenty years later it was agreed that the flavonoids had physiological effects, but only on capillary resistance. In the 1970s the flavonoids were suspected of having carcinogenic and mutagenic effects, while in the last twenty years considerable research has been devoted to their activity as antioxidants and to their anticarcinogenic and antimutagenic properties (Meltzer & Malterud, 1997). There is consistent evidence that quercetin may reduce the risk of lung cancer (Neuhouser, 2004) and quercetin exerts a potent inhibitory effect on *in vitro* bone resorption (Wattel *et al.,* 2003). It has also been discovered recently that quercetin can protect the human oral cavity from damage induced by reactive nitrogen species and that the protective function of quercetin may be significant when the antioxidant capacity of the saliva is decreased by periodontal diseases (Takahama, Hirota & Oniki, 2006).

Coronary heart disease is the leading cause of mortality in industrialised countries and is rapidly becoming a primary cause of death worldwide (Hu & Willett, 2002). Quercetin intake was found to be inversely associated with mortality from coronary heart disease in Dutch elderly men in the so-called 'Zutphen Elderly Study'. The mean flavonoid intake in the study was found to be 26 mg day^{-1} (Hertog *et al.,* 1993). This is comparable to a Danish study where the intake was calculated to be 23 mg day^{-1} (Justesen *et al.,* 2000). The Dutch mean daily intake of onions was 9.4 g, which represented 13% of the total flavonoid intake, while quercetin from different food sources made up 63% of the total flavonoid intake in the diet (Hertog *et al.,* 1993). In a more recent American study, the daily intake of onions was estimated to be almost three times higher, 23.5 g day^{-1} (Chun *et al.,* 2005). In another study it was estimated that quercetin contributed approximately 70% of the total flavonoid intake on a daily basis and that onions and tea were the main sources (de Vries *et al.,* 1997).

Bioavailability of quercetin

At present, flavonoids can be considered to be one of the most important groups of bioactive food components (Rietjens *et al.,* 2002). Bioavailability differs greatly from one polyphenol to another, and therefore the most abundant polyphenols in our diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach *et al.,* 2005). The absorption of quercetin forms from tea has been reported to be half that from onions (de Vries *et al.,* 1998) and the absorption rate from apples one third that from onions (Hollman *et al.,*

1997). The bioavailability seems to be the same for both quercetin monoglucoside and diglucoside (Olthof *et al.,* 2000) and dietary sources containing high amounts of glucose-bound flavonoids are more likely to have potential health effects than foods with other flavonoid glycosides (Rasmussen & Breinholt, 2003).

The losses of onion flavonoids caused by gastric acid and intestinal juices seem to be low (Shon *et al.,* 2004). It was long believed that flavonoid glycosides were not absorbed but hydrolysed to their aglycones by bacterial enzymes in the intestine (Griffiths & Barrow, 1972; Bokkenheuser, Shackleton & Winter, 1987), but later it was proposed that the flavonoid glycosides are in fact absorbed (Hollman & Katan, 1999). The issue of in what form flavonoids are absorbed is still unresolved. The benefits of flavonoids as chemopreventive dietary agents are still only 'potential' and it is largely unknown whether they can reach their multiple intended sites of action, particularly in humans. Although the bioavailability of the aglycones formed after hydrolysis along the aerodigestive tract is considered to be low, conjugates formed during the metabolism of flavonoids, such as sulphate and glucuronic acid conjugates, have been found in relatively high concentrations. The biological importance of these metabolites and of degradation products formed during biological degradation of the flavonoid backbone remains to be determined (Walle, 2004).

If it holds true that quercetin monoglucosides are absorbed in the small intestine but that the diglucosides can only be absorbed after cleavage of the sugar molecules by microflora in the colon, it could be interesting to use glucosyltransferase enzymes from onions to increase the bioavailability of quercetin by genetic engineering of other food crops or to manufacture bioavailable flavonoid glucosides through biofermentation (Kramer *et al.,* 2003; Willits *et al.,* 2004).

Supplements and nutraceuticals

A primary mechanism of cell injury by oxidative damage is liberation of metal ions from metalloproteins. The metal ions are catalysts of free radical damage, especially in the reduced state. This means that addition of a powerful antioxidant after oxidative damage has started could act as a pro-oxidant and promote damage. The more powerful the antioxidant is as a reducing agent, the more problems it might cause. Antioxidants can also inhibit the oxidant-triggered signalling mechanisms that the cell uses to adapt to a free radical insult (Halliwell, 2000). Quercetin could act both pro- and antioxidatively depending on the redox state of its biological environment (Formica & Regelson, 1995) meaning that at high concentrations or under certain conditions it may exert toxic pro-oxidant activity (Rietjens *et al.,* 2002). The use of antioxidant supplements that are commonly recommended in gram rather than milligram doses could result in potentially toxic levels. For example, recommended doses of quercetin supplements range between 500 and 1000 mg day^{-1}, which is 10-20 times what can be consumed in a typical vegetarian diet (Skibola & Smith, 2000). On the Internet, it is common to find companies offering capsules containing 300 mg quercetin with a suggested use of

1-6 capsules per day, which would result in intakes over 100 times higher than the common intake in a Western diet (Mennen *et al.,* 2005).

Nutraceuticals is a term used for purified or concentrated food components in a pharmaceutical formulation with a presumed health effect. Lack of toxicological risk with this kind of compounds should not be taken for granted, so the motto 'the more the better' should not be used in this case because every compound is toxic provided the dose is sufficiently high (Bast & Haenen, 2002). However, the acute LD_{50} for flavonoids in animal experiments is above 1 g kg^{-1} body weight, so as long as the diet is the sole source of flavonoids, the risk of consuming toxic levels seems small (Meltzer & Malterud, 1997).

Fruit and vegetables

An intake of 400-600 g day^{-1} of fruit and vegetables is associated with reduced incidence of many common forms of cancer (Heber & Bowerman, 2001) and reduced risk of cardiovascular disease (Hung *et al.,* 2004). The roles of fruit and vegetables in disease prevention have been attributed in part to the antioxidant properties of their constituent polyphenols (Rice-Evans & Miller, 1995; Rice-Evans, Miller & Paganga, 1997). Phenolics occur at high levels in plant material and given sufficient bioavailability, it seems likely that these compounds will play a major role in the antioxidant potential of foodstuffs (Parr & Bolwell, 2000). Vegetables have an antioxidant quality comparable with that of pure flavonols (Vinson *et al.,* 1998).

'Let your food be your medicine and your medicine be your food', Hippocrates stated in 400 BC. Until more evidence is obtained, the best advice is to consume a greater quantity and variety of naturally occurring antioxidants in the form of fruit and vegetables rather than taking high doses of a few compounds (Lister, 1999). So, intake of natural antioxidants through a balanced diet containing sufficient fruits and vegetables could be the most effective means of protecting the body against various oxidative stressors (Cao, Sofic & Prior, 1996).

Processing - effects on quercetin

The great loss of flavonoids occurs during the pre-processing steps, when the onion is peeled, trimmed and chopped. This is an effect of the fact that the outer flavonoid-rich onion layers are discarded (Ewald *et al.,* 1999). For example after conventional peeling, the edible portion of red onions contains 79% of the total content of quercetin monoglucoside but only 27% of the anthocyanins, due to the flavonoid distribution in the different layers of the bulbs (Gennaro *et al.,* 2002). Further chopping into smaller pieces does not affect the content of either quercetin diglucoside or monoglucoside (Makris & Rossiter, 2001). Neither boiling nor frying results in interconversion of quercetin conjugates or production of free quercetin, although a 25% loss overall by leaching to the boiling water and thermal degradation while frying has been recorded (Price, Bacon & Rhodes, 1997). This is in line with another study, where overall flavonol losses of 21%

during 60 minutes of boiling were found (Crozier *et al.,* 1997). Further warm-holding for up to 2 h after cooking did not influence the flavonoid content in another investigation (Ewald *et al.,* 1999). Quercetin monoglucoside has higher thermostability compared with quercetin diglucoside due to the absence of the hydroxyl group at the C-3 position. Although there is a release of quercetin glucosides to the cooking water and some oxidation occurs, onion bulbs are still an important source of flavonols even after cooking by boiling if not only the scales but also the broth is eaten, by making a soup for example (Hirota, Shimoda & Takahama, 1998). The general conclusion is that onions in ready-made dishes and home-cooked food may be good dietary sources of flavonoids (Ewald *et al.,* 1999).

Compared with the inner edible parts, the dry outer skins of onions are very rich in antioxidant compounds (Suh *et al.,* 1999). To use the full potential of the onion, extracts of the dry outer scales could be used as food ingredients (Ly *et al.,* 2005) and waste onions can be pressed and the juice fermented to vinegar (Horiuchi *et al.,* 2004).

Cultivation method, curing and storage

The role of nitrogen fertiliser level on onion quercetin content is almost unknown, but there are some indications that nitrogen stress could be correlated with flavonol synthesis in onions (Patil, Pike & Hamilton, 1995). Onion cultivar differences in quercetin content are significant (Patil, Pike & Yoo, 1995; Marotti & Piccaglia, 2002). Onions left in field to cure after harvest are known to accumulate more flavonols, but the harvest date has been reported to have almost no effect at all (Patil, Pike & Hamilton, 1995). Grinder-Pedersen *et al*. (2003) found differences in quercetin content between conventionally and organically grown onions, but different cultivars were studied in the two different cultivation systems, so the possibility could not be excluded that the difference were due to cultivar differences. An inconsistent pattern of quercetin content has been reported for onions in different storage conditions (Patil, Pike & Yoo, 1995), but generally quercetin content and composition are reported to be stable during storage (Price, Bacon & Rhodes, 1997).

Methodological aspects

This section gives an overview of Papers I-IV. For specific details, see the respective paper.

Field trials on inorganically fertilised onions

Field experiment design

In all years, the different treatments were randomly placed within each block. In 2002 five blocks were used, and in 2004 and 2005 four blocks were used.

Cultivars

In 2002 two onion cultivars were used: cv. Barito F1, which is described by the seed company as an early harvest cultivar not suitable for long-term storage, and cv. Summit F1, which is described as a late harvest cultivar suitable for long-term storage. In 2003, cv. Barito onions from a commercial grower were used for experiments. In 2004 and 2005 cv. Hyskin F1, described as a long-term storage cultivar, was added to cv. Barito and cv. Summit in the field experiment, thereby making it possible to draw comparisons with organically fertilised onions (Paper IV).

Plant stand density

In this investigation, 50 cm spacing between rows were used all years and in all field trials. This row spacing is used by approximately 50% of Swedish onion growers (Nilsson, 2006), while the remaining 50% use a 25-35 cm spacing between rows. The mean value for Swedish commercial growers is 30 onions per row metre, and this was achieved in all years in the field experiments.

Water regimes

Water was applied to the field experiments when required, based on evaporation and rainfall data, until the middle of July each year. About 10% of the onion growers on the island of Öland do not have irrigation capacity on all fields (Nilsson, 2006) and this lack may lead to poor and uneven seed germination and seedling growth. Additional water could also allow the onion crop to utilise mineralised nutrients in a more efficient way.

Sowing

Commercial growers try to put the seeds in the soil as early as possible in the season, in the end of March to the middle of April. Thanks to the sandy soil at the experimental station in Torslunda, where most of the trials were performed, there were no problems following the commercial standards for sowing time. In the inorganically fertilised onion field experiments, seeds were used in all years.

Weed and pest management

The common procedure in commercial onion growing is to apply both soil and foliar herbicides before onion seedling emergence and 1-2 additional applications of foliar herbicides later. In the field experiments this strategy was followed, mechanical row cultivation was used for the later stages and manual hand-weeding when needed to keep the weed population at a low level. The only pest chemical used (Acrobat) was to combat onion downy mildew (*Peronospora destructor*) based on a climate data warning model used by commercial growers.

Fertilisers

Every grower has his own fertiliser strategy based on previous years and soil type. However, as a rule of thumb the common amounts of NPK given are 120:60:300 and of this, about 60:60:200 are applied at sowing and the rest of the NK not later than the middle of June (Nilsson, 2006). Approximately these levels of PK were given (Paper I) and the same amounts were applied to all experimental plots. Two levels of nitrogen (N) fertiliser were used in the fertiliser field experiments, one where the application rate was minimised based on soil analyses (Gertsson & Björklund, 2002) and one where 80 kg ha^{-1} more was added at the second fertiliser application time in the beginning of June (Paper I).

Lifting times

Early cultivars are normally lifted in the first part of August and late cultivars in the beginning of September, when about 50% to 80% of the onions on the field have fallen leaves (Nilsson, 2006). Early lifting generally improves storability of onions (Fustos, Pankotai Gilinger & Ombodi, 1994). Lifting at 50% fallen leaves postpones the onset of sprouting by 7-10 days compared with 80% for onions stored in cool storage for 6 to 9 months (Grevsen & Sorensen, 2004). Outer dry scale quality is one of the determinant factors for the storability of onions and it plays a significant role in maintaining dormancy (Fustos, 1997). Early lifted onions have a higher proportion of bulbs with three or more intact skins, which is commercially desirable (Wright & Grant, 1997). In the lifting time experiments with inorganically fertilised onions (Paper I, II, III), two different lifting times (50% and 80% fallen leaves) were used every year. In an extended lifting time experiment in 2005, very early lifting in July (0%) and very late lifting (100%) were added to the experiment (Fig. 2.)

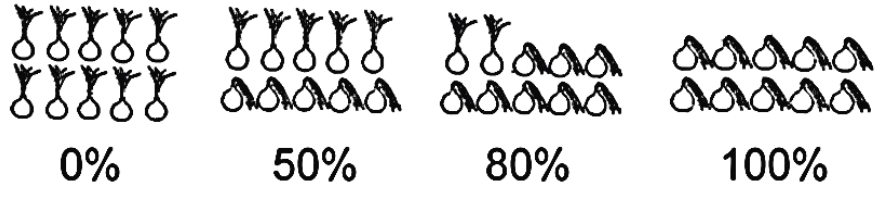

Figure 2. Illustration of the four different lifting times (percentage fallen leaves) in the extended lifting time experiment in 2005. In all the other years, only onions from the two middle lifting times, 50% and 80% fallen leaves, were analysed.

Curing

Common commercial practice is to leave the lifted onions on the field for approximately 10 days for curing (Fig. 3), as longer periods lead to increased risk of quality disorders (Nilsson, 2006). Field curing and removing the foliage after curing, not before, is the best way to ensure good postharvest onion quality and successful storage (Wright, Grant & Triggs, 2001). There were no problems with rainfall during the field curing days in any of the years and the onions were left to cure in field for 10-14 days. After that time, until the start of the storage period, the onions were stored in wooden bins (Papers II, III).

Figure 3. Field curing of onions.

Storage

The most common way for commercial growers to store onions is in loose heaps with forced air ventilation. For long-term storage, temperatures between 0-5°C and a relative humidity between 65-70% is common (Nilsson, 2006). In 2002 and 2004, comparative studies were performed on storage in a climate chamber at constant temperature and storage in the grower's central storage at more varying temperatures (Paper III).

Soil water conditions

Total onion yield is reduced by soil water stress imposed at any growth stage (Pelter *et al.,* 2004). In 2004, individual measurements of the soil water content was performed using a TDR measuring technique, a Field scout TDR 300 (Spectrum Technologies). By using 20 cm rods, the percent volumetric water content in the soil volume where the main onion root volume is situated could be estimated. In 2005, monitoring of soil water content was performed at regular intervals. The two inorganically fertilised onion experiment soil beds were placed a few metres apart in a south–north direction in the field and they were divided

into 12 experimental plots each (3 cultivars, 2 lifting times and 2 replicates). The field had no visible differences in texture or water-holding ability. The distance between the southern and northern end of the field was less than 100 metres.

Field trials on organically fertilised onions

The field trials on organically fertilised onions were performed in the same way as described above for inorganically fertilised onions with the following exceptions:

Cultivar

In the organically fertilised field trials, cv. Hyskin was used exclusively.

Seeds and transplants

The ratio of area cropped with seed onions compared with sets is about 100:1 in southern Sweden. Seed onions are better suited for long-term storage, but sets are ready to harvest earlier in the season (Nilsson, 2006). Transplants are an expensive way to establish an onion culture and are almost solely used in organic onion growing due to their better weed competition abilities. In both 2004 and 2005, seeds were used in the organically fertilised onion field trials in the same way as in the inorganically fertilised field trials. In 2005, a field trial of organically fertilised transplanted onions was added.

Fertiliser method and mycorrhiza inoculation

The fertiliser Biofer (6:3:12), an organic commercial pellet fertilizer product that is approved for usage in certified organic growing, was used exclusively (Paper IV). In 2004, apart from being broadcast and harrowed down into the soil just as the NPK in the inorganically fertilised field trial, Biofer was also broadcast followed by rotary cultivation and in another treatment placed in the rows. One treatment was given no fertiliser at all. One treatment got placed fertiliser combined with inoculation of mycorrhiza (Paper IV). In 2005, the treatments were reduced to broadcast fertiliser application followed by harrowing, placed fertiliser and no fertiliser at all. In 2005, the field trial with transplants was divided into three treatments, one that was given mycorrhiza inoculum in the form of inoculated *Sorghum* roots, one that received addition of *Sorghum* roots with no inoculum, and one that received no addition to the substrate at all (Paper IV).

Weed and pest management

In the organically fertilised onion trials, no chemical herbicides or pesticides were used. Weeds were controlled by mechanical means and by hand.

Lifting time

The organically fertilised onions, both seed and transplant, were lifted at 80% leaf fall in both years.

Analyses of quercetin

Onions that have been freeze- or vacuum-dried contain more quercetin glucosides, whereas in hot air-dried onions the aglycone dominate (Fu, 2004). This means that there is always a risk that the levels and forms of different compounds may be altered during the drying process. In this project, extractions and analyses were performed exclusively on fresh, raw onion samples to reduce the risk of loss and transformation of quercetin compounds.

Extraction

A common way to analyse onion flavonoids is to use freeze-dried onion powder and extract it in methanol (Hertog, Hollman & Katan, 1992; Crozier *et al.,* 1997; Justesen, Knuthsen & Leth, 1998; Sakakibara *et al.,* 2003) or ethanol (Patil, Pike & Hamilton, 1995; Lombard *et al.,* 2005). When extracting fresh onion samples methanol has been used (Bilyk, Cooper & Sapers, 1984; Ferreres, Gill & Tomas Barberan, 1996; Bonaccorsi *et al.,* 2005) as well as ethanol (Trammell & Peterson, 1976), while methanol has sometimes been used for extraction of raw, frozen onions (Sellappan & Akoh, 2002). Quercetin can also be extracted from powdered outer dry onion scales (Horbowicz, 2002) but this was not done in this project. In the initial phase of this project, comparisons of different extraction solvents were performed. Ethanol (166 ± 28 mg quercetin glucosides kg^{-1} fw) resulted in approximately the same extraction efficiency as methanol (153 ± 33 mg quercetin glucosides kg^{-1} fw) so ethanol was chosen because it is easier to handle at a research station with sparse laboratory equipment. Comparisons were also made between extracts from onion pieces that had been:

- chopped
- chopped and crushed in a mortar
- chopped and homogenised in a blender.

The extractions of homogenised onions had the best repeatability. Comparisons of three different extraction conditions were performed: A fast method using an ultrasonic bath at room temperature where the extracts were put for one hour; a short-term method using a shaker where the extracts were left for 24 hours at 4°C; and finally a long-term method where the extracts were stored in a freezer at -20°C for at least 2 weeks. The long-term method had best extraction yield and best repeatability and seemed easy to perform at the research station. Based on these results, the extraction method with fresh homogenised onion tissue in ethanol at -20 °C for at least 2 weeks was chosen throughout the whole project. Hydrolysis never yields the exact number of glycosides involved and requires time-consuming sample treatment prior to analysis (Escarpa, Perez-Cabrera & Gonzalez, 2000). Therefore only analyses of raw onion extracts without previous hydrolysis were performed.

Sample preparation

To get a representative sample, 10 onions from each field trial plot were mixed together to a homogeneous onion pulp before each extraction. To fit the mixer, the onion tissue volume had to be reduced. The sides of onions that had received the

most sunlight exposure during growth and field curing could be thought to have higher content of flavonoids, so longitudinally opposed parts were used (Fig. 4).

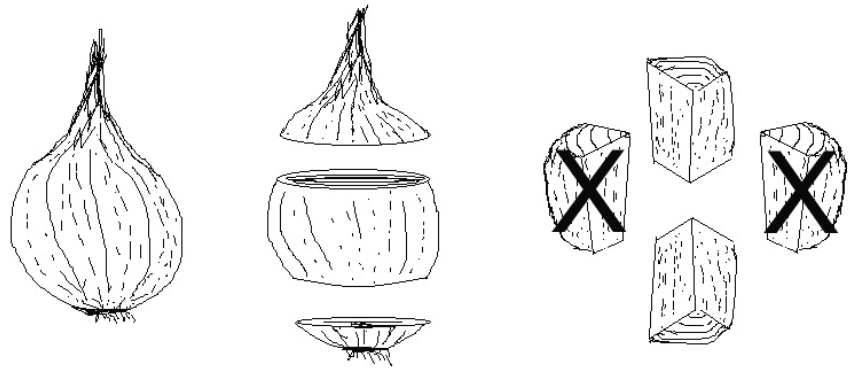

Figure 4. Each onion was peeled, the top and bottom were cut off and the bulb was cut into four wedge-shaped pieces. Two opposite wedges were used for mixing and extraction while the other two (marked X) were discarded.

Statistics

The different results from the analyses described in Papers I-IV were statistically analysed in Minitab Release 14.1 (Minitab Inc.). When suitable, results were subjected to ANOVA. If nothing else is stated in the text, no interaction between the analysed factors was significant. Significance was determined at $p<0.05$ and the results reported were significantly different at this level unless otherwise stated.

Results and discussion

Annual variation

Global radiation (amount of sunlight) in August seemed to be one of the major determinants of mean annual quercetin glucoside content in the onions (Papers I, II). In 2005 there was high global radiation in June and July, but the least amount of global radiation of all four years in August, resulting in the lowest levels of quercetin glucosides of all years both at lifting and after field curing (Fig. 5).

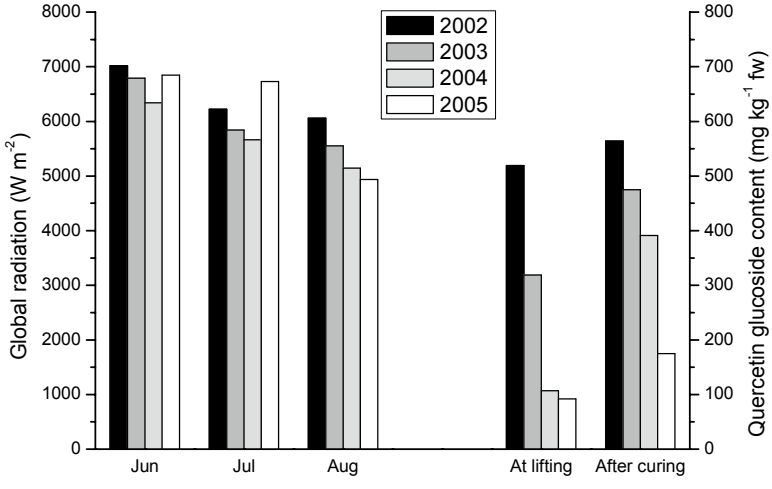

Figure 5. Total global radiation in June, July and August in the different years and total quercetin glucoside content at lifting and after curing each year.

After the increase in quercetin glucoside content during field curing, the levels were stable throughout the whole storage period (Fig. 6A). It was known from an earlier study that onions left in the field after harvest accumulate more flavonols (Patil, Pike & Hamilton, 1995). However in the present study case the increase was dramatic, between 100-300%, during the 10-14 days of curing (Fig. 6A, Table 1). The only exception was 2002, which was the year with the highest global radiation in August and therefore high levels of quercetin glucosides in the onions already at lifting (Fig. 5).

Table 1. *Changes in quercetin glucoside content from lifting to start of storage in the inorganically fertilised onions, 2002-2005*

		Content at lifting (mg kg^{-1}fw)			Content after curing (mg kg^{-1}fw)			Content at start of storage (mg kg^{-1}fw)			Increase during curing (%)			Increase from lifting to start of storage (%)		
Year	Treatment	Q_{DG}	Q_{MG}	Total	Q_{DG}	Q_{MG}	Total	Q_{DG}	Q_{MG}	Total	Q_{DG}	Q_{MG}	Total	Q_{DG}	Q_{MG}	Total
2002	Low nitrogen	174 a	351 a	525 a	177 a	384 a	561 a	181 a	313 a	494 a	2	9	7	4	-11	-6
	High nitrogen	165 a	346 a	511 a	180 a	387 a	567 a	178 a	317 a	496 a	9	12	11	8	-8	-3
2004	Low nitrogen	38 a	80 a	118 a	119 a	278 a	397 a	175 a	318 a	493 a	213	248	236	361	298	318
	High nitrogen	33 a	62 b	95 b	119 a	265 a	384 a	168 a	317 a	485 a	261	327	304	409	411	411
2002	Early lifting	171 a	342 a	513 a	180 a	394 a	574 a	179 a	312 a	491 a	5	15	12	5	-9	-4
	Late lifting	169 a	356 a	525 a	177 a	377 a	554 a	181 a	318 a	499 a	5	6	6	7	-11	-5
2003	Early lifting	98 a	196 b	294 b	147 a	344 a	491 a				50	76	67			
	Late lifting	98 a	245 a	343 a	116 b	344 a	460 a				18	40	34			
2004	Early lifting	34 a	69 a	103 a	114 a	270 a	384 a	166 a	311 a	477 a	235	291	273	388	351	363
	Late lifting	37 a	73 a	110 a	124 a	273 a	397 a	176 a	324 a	500 a	235	274	261	376	344	355
2005	Early lifting	25 b	50 b	75 b	50 b	106 b	156 b	88 a	158 a	246 a	100	112	108	252	216	228
	Late lifting	35 a	74 a	109 a	62 a	131a	194 a	84 a	155 a	239 a	77	77	78	140	109	119
2002	Barito	163 a	320 b	483 b	183 a	404 a	587 a	184 a	336 a	520 a	12	26	22	13	5	8
	Summit	177a	377 a	554 a	174 a	367 b	540 b	175 a	294 b	469 b	-2	-3	-3	-1	-22	-15
2003	Barito	98	221	319	132	344	475				35	56	49			
2004	Barito	37 a	82 a	119 a	114 b	278 b	392 b	177 ab	337 a	514 a	208	239	229	378	311	332
	Hyskin	36 a	66 a	102 a	142 a	318 a	460 a	180 a	334 a	514 a	294	382	351	400	406	404
	Summit	34 a	66 a	100 a	101 c	219 c	320 c	157 b	281 b	438 b	197	232	220	362	326	338
2005	Barito	28 a	59 a	87 a	53 a	116 ab	169 ab	84 b	152 b	236 b	89	97	94	200	158	171
	Hyskin	33 a	68 a	101 a	63 a	135 a	198 a	96 a	178 a	274 a	91	99	96	191	162	171
	Summit	29 a	59 a	88 a	52 a	106 b	158 b	77 b	139 b	216 b	79	80	80	166	136	145

Content values within columns, treatment groups and years followed by the same letters are not significantly different ($p<0.05$).

Fertiliser treatment data are means of all lifting times and cultivars. Lifting time data are means of all fertiliser treatments and cultivars. Cultivar data are means of all fertiliser treatments and lifting times.

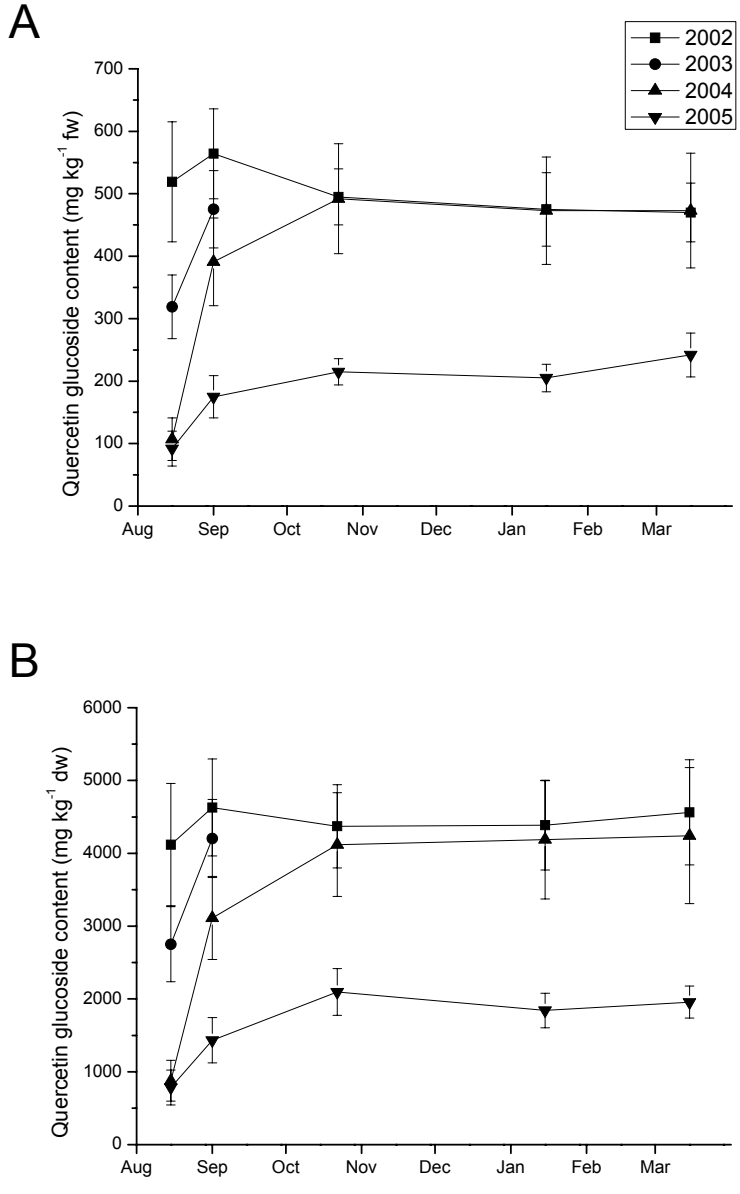

Figure 6. A. Mean total content of quercetin glucosides per kg fresh onions at different stages in all years. *B*. Mean total content of quercetin glucosides per kg dried onions at different stages in all years. Lifting = August, After field curing = September, At start of storage = October, After 3 months of storage = January, After 5 months of storage = March. Error bars denote standard deviation.

Lifting time

Antioxidant capacity of vegetables is generally dependent on both species and harvest time (Ou *et al.,* 2002). However, harvest date has been reported to have almost no effect on quercetin levels in onion (Patil, Pike & Hamilton, 1995).

In the field experiments, the difference between lifting at 50% or 80% fallen leaves was small in most of the years (Table 1). In 2003, the quercetin content at lifting in individual onions with fallen and erect leaves was analysed (Fig. 7). Individual onions with fallen leaves had almost double the quercetin content of individual onions with still erect leaves (281 mg kg^{-1} fw compared with 163 mg kg^{-1} fw). Based on this, one could expect lifting time to be a major determinant of quercetin content in fresh onions and indeed this was confirmed in the extended lifting time experiment in 2005. Quercetin was almost absent in the end of July when all onions had erect leaves (0% fallen leaves, Fig. 2). It was found that the later the lifting time, the higher the quercetin content in the fresh onions at lifting (Fig. 8).

After field curing, the effect of erect or fallen leaves on quercetin content was absent (Paper II). This means that during the 10-14 days when the onions were lying on the field, the increase in quercetin content in onions with erect leaves was much higher. After curing, no difference in quercetin glucoside content between early and late lifting could be found in three out of four years (Table 1).

At start of storage, lifting time had almost no influence on quercetin glucoside content in the onions (Table 1). No differences in quercetin glucoside content between lifting times were found during and after storage (Paper III). However, the rate of sprouting, which is an important parameter of quality for stored onions, was higher for late lifted onions. Early lifting resulted in slightly reduced yields; early lifted 43 ± 6 ton ha^{-1}, late lifted 48 ± 6 ton ha^{-1} (Paper I). In conclusion, lifting time does not significantly influence quercetin content in onions after storage. Late lifting resulted in approximately 10% higher yield in all years and could be recommended if the onions are intended to be consumed within a few months. However, the lower yield for early lifted onions could probably be compensated by better keeping quality if the onions should be stored for long time (Paper III).

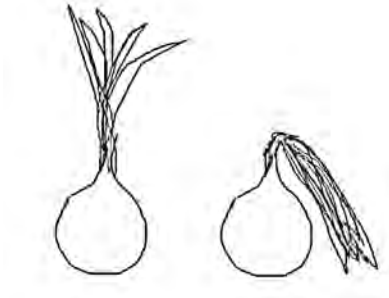

Figure 7. Individual onions with erect and fallen leaves respectively.

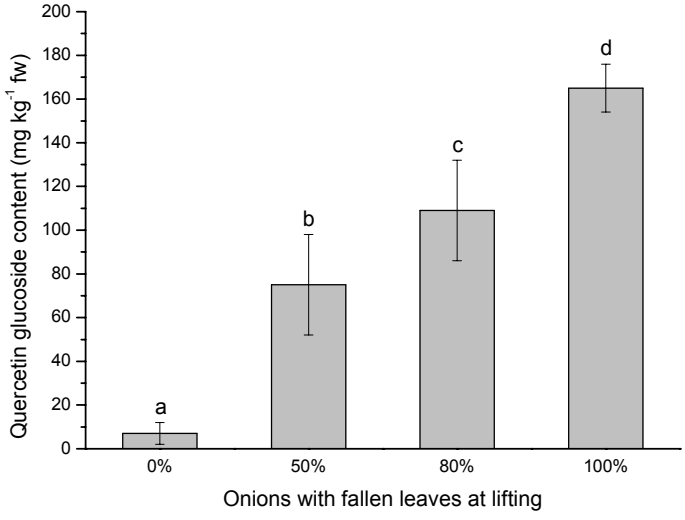

Figure 8. Total quercetin glucoside content at lifting, before curing and drying, in onions lifted at different stages in 2005. Bars with different letters are significantly different at $p<0.05$.

Cultivars

In a study in 1992 of 55 cultivars of yellow onions, the total quercetin content varied from 54-286 mg kg^{-1} fw (Patil, Pike & Yoo, 1995). Furthermore, it has been concluded that onion cultivars show great variability in flavonoid content due mainly to genetic factors (Marotti & Piccaglia, 2002).

There were small, but in some years significant, differences in size and yield between the cultivars (Paper III). In the field experiment, some cultivar differences in quercetin content were found in some of the years, but compared with the annual variations the differences were small (Table 1). Quercetin monoglucoside was the most predominant of the quercetin compounds in all cultivars analysed (Table 1). The monoglucoside has also been reported to be the major compound in other investigations (Herrmann, 1976; Patil, Pike & Yoo, 1995; Marotti & Piccaglia, 2002). However, there are studies in which other cultivars were analysed where the diglucoside content was slightly higher than the monoglucoside content (Bonaccorsi *et al.,* 2005) whereas in another investigation, quercetin diglucoside was found to be the main quercetin component (Price & Rhodes, 1997).

Nitrogen fertiliser level

It is known that for onion plants grown at very low N supply, the harvest date is postponed and yield reduced, but a surplus of nitrogen does not influence the

harvest date or the yield (Sorensen & Grevsen, 2001). In Estonia, nitrogen fertiliser levels over 80 kg N ha^{-1} did not seem to have effect on yield (Poldma & Merivee, 2005). There are some indications that nitrogen stress could be correlated with flavonol synthesis (Patil, Pike & Hamilton, 1995).

In the low nitrogen treatments in the field experiments, based on soil samples, a total of 60-80 kg N ha^{-1} was added during the season (Paper I). The higher level of nitrogen, 80 kg ha^{-1} more, did not affect the yield or the content of quercetin glucosides in the onions after curing any of the years, or at later stages during storage (Table 1). Placing of nitrogen fertiliser in the row instead of broadcast application does not seem to have an effect on yield (Sorensen, 1996). In 2004 comparisons were made of different ways of applying organic fertiliser (Paper IV) and it was confirmed that placement of the nitrogen fertiliser did not affect either yield or quercetin glucoside content. Interestingly, no significant difference in quercetin glucoside content could be found between unfertilised onions and onions that received nitrogen fertilisers (Paper IV). The environmental implications of this finding could be that the nitrogen fertiliser levels can be minimised if given at the appropriate time and in the correct amounts, without negative effect either on yield or quercetin glucoside content.

Onion size

It has been reported that size and bulb weight are not correlated with quercetin concentration and that small bulbs contain the same concentration of quercetin as larger bulbs (Patil, Pike & Hamilton, 1995). This was confirmed by the field trial results that showed minor or no differences in quercetin glucoside content between small-, medium- or large-sized onions (Paper III).

Different scales

Due to the role of UV light for quercetin synthesis, it is not surprising that a gradient in total quercetin content in the edible onion from outer to inner parts has been reported (Bilyk, Cooper & Sapers, 1984; Patil & Pike, 1995; Chu, Chang & Hsu, 2000). A distinct gradient between the outer two edible scales, the two next middle scales, and the rest of inner scales was found when medium sized onions (diameter 55-70 mm) were analysed in the present study (Fig. 9) (Papers I, II).

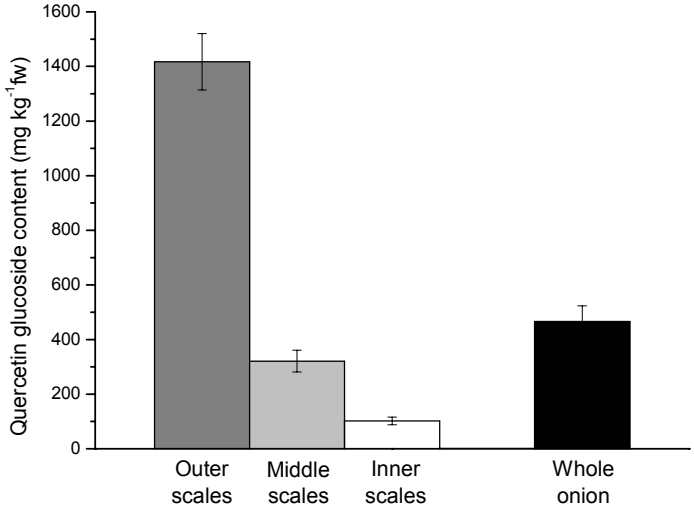

Figure 9. Variation in quercetin glucoside content in different scales of inorganically fertilised medium sized onions in 2003 after field curing. Error bars denote standard deviation.

Storage

An early report stated that quercetin diglucoside in onion is formed during storage and that the content increases continuously (Herrmann, 1976). However, later studies have not found any increasing trend in quercetin diglucoside content at the expense of monoglucoside during storage (Patil, Pike & Yoo, 1995). In some onion cultivars, an initial loss of quercetin monoglucoside has been found during the drying phase, but apart from that only small changes in content and composition have been observed during 6 months of storage (Price, Bacon & Rhodes, 1997). An inconsistent pattern of quercetin content in different storage conditions has been reported, and the conclusion was that it could be due to bulb sprouting, because in controlled atmosphere storage no sprouting and no changes in quercetin content occurred (Patil, Pike & Yoo, 1995). In the present study, the ratio of quercetin diglucoside to quercetin monoglucoside was unchanged during storage and the levels remained stable during five months of cold storage (Fig. 6, Paper III).

Organically fertilised versus inorganically fertilised onions

Different food production methods, conventional or organic, may result in differences in the content of secondary metabolites such as polyphenolic compounds (Grinder-Pedersen *et al.,* 2003). However, differences between cultivars tend to be greater than those found between the two cultivation systems (Brandt & Mølgaard, 2001). Differences in polyphenol content between resistant

and susceptible cultivars have been found, suggesting that these compounds play an important role in the defence mechanism (Lachman, Orsak & Pivec, 1999).

In the organically fertilised onion field study, one of the cultivars grown in the inorganically fertilised field trials was grown. No significant effects on quercetin glucoside levels were found between onions that received organic fertilisers (but no chemical herbicides or fungicides) or inorganic fertilisers (Paper IV). One conclusion could be that the form of nitrogen, organic or inorganic, seemed to have no effect on quercetin synthesis. Another conclusion could be that the extra stress that could be expected from the absence of chemical fungicides did not seem to induce extra quercetin biosynthesis. The yield is generally somewhat lower for organically grown vegetables (Fjelkner-Modig *et al.,* 2000). This was the case in this study in 2005, but in 2004 it was only a tendency that was not significant (Paper IV). The reasons for the small differences found in this study may be that the organically fertilised onions were fertilised up to a conventional level, no serious fungus infections occurred in either of the two seasons and the weed population was kept at a low level.

Inoculation with mycorrhiza

There were no significant effects of mycorrhiza inoculation on the root colonisation any of the years (Paper IV), and as could be expected, no effect of mycorrhiza inoculation on quercetin glucoside content was found either in sown or transplanted onions.

Dry matter content

When analysing dried onion material, it is common to present quercetin glucoside data based on dry weight. In this study, differences in quercetin levels from a consumer's point of view were studied and therefore presenting the data based on fresh weight was found to be more suitable. The dry matter content showed minor differences between years and treatments but was significantly reduced during storage (Table 2). Moisture loss in sweet onions can be up to 10% during curing (Maw & Mullinix, 2005) but in our experiments dry matter content changes during curing were small (Table 2). In vegetables in general, a higher dry matter concentration can be observed in organically grown or organically fertilised products than in conventionally grown or mineral fertilised products (Woese *et al.,* 1997). However, in the field study, no significant differences in dry matter content between onions that received inorganic or organic fertilisers could be found (Paper IV). The influence of dry matter changes on quercetin content was minor and had almost the same pattern as for results based on fresh weight (Fig. 6B).

Table 2. *Dry matter content (%) of the inorganically fertilised onions in the different treatments on the different analysis occasions in each year*

Year	*Treatment*	*At lifting*	*After curing*	*At start of storage (October)*	*After 3 months of storage (January)*	*After 5 months of storage (March)*
2002	Low nitrogen	12.6 a	12.5 a	11.6 a	10.8 a	10.3 a
	High nitrogen	12.5 a	12.5 a	11.3 a	10.6 a	10.2 a
2004	Low nitrogen	12.3 a	12.5 a	12.0 a	11.4 a	11.3 a
	High nitrogen	12.0 b	12.7 a	11.8 a	11.2 a	11.1 a
2002	Early lifting	12.5 a	12.5 a	11.4 a	10.7 a	10.3 a
	Late lifting	12.7 a	12.5 a	11.5 a	10.7 a	10.3 a
2003	Early lifting	11.3 a	11.5 a	-	-	-
	Late lifting	10.9 b	11.1 b	-	-	-
2004	Early lifting	12.2 a	12.7 a	12.0 a	11.4 a	11.3 a
	Late lifting	12.2 a	12.5 a	11.9 a	11.1 a	11.1 a
2005	Early lifting	11.6 a	12.3 a	11.6 a	11.3 a	11.1 a
	Late lifting	11.8 a	12.2 a	11.6 a	11.1 a	11.0 a
2002	Barito	12.1 a	12.1 a	11.0 a	10.3 a	9.7 a
	Summit	13.1 b	12.9 b	11.9 b	11.1 b	10.9 b
2003	Barito	11.1	11.3	-	-	-
2004	Barito	11.6 a	11.6 a	11.6 a	10.9 a	10.8 a
	Hyskin	12.4 b	12.7 b	12.0 b	11.5 b	11.3 b
	Summit	12.6 b	12.9 b	12.2 b	11.5 b	11.5 b
2005	Barito	11.0 a	11.9 a	11.1 a	10.6 a	10.5 a
	Hyskin	12.1 b	12.2 b	11.7 b	11.3 b	11.2 b
	Summit	12.0 b	12.7 c	12.0 c	11.6 c	11.4 c
2002	Constant T	-	-	11.4 a	10.9 a	10.4 a
	Variable T	-	-	11.4 a	10.6 a	10.1 a
2004	Constant T	-	-	11.9 a	11.3 a	11.2 a
	Variable T	-	-	11.9 a	11.3 a	11.2 a

Values within columns, treatment groups and years followed by the same letter are not significantly different ($p<0.05$).
Fertiliser treatment data are means of all lifting times and cultivars.
Lifting time data are means of all fertiliser treatments and cultivars.
Cultivar data are means of all fertiliser treatments and lifting times.

Soil water conditions

In 2004, some fluctuations in soil water content occurred during the season. In 2005, the mean soil water content during the bulb growth period (June-August) was found to be significantly different in the northern part of the field compared with the southern part (Fig. 10).

No correlation was found between the quercetin glucoside content in the onions from each experimental plot and the mean soil water content in each plot. One conclusion could be that moderate water stress does not affect onion quercetin biosynthesis. The soil water data are not included in any of the papers.

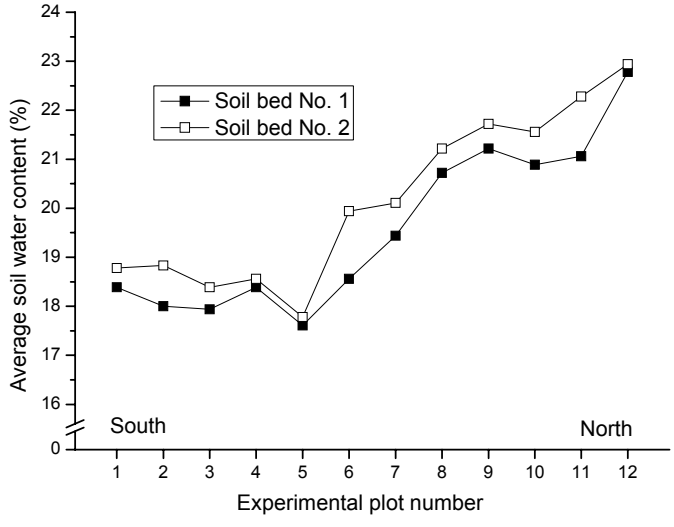

Figure 10. Average soil water content in the soil profile (0-20 cm depth) in the two inorganically fertilised soil beds in 2005. The soil water content was measured at 12 points from south to north in the field in two parallel soil beds. Bed number 1 was situated east of bed number 2.

Conclusions

- The main factor determining quercetin levels in onion is suggested to be the amount of global radiation at the end of the growth period, in this study in August.
- An effect of this is that very early lifting, when the onions still are growing in the end of July, results in onions with very low content of quercetin.
- During field curing the quercetin levels increase significantly.
- After field curing, no effect of lifting time on quercetin content is found, meaning that early lifted onions, that are better suited for long term storage, does not have lower levels of quercetin than late lifted either at start, during or after storage
- Cold storage at constant temperature maintains high quercetin levels in onions for at least six months.
- Cultivar differences in quercetin content are confirmed in this study. However the differences can be inconsistent between years.
- Quercetin content in onion is almost unaffected by nitrogen fertiliser level, nitrogen source (organic or inorganic fertiliser) and soil water content.

Future perspectives

For the future, it would be of interest to analyse other kinds of coloured onions and determine whether red onions react in the same way as yellow to global radiation.

No sensory analyses were performed within this project but there may be a correlation between sugar content/composition, taste and colour of the onions.

Nitrogen fertilisers are of great importance for growth and are the focus of attention due to the debate of nutrient leaching from soils. However, other macro- and micro-nutrients might affect the quercetin content in different ways.

Field curing proved to be a good way to increase quercetin content. Due to the fact that the Swedish weather is not always ideal for field curing, additional investigations of effects of forced hot air drying as a substitute or complement to field curing would be of interest.

There are cultivar differences in onion quercetin content, and they reacted differently to the same environmental conditions, which could mean that plant breeding could be a way to obtain more health-promoting onions.

Practical implications

From a grower's point of view, it is good news that minimising the nitrogen fertiliser level, and thereby probably lowering the fertiliser cost, has no negative effects on yield or the content of bioactive quercetin compounds in onions. Early lifting, which promotes a lower sprouting rate, does not lower the quercetin content. However, if the onions are not to be long term stored, late lifting results in slightly higher yield. Keeping the onions at constant temperature in a cold storage maintains the quercetin content and the onions are almost as healthy after 6 months of storage as they were at harvest. In short, it can be concluded that most efforts that are made to achieve and retain a good outer onion quality are also positive for the inner quality measured as the content of quercetin.

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Fältförsök är chansartade. 2003 blev det ingen skörd, men jag räddades av lökodlaren **Sivert Gottfridsson** som tillhandahöll lök. Detta är jag mycket tacksam för. Faktum är att många av de resultat som redovisas i denna avhandling hade jag nog aldrig kommit fram till om jag inte tvingats jämföra många olika lossnings- och fälttorkningsmetoder istället för rutinartad lökhackning som planerat.

Forskningsprojektet har fokuserat på lökanalyser, så jag är mycket tacksam att **HS Miljölab** i Kalmar har varit hjälpsamma med alla jordanalyser så jag slapp göra dem.

Ett speciellt tack vill jag framföra till **Sven-Åke Nilsson** och övrig personal på Kalmar-Ölands Trädgårdsprodukter i Färjestaden för att jag fått lagra lök under samma förhållanden som de kommersiella odlarna, och för all information om lökodling jag fått del av.

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Jag vill också tacka **Jan-Eric Englund** för allmänna försöksuppläggstips och för att du med hjälp av dina statistikkunskaper lyckades avstyra min plan att ägna mycket tid och möda åt ett sortförsök som redan på pappret visade sig omöjligt att få ut några signifikant säkerställda behandlingseffekter från.

Ett särskilt tack till alla de underbara doktorandkollegor jag mött under de här åren, och av olika skäl ett särskilt tack till:
Sara. Bäbisspenatens förkämpe! Min tvillingdoktorand. ”Vi har rest en lång väg tillsammans…”
Rakel. Våga inte klaga! Nyplockade jordgubbar är mycket godare än färsk, rå lök!
Margit. Tripsfällornas mästare, vilken PET-flaskmodell fungerade bäst?
Andrea. Vad tror du om växthusodlade rödlökar att hänga i dina jul-järnekar?
Klara. Eller heter du Klaa utan “r” egentligen? Denna lilla bokstav gör ju en viss skillnad när man sysslar med krukväxtodling…
Sanna. ”Hej Gröna hjälpen! Hur gör man för att bli en riktig lökdoktor?”
Victoria. Gröna rum och svarta fiskar. Du sätter färg på tillvaron!
Åsa. Vattenspridare och glädjespridare.

Skåne är inte så platt som man ofta tror, och jag vill aldeles särskilt tacka **Linnéa**, **Åsa**, **Ulrika** och **Elisabeth**, mina hortonomsupporters med tillhörande män och barn som hjälpt mig hålla kontakt med livet utanför Alnarp. Jag vill även framföra ett stort tack till **Peter**, **Stina**, **Anna** och **Martin** som alltid har nya överraskningar på gång…

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Slutligen ett stort TACK till min familj; **Miles Barbro**, **Anna** och **Otto** samt **Knut**, **Florence** och **Håkan**. Och alla djuren, Ebba, hönorna, sköldpaddorna, fåren, trolleriduvorna, schackrutiga strumpebandssnokarna, klockgrodorna… ja helt enkelt HEMMA, ni har alla på ett underbart sätt hjälpt mig tänka på annat än lök under mina semesterdagar.

Och allra sist ett tack till ***Allium cepa*** – utan dig hade detta projekt aldrig blivit av!