Factors affecting the postharvest performance of fresh-cut lettuce

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Contents

Abbreviations

AAPH	2,20-Azobis (2-amidino propane) dihydrochloride
A_{net}	net assimilation
AO	ascorbate oxidase
AsA	L-ascorbic acid
ATP	adenosine triphosphate
C	control
Chl(s)	chlorophyll(s)
DHA	L-dehydroascorbic acid
DMF	dimethylformamide
DTT	dithiothreitol
DW	dry weight
EL	electrolyte leakage
FL	fluorescent tubes
FW	fresh weight
GB	green butterhead genotype
LCP	light compensation point
LED(s)	light emitting diode(s)
ORAC	oxygen radical absorbance capacity
OVQ	overall visual quality
PAR	photosynthetically active radiation
RB	red butterhead genotype
R_{dark}	dark respiration
RL	reduced light
RMCD	randomly methylated b-cyclodextrin
ROS	reactive oxygen species
SLW	specific leaf weight
TE	Trolox equivalents

CHAPTER 1

General Introduction

The Unites Fresh Produce Association defines fresh-cut products as fruits or vegetables that have been trimmed, peeled and/or cut into 100% usable product that is bagged or pre-packed to offer consumers high nutrition, convenience, and value while still maintaining its freshness (UFPA, 2010; http://www.unitedfresh.org/). The industry of fresh-cut fruits and vegetables is constantly growing as a result of convenience of these products and consumer awareness of the importance of the healthy diet (Steinmetz and Porter, 1991; Willey, 1994; Parish et al., 2003).

The quality of fresh-cut fruits and vegetables is a combination of attributes such as texture, appearance, nutritive value and flavour that change over time (Kader, 2002; Rico et al., 2007). The end of shelf-life is the time when the product's attributes drop below the acceptance limit under standardised storage conditions (Tijskens, 2000). The term acceptability is a practical approach to quality by comparing it to a criterion, the quality limit. Below that limit the product is rejected (Tijskens, 2000). For consumers acceptable and appealing product is the one with fresh-appearance, which is consistent through the package, almost free of defects and of excellent nutritional quality (Steinmetz and Potter, 1991; Willey, 1994; Watada et al., 1996; Parish et al., 2003).

The quality of fresh-cut fruits and vegetables is often offset by a rapid deterioration, due to processing operations, and a shelf-life of these products is seriously reduced in the market place. Hence, developing new, effective, non-invasive and non-chemical techniques for improving and maintaining quality are the timely questions of the industry.

The subjects covered in this thesis focus on pre- and post-harvest methods with potential to improve the quality of fresh-cut butterhead lettuce (*Lactuca sativa* L.). In particular, this study is focused on how the lettuce quality retention might be enhanced by:

1. Growth irradiance and genetic selection aiming at increasing antioxidant status of the tissue,
2. Harvesting plants at optimal physiological maturation,
3. Using low light during pre- and post-processing storage,
4. Improving conditions and duration of storage prior to processing.

1.1 The physiology of fresh-cut tissue

The physiology of fresh-cut (minimally processed) tissue is basically the physiology of wounded tissue (Brecht, 1995). The wounding, due to, for example, detachment, cutting, cracking or breaking, is perceived by the plant, and subsequently a signal is generated, which leads to changes in physiological and biochemical processes. Consequently, a number of wound-induced symptoms develop, such as discoloration (Ke and Saltveit, 1988, 1989; Peiser et al., 1998; Tay and Perera, 2004; Martín-Diana et al., 2005a, 2005b), increased respiration and ethylene production (Peiser et al., 1998; Fan and Matthies, 2000; Saltveit, 2004; Tay ad Perera, 2004; Martínez-Romero, 2007), loss of flavour and texture (Tay and Perera, 2004; Martín-Diana et al., 2005a, 2005b, 2006), loss of weight (Ihl et al., 2003; Agüero et al., 2008), decline in levels of nutrients such as ascorbate (Ihl et al., 2003, Murata et al., 2004; Degl'Innocenti et al., 2005, 2008; Martín-Diana et al., 2005b, 2006) and/or carotenoids (Martín-Diana et al., 2005a, 2006), development of off-odours (Beaulieu, 2006), and membrane disintegration (Hodges et al., 2000).

1.1.1 Discoloration

In lettuce, two types of discoloration are distinguished, i.e. enzymatic browning represented by wound-induced browning and russet spotting (RS), and senescent browning (SB) (Saltveit, 1997). The wound-induced browning refers to browning of the tissue due to cutting or bruising (Campos-Vargas and Saltveit, 2002; Saltveit, 2004; Martín-Diana et al., 2005a, 2005b), whereas the RS is associated with exposure to ethylene leading to appearance of small, reddish-brown spots or lesions on the achlorophyllous midribs of leaves (Link and Gardner, 1919; Ke and Saltveit, 1988, 1989; Couture et al., 1993; Peiser et al., 1998; Tay and Perera, 2004; Adams and Brown, 2008). Enzymatic and non-enzymatic reactions with phenolic compounds produce brown pigments in plant tissue. Wounding produces a signal that migrates through the tissue, and induces the synthesis of phenylalanine ammonia lyase (PAL) (Ke and Saltviet, 1988, 1989; Tomás-Barberán et al., 1997). The increased PAL activity promotes synthesis of cinnamic acid and derivatives via the shikimic pathway (Fan and Mattheis, 2000). In the next step, the phenolic compounds can be oxidized by polyphenol oxidase (PPO; EC 1.10.3.1) and peroxidase (POD; EC 1.11.1.7) to quinones, which ultimately polymerize to produce the brown appearance (Degl'Innocenti et al., 2005).

Moreover, vegetables containing chlorophyll during storage also undergo changes or loss of green colour (Abe and Watada, 1991; Bolin and Hussoll, 1991; Haard, 1993; Ihl et al., 2003; Tay and Perera, 2004). Factors responsible for loss of chlorophyll during storage are light, temperature, O_2, C_2H_4, humidity, but also chlorophyllase and magnesium dechelactase activity (Shioi et al., 1996, Jacob-Wilk et al., 1999).

1.1.2 Increased respiration and ethylene evaluation

Wounding has been shown to induce changes in mitochondrial structure and increase in their number and function (Asahi, 1978). In wounded tissue an increased respiratory activity occurs due to enhanced aerobic mitochondrial respiration (Laties, 1978; Brecht, 1995). Also the increase in respiration seen in wounded plant tissue is thought to be a consequence of elevated ethylene (Fan and Mattheis, 2000). Respiration rates of iceberg and romaine lettuce fresh-cuts were found to be 20 to 40 percent higher than that of the

respective intact heads (Cantwell and Suslow, 2002). The wound induced increased respiratory activity is a well-established phenomenon in lettuce tissue (Peiser et al., 1998; Fan and Mattheis, 2000; Tay and Perera, 2004). The increased respiration leads to depletion of energy reserves in tissue.

Wounding of plant tissues also induces elevated ethylene production (Brecht, 1995; Tay and Perera, 2004), which may accelerate deterioration and senescence in vegetative tissue. The level of ethylene has been shown to increase in proportion to the amount of wounding in several vegetables and fruits. Still, the level of the wound-induced increased ethylene synthesis in lettuce is very low as compared with other species, and these levels have been reported to return to non-stressed levels within 24 hours after stress application (Ke and Saltveit, 1989; Saltveit, 2004).

1.1.3 Dehydration, loss of flavour and texture, and development of off-flavours

Any reduction of water vapour pressure in the atmosphere below 99% to 99.5% relative humidity (RH) results in water loss from plant tissue (Burton, 1982). Water in intercellular spaces of whole organs is not directly exposed to the outside atmosphere, however, wounding exposes interior tissues and drastically increases the water evaporation rate. The difference in rate of water loss between intact and wounded tissue is 10- to 100-fold for organs with cuticularized surfaces (Burton, 1982). Thus, avoiding desiccation at the cut surface of fresh-cut product is critical for maintaining acceptable visual appearance. Dehydration equals decrease in turgidity pressure in the cells and cellular wall degradation, and therefore, affects texture, turgidity and colour. Textural changes in vegetables are related to certain enzymatic and non-enzymatic processes, and have been previously been presented for lettuce samples (Martín-Diana, et al. 2005a, 2005b, 2006). A water loss during storage has been shown to enhance the loss of both ascorbic acid and total carotene (Barth and Zhuang, 1996; Barth et al., 1990; Nunes et al., 1998) either directly or through induction of ethylene (Yang, 1985).

In response to wounding, plants synthesize an array of secondary compounds, such as phenylpropanoid phenolics, polyketide phenolics, flavonoids, terpenoids, alkaloids, tannins, glucosinolates, and long-chain fatty acids and alcohols (Miller, 1992). Many of these compounds are related to the wound healing or defence against microorganisms and insects, but in some cases, these compounds may affect the aroma, flavour, appearance and nutritive value or safety of fresh-cut vegetables (Brecht, 1995). Some compounds may result in poor or persistent off-odours and -flavours.

1.1.4 Changes in levels of nutrients and antioxidants

Processed foods are perceived as being of lower nutritional value, and the preservation methods as a reason for depletion of natural antioxidants in these products. Klein (1987) and McCarthy and Matthews (1994) suggested that the tissue wounding due to, for example, peeling, slicing, dicing and shredding of vegetables causes not only physiological and biochemical reactions leading to undesirable quality changes such as discoloration and a loss of texture, but also accelerates loss of nutritional value. This assumption has never been rigorously tested and, in fact, plants respond to numerous abiotic and biotic stresses for example by up-regulation of antioxidant compounds (Baker and Orlandi, 1995; Wang et al., 2003). It appears that the change in nutritional properties is product and procedure dependent, and therefore no change, increase or decrease might be observed

(Nicoli et al., 1999). The knowledge and available data on the nutrient content and retention of fresh-cut vegetables are generally sparse and needed (Lindley, 1998).

1.1.4.1 Carbohydrates

Soluble sugars and starch are the primary energy reserves in the tissue, and their levels as well as the rate of their loss during storage have been suggested to define the storability of the product (Lipton, 1987; Brecht, 1995; Klieber et al., 2002; Kays and Paull, 2004; Clarkson et al., 2005). Eskin (1990) has demonstrated that tissues with high respiratory rates and/or low energy reserves exhibit shorter storability. Although the wound induced increase in respiratory activity is a well-established phenomenon in lettuce tissue (Peiser et al., 1998; Fan and Mattheis, 2000; Tay ad Perera, 2004), and this increase leads to depletion of energy reserves in the tissue, limited number of researchers have looked into the relationship between these energy reserves and the storability of fresh-cut vegetables. Besides, the available and scarce results appear to be contradictory, i.e. while Toledo et al. (2003) showed a decrease in total soluble carbohydrates of *Spinacia oleracea* L., Noichinda et al. (2007) showed no decrease in glucose, fructose or sucrose of *Brassica oleraceae* var. *Alboglabra* during dark storage.

1.1.4.2 Antioxidants

Carotenoids, polyphenolics compounds, and ascorbic acid represent the most important antioxidants, which contribute to the total antioxidant capacity of leafy vegetables (Figure 1) (Larson, 1988; Yamasaki, 1997; Brecht et al., 2004; Rico et al.., 2007; Vincente et al., 2009). Antioxidants present in plants can act as reducing agents, free radical terminators, metal chelators, and singlet oxygen quenchers and mediate the activity of various oxidizing enzymes (Ho, 1992; Okuda, 1993). Plants respond to numerous abiotic and biotic stresses by up-regulation of antioxidant compounds (Baker and Orlandi, 1995; Wang et al., 2003).

Carotenoids

Carotenoids are liposoluble pigments responsible for the yellow, orange and red colour of several fruits and vegetables, and represent (together with tocopherols) the most abundant lipophilic antioxidants in plant tissue (Munné-Bosch and Folk, 2004). These pigments in plants play a role in radiation interception, mainly in the blue–green region of the spectrum (Kopsell and Kopsell, 2006), protect the photosynthetic structures from excessive energy (Grusak and Della Penna, 1999), and scavenge reactive oxygen species (ROS) (Cazzonelli et al., 2011). They are usually present in low concentrations and their levels are highly variable among species. Carotenoids are perceived as relatively stable compounds as compared to other antioxidants (Buescher et al., 1999). During post-harvest storage of leafy vegetables unchanged or decreased carotenoid concentration has been reported (Ezell and Wilcox, 1962; Kopas-Lane and Warthesen, 1995; Pandrangi and Laborder, 2004; Martín-Diana et al., 2005a).

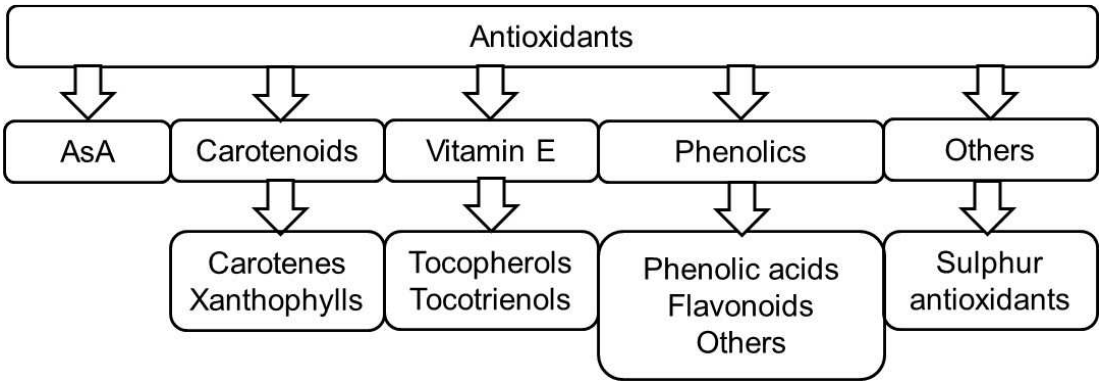

Fig. 1. Main antioxidants in fruits and vegetables, adapted from Vincente et al. (2009).

Polyphenolic compounds

A large number of phenolic compounds have been identified in plants (Tsao and Deng, 2004), and subdivided into different subclasses, such as phenolic acids, flavonoids and other compounds (e.g. lignans, stilbenes, tannins, coumarins and lignin). The general characteristics of phenolic compounds are aromatic rings with variable degreed of hydroxylation (Mattila et al., 2006). The main function of these compounds is preventing attacks of potential predators or antimicrobials, protecting against UV-radiation and contributing to the pigmentation of fruits and flowers. Phenolic compounds are the most investigated antioxidants during storage of fresh-cut fruits and vegetables (Ke and Saltveit, 1988, 1989; Couture et al., 1993; Loaiza-Velarde et al., 1997; Campos-Vargas and Saltviet, 2002; Kang and Saltveit, 2002, 2003; Loaiza-Velarde and Saltveit, 2002; Saltveit, 2000, 2004; Saltveit et al., 2005a, b; Saltveit and Choi et al., 2007; Saltveit and Qin, 2008; Degl'Innocenti et al., 2005, 2008; Martínez-Sánchez et al., 2011), and wounding of vegetables and fruits has been shown to enhance tissue antioxidant levels (Ferreres et al., 1997; Tomas-Barberan et al., 1997a, b; Kang and Saltveit, 2002; Campos-Varags and Saltveit, 2002; Cisneros-Zevallos, 2003; Reyes et al., 2007). Most studies on leafy vegetables have been conducted on non-photosynthetic tissue, i.e. mid-rib tissue; and the changes in antioxidants were limited to polyphenolic compounds, or followed for no more than 48h. Since photosynthetic tissue comprises a bulk of the fresh-cut material prepared from most of lettuce varieties, and vascular and photosynthetic tissues are not alike in respect to biochemical composition, the knowledge on antioxidant changes in this type of tissue during storage is still missing. Besides, since polyphenolic compounds are not the only antioxidant compounds responsible for scavenging of ROS, changes in other main antioxidants during cold storage should also be investigated during the period of commercial storability of fresh-cut lettuce (10 days).

Ascorbic acid (AsA)

Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-ascorbic acid (AsA). AsA is a principle biologically active form, but its oxidation product, L-dehydroascorbic acid (DHA) also exhibits biological activity. Vitamin C is a multifunctional metabolite in plants influencing nearly every aspect of plant biology (Noctor and Foyer, 1998; Smirnoff, 1996). After harvest, conditions and duration of

transport and storage affect the most the retention of AsA content (Lee and Kader, 2000). An extended storage and high temperature accelerate the AsA losses, while high RH prevents these losses. The AsA content is negatively affected by the cutting and the method used. The decline in AsA during dark storage has been reported for example in *Lactuca sativa* L. var. *crispa* (Ihl et al., 2003), *Lactuca sativa* L. var. *capitata* L. (Murata et al., 2004), *Spinacia oleracea* L. (Bergquist et al., 2006) and *Eruca sativa* (Degl'Innocenti et al., 2007), but not in *Lactuca sativa* L. var. *capitata* L. (Degl'Innocenti et al., 2007). In contrast, low light intensity during storage of *Brassica oleracea* var. *alboglabra* has shown potential to improve the retention of AsA during storage of leafy vegetables (Noichinda et al., 2007). The higher the severity of damage, i.e. size of leaf pieces (small vs. big) and sharpness of blade (dull vs. sharp), the higher AsA losses occur (Barry-Ryan and O'Beirne, 1999).

1.1.4.3 Accumulation and detoxification of ROS

Stresses, such as physical detachment of leaves and wounding promote not only oxidative processes in the tissue, but might also accelerate leaf senescence (Philosoph-Hadas et al., 1991; Bartoli et al., 1996). It has been stated by other authors that the differences in post-harvest behaviour of wounded tissue is a function of the oxidative processes due to stress imposed in the pre-harvest stage (Galvis-Sanchez et al., 2004; Calderon-Lopez et al., 2005) and in the post-harvest stage. The pre-harvest stage gives tissue certain potential with respect to ROS and antioxidants (Mckersie and Leshem, 1994; Foyer et al., 1994; Baker and Orlandi, 1995; Dixon and Paiva, 1995; Hodges et al., 1996, 1997a, b; Hodges and Forney, 2000; Wang et al., 2003), and the detachment and wounding of the plant adds to the pool of oxidative stress. Since in detached and wounded tissue remobilisation of metabolic components is not possible, a hyper accumulation of toxic molecules, such as free radicals, might occur (Wagstaff et al., 2007).

1.1.4.4 Membrane disintegration

Wounding of plant tissues resulting from the preparation of fresh-cut products might cause membrane lipid degradation (Rolle and Chism, 1987; Deschene et al., 1991; Picchioni et al., 1994; Zhuang et al., 1997). In damaged membrane systems, an extensive enzymatic degradation occurs causing loss of lipid components and loss of compartmentation of enzymes and substrates (Brecht, 1995; Marangoni et al., 1996). The wound induced ethylene production may play a role in this process by increasing the permeability of membranes and reducing phospholipid biosynthesis (Watada et al., 1990). There is limited evidence that there are cultivar differences in rates of membrane deterioration in response to minimal processing (Lamikanra, 2002). The losses in membrane integrity are commonly assessed by the electrolyte leakage method (Murata and Tatsumi, 1979; Hariyadi and Parkin, 1991; Carlin et al., 1990; Varoquaux et al., 1996; Saltveit, 2000).

1.1.4.5 Storage in darkness and dark-induced senescence

Most of these wounding-associated symptoms in fresh-cut lettuce leaves may also occur as a consequence of detachment from the roots and dark storage, factors which promote senescence (Wangermann, 1965; Trippi and Thimann, 1983; Biswal and Biswal, 1984; Hodges and Forney, 2003; Buchanan-Wollaston, 1997). Senescence symptoms such as chlorophyll degradation and membrane disintegration have been identified as important factors in postharvest longevity of plant tissue (Noodén et al., 1997). Consequently,

delaying senescence symptoms, especially the loss of green colour and loss of turgescence, has become one of the most important goals in the postharvest technology.

1.2 The suitability of raw material for fresh-cut industry

In order to obtain a high quality fresh-cut product, it is necessary to start with a high quality raw material (Watada et al., 1996).

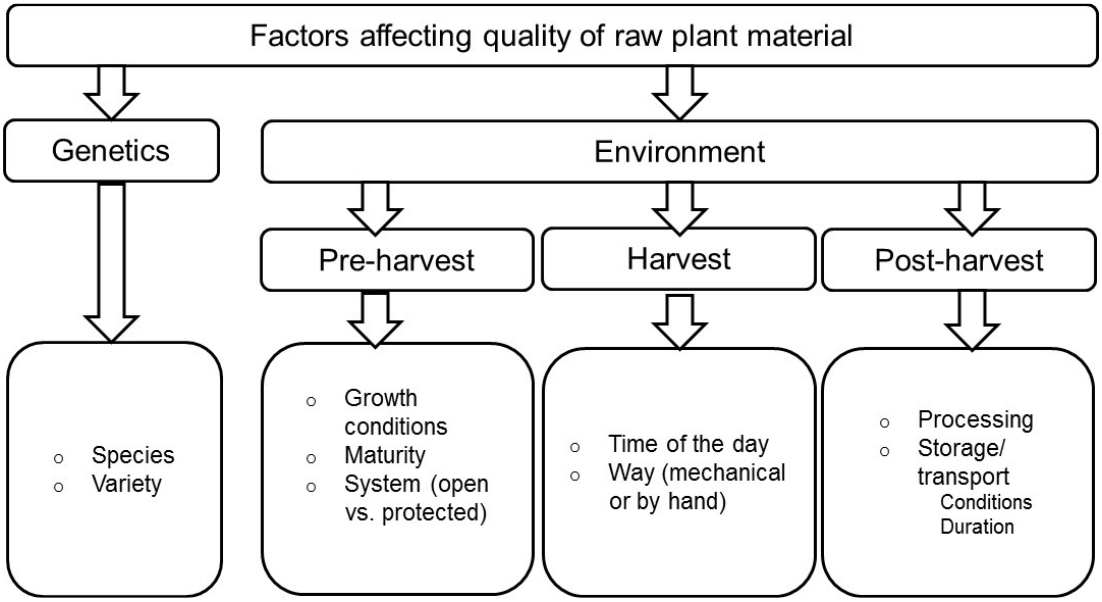

Fig. 2. Factors affecting quality of raw plant material in the supply chain of fresh-cut product, adapted from Vincente et al. (2009).

Any pre-harvest condition that stresses the plant also will affect the quality and shelf-life of the associated postharvest crop (Monselise and Goren, 1987; Nigh, 1990). Therefore, the knowledge of pre-harvest conditions is important for assessing the postharvest potential of fresh-cut product (Kim et al., 1993; Gorny et al., 1998; Blacharski et al., 2001). To this date, genetic background (Varoquaux et al., 1996; Degl'Innocenti et al., 2007), cultivation conditions (Voipio et al., 1995; Eskins, 1996; Krizek et al., 1998; Romani et al., 2002; Kleinhenz et al., 2003; Gazula et al., 2005; Gruda et al., 2005; Garcia-Marcias et al., 2007; Tsormpatsidis et al., 2008) and plant age at harvest (Couture et al., 1993; Pandjaitan et al., 2005; Bergquist et al., 2006; Zhao et al., 2007) has been suggested as possible factors in affecting some of the quality attributes of leafy vegetables. The primary focus of these studies was on obtaining a nutritionally rich product, while the link was not made to the storability and visual quality of the end product.

The conditions and duration of post-harvest period (pre- and post-processing) are also main determinants in maintaining the raw product as close as possible to its harvest condition. Parameters such as temperature, RH, composition of the atmosphere, and light influence physiological and biochemical processes in the harvested tissue (Watada et al., 1996). The first three parameters have been numerously and in depth investigated, and optimal conditions for leafy vegetables are known (Kader et al., 1989; Lopez-Galvez et al.,

1996; Saltveit, 2003). Therefore, these parameters, as well as their importance on the fresh-cut quality are not going to be discussed to a full detail in this thesis.

1.2.1 Genetics

Fruits and vegetables are diverse in their physiology, and they represent numerous morphological structures and tissues (Brecht, 1995). Therefore, it is not surprising that various species and varieties of leafy vegetables react differently to processing and storage. For instance, Varoquaux et al. (1996) presented a response of leaves of five *Lactuca sativa* L. varieties to storage under modified and controlled atmosphere. The changes in parameters such as membrane integrity, browning of cut edges or respiration rate were different between the varieties and this indicated that some varieties are more suitable for fresh-cut industry than other varieties. Hodges et al. (2001) showed altered antioxidant responses in harvested leaves of two cultivars of *Spinacia oleracea* differing in their senescence rates. Degl'Innocenti et al. (2007) presented different responses in a number of physiological and biochemical parameters, including the activities of key enzymes involved in the metabolism of phenols and ascorbic acid in three species: *Lactuca sativa* var. *capitata* L., *Cichorium indivia* var. *latifolium* and *Eruca sativa* upon cold storage as fresh-cuts. These investigations have clearly indicated that the quality and storability of leafy vegetables can be improved through genetic selection.

1.2.2 Pre-harvest

1.2.2.1 Conditions during growth

Many studies have been devoted to the effects of growth conditions on the quality of fruits and vegetables. In recent years, the main interest of these studies has been on the effects of various growth conditions on the antioxidant composition of the tissue. For various types of lettuce numerous studies have been conducted, for example on the effects of UV lighting (Voipio et al., 1995; Krizek et al., 1998; Romani et al., 2002; Garcia-Marcias et al., 2007; Tsormpatsidis et al., 2008), increased light intensity (Kleinhenz et al., 2003; Gazula et al., 2005), nitrogen (Zhao et al., 2007), or growing temperature (Kleinhenz et al., 2003; Gazula et al., 2005) on the antioxidant content. Although a high level of antioxidants is generally believed to be beneficial in coping with various biotic and abiotic stresses, it is not known if increased antioxidant levels are also protective against postharvest stresses such as the wounding induced by cutting, and senescence induced by dark storage.

1.2.2.2 Physiological maturity

The negative consequence of wounding depends on the physiological maturity of the plant, and it has been suggested to be higher early and late in fruit and vegetable development (Brecht, 1995). As mentioned above (1.4.1.), it is generally assumed that the tissue with low storage reserves deteriorates quicker. Therefore, plants harvested very early in development, with meagre storage reserves and high metabolic rate might deteriorate rapidly, while more mature plants with high storage reserves and low metabolic rate might have longer storability (Brecht, 1995). However, this theory has not been well tested for the leafy vegetables (Couture et al., 1993; Pandjaitan et al., 2005; Bergquist et al., 2006; Zhao et al., 2007). Among these few reports, only a small number has been devoted to lettuce, and its properties and quality as a fresh-cut product (Couture et

al., 1993). The significance of the maturity on quality of leafy vegetables was inconsistent between studies. For example, increased levels of total antioxidants by harvesting young tissue were reported for *Spinacia oleracea* (Bergquist et al., 2006; Zhao et al., 2007), but not for *Lactuca sativa* L. (Zhao et al., 2007). In contrast, Pandjaitan et al. (2005) reported that mid-size leaves of *Spinacia oleracea* were significantly higher in antioxidants than immature and fully-mature leaves harvested from the same plant. The discrepancies in results may be partly explained by the interaction of plant age with weather conditions during different growing times. Therefore, there is a need to investigate the age-dependent differences in the quality and storability of fresh-cut leafy vegetables without interaction of growth conditions. Since the majority of these investigations focused only on the effect of crop maturity on the content of antioxidants at harvest, the relationship between age-dependent improvements in the nutritional quality, and the shelf-life of the products still need to be established.

1.2.3 Harvest

The timing of harvest can influence the storability of the fresh-cut product. The content of chemical compounds, such as sugars, starch and antioxidants, or water content change with the season, and time of the day in relation to light, temperature and humidity. The method of harvest, i.e. mechanical or by hand, may also determine the extent of variability in maturity and physical injuries, and consequently influence nutritional composition of vegetables (Kader and Lee, 2000). The incidence and severity of mechanical injuries such as bruising, surface abrasions, and cuts are influenced by the method of harvest and handling operations. Proper management to minimize physical damage to the commodity is a must for manual or machine harvesting (Kader and Lee, 2000).

1.2.4 Post-harvest

1.2.4.1 Processing (wounding)

Quality of fresh-cut products and its deterioration rate is highly dependent on the injury done to the product. It has been clearly shown that the higher degree of injury incurred during the processing the faster the quality deterioration, and the shorter the shelf-life (Toivonen et al., 2005). The severity of wounding depends on kind and sharpness of used blades, direction of cutting, cutting technique (slicing, chopping, tearing), and the size of leaf pieces (Bolin et al., 1977; Bolin and Huxsoll, 1991; Pereyra et al., 2005).

1.2.4.2 Pre- and post-processing storage

Storage of the raw product after harvest, and fresh-cut product after processing is common practice, during which physiology and composition of the harvested tissue continues to change. The conditions and duration of storage are main important factors in quality retention of fresh-cut product.

Duration of storage

The processing of raw product is seldom done on the production side, but mainly by regional or national processors. The time between harvest and processing depends on the distance from the harvest site to the processor, on the current demand and on possible speculative storage of the products. Up to date, the impact of storage prior to processing

on the quality of the processed product (fresh-cut leafy vegetables) has not been quantified.

The duration of post-processing storage depends on the type of produce, and conditions during storage. *Lactuca sativa* L. is commonly stored for up to 10 days, before the product quality reaches the end of shelf-life.

Temperature and relative humidity

Keeping intact and fresh-cut fruits and vegetables within their optimum range of temperature and relative humidity (RH) is very important in maintaining the quality and minimizing postharvest losses (Kader, 2002). Every 10°C increase in temperature accelerates deterioration and rate of loss in nutritional quality by two- to three-fold (Kader, 2002b). Although, the optimal storage range of vegetables is 0-2°C, a temperature range of 1 to 10°C is often use in the market chain of fresh-cut lettuce. A brief exposure (1 or 2 hour) to high storage temperature may already cause a dramatic drop in the visual quality of the product (Jedermann and Lang, 2007), due to high respiration (Schilmme, 1995; Watada et al., 1996), physiological deterioration, microbial growth (Schlimme, 1995; Behrsing, 1993) and yellowing (Yamauchi and Watada, 1991). In contrast, low temperature preserves quality thanks to slowing down metabolisms by decreasing respiration, ethylene production, enzymatic processes and microbial activity (Nicola et al., 2009). However, it is always important to keep the temperature above the freezing and chilling point, which can damage the product (Bolin and Huxsoll, 1991).

The RH for the storage of fresh-cut vegetables should be at a level where water pressure deficit is maintained close to that of the products' internal atmospheric environment. Although RH of 99% to 99.5% is recommended for leafy vegetables like fresh-cut lettuce (Wills et al., 1998; Kays and Paull, 2004), a RH lower than the optimum is often used in the market chain (Cantwell and Suslow, 2002). A small moisture loss from the optimal level is enough to cause wilting, shrivelling and dryness of lettuce which is directly affecting the visual quality (Aguero et al., 2010).

Atmosphere

Fresh-cut lettuce is often packed in polypropylene plastic film with a high barrier to oxygen diffusion. Produce is either packed under atmospheric conditions (Modified Atmosphere) or under a mixture of decreased O_2 and increased CO_2 (Active Modified atmosphere). Due to the respiration of the produce, in both packaging concepts the O_2 concentration will decrease to virtually zero and the CO_2 concentration will rise to 15-20% within a couple of days, depending on the storage temperature. The low oxygen concentration will effectively block cut-edges discoloration, but in the longer term the anaerobic conditions may cause excessive fermentation and deterioration of the produce. Regarding the atmospheric composition during storage, within the present investigations, we packed the fresh-cut produce under ambient conditions in boxes allowing sufficient ventilation to prevent any oxygen depletion. In this way we avoided any interference of the changing gaseous composition in the package with the physiological processes under study. We believe that observed effects of applied treatments might, in commercial packaging, be masked by the effects of the modified atmosphere that often leads to anaerobic conditions during storage.

Light

Retail grocery markets currently display fresh-cut vegetables in light-transmissible packages, which are exposed to 12 to 24 h continuous artificial low intensity lighting. Therefore, the exposure of fresh-cut produce to light is unavoidable in parts of the supply chain. A possible positive effect of light has been attributed to delaying senescence during the postharvest storage of intact leaves of leafy vegetable (Hosoda et al., 2000) and ornamental plant (Ranwala and Miller, 2000). The delaying effect of light on the senescence has been attributed to maintaining photosynthetic activity of the tissue, and therefore its energy reserves, and the retention of important antioxidants, such as carotenoids (Noichinda et al., 2007; Lester et al., 2010), phenolics (Zhan et al.., 2009), and ascorbic acid (Noichinda et al., 2007; Zhan et al., 2009). The effect of light on the quality of vegetable products, however, has been contradictory. There are a number of reports that show a negative effect of light on the quality retention in vegetables such as *Brassica oleraceae* L. var. *italica* (Kasim and Kasim 2007; Olarte et al., 2009), *Brassica oleracea* L. var. *capitata* (Perrin et al., 1982), *Brassica oleracea* L. var. *botrytis* L. (Sanz et al., 2007; Olarte et al., 2009), *Beta vulgaris* L. var. *vulgaris* (Sanz et al., 2008), *Lepidium sativum* L. (Zhan et al., 2009), *Allium porrum* L. (Ayala et al., 2009), *Lactuca sativa* L. (Martínez-Sánchez et al., 2011), and *Asparagus officinalis* L. (Sanz et al., 2009). On the contrary other authors have found a positive effect of light on the post-harvest quality retention in vegetables such as *Brassica oleraceae* var. *gemmifera* (Kasim and Kasim, 2007), *Brassica oleracea* var. *alboglabra* (Noichinda et al., 2007), *Brassica rapa* L. subsp. *sylvestris* (Barbieri et al., 2009), and *Spinacia oleracea* L. (Toledo et al., 2003; Lester et al., 2010). These discrepancies in the effect of light can be explained by differences in the type of vegetable used, i.e. leaf, inflorescence, root or stalk; the type of tissue used, i.e. intact vs. wounded; tissue coloration; the duration of storage; the intensity and spectrum of the applied light; and the type of packaging. Moreover, in these investigations the light intensities used ranged between 4 and 45 μmol m^{-2} s^{-1}, often being below or just at the LCP of these vegetables, and the limited exchange of gases through the packaging material during storage led to unfavourable treatment-dependent changes in the atmosphere, which may have masked the effects of light per se.

Within these studies only few have been devoted to the effect of light on the quality of fresh-cut produce (Sanz et al., 2009; Zhan et al., 2009; Martínez-Sánchez et al., 2011) and, surprisingly, only one study was devoted to fresh-cut lettuce (Martínez-Sánchez, et al., 2011). Therefore, data are scarce on effects of light at levels maintaining photosynthesis in fresh-cut leafy vegetables, as well as relationship between energy reserves, antioxidants and shelf-life of the fresh-cut product.

Contents of this thesis

The aim of this thesis is to enhance the knowledge and understanding on pre- and postharvest factors affecting the storability of fresh-cut leafy vegetables.

Chapter 2 focuses on the effects of the most abundant antioxidants in the fresh-cut lettuce photosynthetic tissue and them contributing to protecting harvested tissue against the stress caused by wounding.

Chapter 3 describes the age-dependent differences in the quality and storability of fresh-cut lettuce. The shelf-life of fresh-cuts was related to a number of physiological processes associated with senescence, such as a decrease in photosynthesis, chlorophyll degradation, and loss of antioxidants and cellular integrity.

Chapter 4 shows the relationship between the energy reserves in lettuce photosynthetic tissue and the storability of fresh-cuts by comparing tissue stored in darkness or at low light irradiance.

Chapter 5 demonstrates how physiology and composition of the harvested tissue changes during storage prior to processing consequently affecting the storability of fresh-cuts.

Chapter 6 is the general discussion. The research results described in chapter 2-5 are placed in a more general physiological perspective and practical implications of this study are discussed.

CHAPTER 2

The shelf-life of fresh-cut *Lactuca sativa* L. is not determined by the level of non-enzymatic antioxidants at harvest

Abstract

In response to stress, such as high light, temperature extremes or wounding, antioxidants accumulate to detoxify reactive oxygen species (ROS) in plant cells. It remains unknown whether this role of antioxidants is preserved in the plant after harvest and, if high initial levels of antioxidants may extend storability of harvested products. In the present study we investigated the performance during dark-stored excised (wounded) *Lactuca sativa* L. leaf discs with contrasting initial levels of non-enzymatic antioxidants. We chose two related genotypes, i.e. green (GB, *cv.* Troubadour) and red butterhead lettuce (RB, *cv.* Teodore) differing in antioxidant concentration, and we exposed them to quantitative or qualitative changes in light during growth to introduce further variation in antioxidant content. 40 days after transplanting lettuce heads were harvested, leaves were processed, and discs were monitored for changes in overall visual quality, pigmentation, and levels of main non-enzymatic antioxidants, i.e. carotenoids, ascorbic acid, polyphenolic compounds, as well as oxygen radical absorbance capacity (ORAC). The levels of main non-enzymatic antioxidants were higher in RB than GB. Compared to high light, reduced light resulted in lower levels of main non-enzymatic antioxidants in both genotypes, whereas the use of LED lighting with high percentage blue light increased these levels. The evaluation of postharvest quality during dark storage revealed no consistent relation with the initial amount of antioxidants in the tissue. Samples with an ORAC value of about 3000 μmol TE 100g FW^{-1} displayed a shelf-life in the range of 6 to 20 days. Red tissue, high in antioxidants showed a similar shelf-life as green tissues with much lower antioxidant levels. The use of blue light increased the amount of antioxidants in green tissue, but severely compromised the shelf-life. We hypothesize that, instead of the level of antioxidants, the initial oxidative status and the response to wounding are more important determinants for predicting fresh-cut storability.

Witkowska, I.M., Harbinson J., Woltering, E.J. 2013. The shelf-life of fresh-cut *Lactuca sativa* L. is not determined by the level of non-enzymatic antioxidants at harvest (*submitted)*

Introduction

Fruits and vegetables are an excellent source of nutritionally important compounds (Simonne et al., 1997; Steinmetz and Potter, 1996; Arai et al., 2000; Birt et al., 2001). Mounting evidence suggests that consumption of fruits and vegetables results in long-term health benefits, and may prevent or reduce the risk of several chronic diseases (Jang et al., 1997; Arai et al., 2000; Berger, 2005). Carotenoids, polyphenolic compounds, and ascorbic acid represent the most important antioxidants contributing to the total antioxidant capacity of leafy vegetables (Brecht et al., 2004; Rico et al., 2007). The levels of antioxidants in fresh products can be increased through breeding and selection (Nicolle et al., 2004b; Neill et al., 2002) and through cultivation under "unfavorable" conditions (Voipio and Autio, 1995; Eskins, 1996; Krizek et al., 1998; Romani et al., 2002; Kleinhenz et al., 2003; Gruda et al., 2005; Gazula et al., 2005; Garcia-Marcias et al., 2007; Tsormpatsidis et al., 2008). Stress, such as high light, temperature above or below optimal range and water-deficiency affect the physiology of plants leading to, among others, the accumulation of antioxidants (McKersie and Leshem, 1994; Dixon and Paiva, 1995). Hydrophilic and lipophilic antioxidants accumulate to detoxify reactive oxygen species (ROS) within different compartments of the plant cell (Baker and Orlandi, 1995; Hodges et al., 1996, 1997a, b; Wang et al., 2003). Consequently, as reported in numerous studies (Oh et al., 2009 a, b), inducing the accumulation of antioxidants in plant tissue has become an attractive tool for enhancing nutritional quality of fresh fruits and vegetables. Although a high level of antioxidants is generally believed to be beneficial in coping with various biotic and abiotic stresses, it is not known if increased antioxidant levels are also protective against postharvest stresses such as the wounding induced by cutting and senescence induced by dark storage. Wounding, e.g. in fresh-cut production, is thought to be associated with the production of ROS that may be responsible for the rapid deterioration of the product. As well as enhancing tissue antioxidant levels, this wounding may also induce degradative and oxidative processes (Ferreres et al., 1997; Tomas-Barberan et al., 1997a, b; Kang and Saltveit, 2002; Reyes et al., 2007). Most studies on the effects of wounding on antioxidants in lettuce have been conducted on non-photosynthetic tissue, i.e. mid-rib tissue; and the changes in antioxidants were mostly limited to measurements of polyphenolic compounds, or followed for no more than only 48h. Since, i) photosynthetic tissue comprises a bulk of the fresh-cut prepared from most of lettuce varieties, ii) vascular and photosynthetic tissues are not alike in respect to biochemical composition, iii) polyphenolic compounds are not the only antioxidant compounds responsible for scavenging of ROS, and iv) the commercial shelf-life of fresh-cut lettuce is approximately 10 days, we aim in the present study to investigate the performance of wounded photosynthetic tissue with contrasting initial levels of main non-enzymatic antioxidants during a prolonged cold storage. It has been hypothesized that the specific circumstances that lead to higher levels of antioxidants may partly protect against the stress caused by wounding in the preparation of fresh cut products. The model species used for the experiment was butterhead lettuce (*Lactuca sativa* L.), which is an important leafy vegetable available worldwide over the whole year. Besides, lettuce also contains appreciable amounts of nutritionally important compounds (Caldwell, 2003; Cao et al., 1996; Chu et al., 2002; Vinson et al., 1998), and appears to exert a diversity of beneficial health effects (Caldwell, 2003; Llorach et al., 2004; Nicolle et al., 2004a, b). We chose two

related lettuce genotypes, green (GB) and red butterhead (RB), as it has been reported that red, anthocyanin containing tissues have higher levels of antioxidants (Caldwell, 2003; Ferreres et al., 2007; Zhao et al., 2007; Liu et al., 2007; Llorah et al., 2008). Plants of both genotypes were grown under different lighting (quality and intensity) conditions to introduce further variation in antioxidant concentration. The obtained photosynthetic, leaf tissue was processed by cutting similar to that done commercially, and monitored for quality changes during storage.

Materials and methods

Plant material and growth conditions

Two related genotypes of *Lactuca sativa* L., green butterhead genotype (GB) *cv.* Troubadour and red butterhead genotype (RB) *cv.* Teodore (Rijk Zwaan B.V., The Netherlands) were used in two experiments. The seeds were sown in boxes filled with vermiculite, and after one week seedlings were transplanted to a hydroponic system (Hoagland's solution, pH 5.9±0.2; EC=1.2 mS cm^{-1}) in a climate chamber located at Wageningen University, The Netherlands. Temperatures were maintained at 20°C during the day and at 15°C during the night, and the relative humidity was 70%. Three light conditions (12h photoperiod), differing either in light quantity or quality, were used to influence antioxidant levels in the plants. For the control (C) 250 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) was provided by white fluorescent tubes (TLD 50 W 840 HF, Philips, The Netherlands). For reduced light treatment (RL) PAR was reduced by 75% (experiment 1) or 50% (experiment 2) as compared to the control, using a neutral shade filter (210.06ND and 210.03ND; Lee Filters, Andover, UK). LED lighting was provided by light emitting diodes (LEDs) at the same intensity as the control (250 μmol m^{-2} s^{-1}). The LEDs arrays consisted of 30% of red (peak wavelength at 667 nm), and 70% of blue (peak wavelength at 465 nm) LED modules (Greenpower LED modules HF, Philips, Eindhoven, The Netherlands). The seedlings and lettuce plants were not chemically treated before or during the experiment. Forty days after transplanting plants were harvested and used for the postharvest storage experiment.

Storage experiment with excised discs and intact leaves

Leaf discs excised with a stainless steel cork borer (18 mm diameter) were used as mimic of commercial fresh-cut lettuce. The leaf discs were cut from leaf lamina avoiding the inclusion of the midrib and major veins. This method of cutting was used to assure similar wounding for all samples and treatments. Randomly selected leaf discs and intact leaves were placed in plastic Petri dishes (25×100-mm), with vents, lined with wet filter papers (Whatman, #3) to prevent desiccation of leaf discs. The dishes were placed in temperature controlled storage units (Elbanton, Kerkdriel, The Netherlands) at 12°C in the dark, and relative humidity of 90%.

At every sampling time, 9 leaf discs were randomly selected from different Petri dishes (replicates), weighed, and specific leaf weight on fresh weight basis (SLW) was calculated. Per each replicate, two leaf discs were used directly for determination of pigments, two leaf discs were used directly for acid extraction of AsA, and two (phenolics) or three (ORAC) leaf discs were frozen in liquid nitrogen, dried using a freeze-dryer

(Modulyo, Pirani 501, Edwards, UK) and ground into fine powder with a ball-mill (Retsch, Germany).

Overall visual quality (OVQ) and shelf-life

The quality of both the lettuce discs was evaluated using overall visual quality (OVQ) ratings modified from Kader et al. (1973). The OVQ was evaluated on the basis of leaf characteristics such as color (yellowing, browning, and brightness), wilting, and presence or absence of defects. Quality ratings were made on a scale from 9 to 1, where 9 = excellent, essentially free from defects, and 1 = extremely poor, not usable. An OVQ rating of 6 was considered the lower limit of consumer acceptance.

Determination of pigments

Pigments were extracted in dimethylformamide (DMF) in the dark at -20°C. The absorbance of the extract was measured in the range 400–750 nm using a Cary 4000 spectrophotometer (Varian Instruments, Walnut Creek, CA, USA), and the pigment concentration was calculated using the equations provided by Wellburn (1994).

Determination of ascorbate (AsA)

Ascorbate was measured according to the method of Foyer et al. (1983). This assay measures the A_{265} that is specifically removable by ascorbate oxidase (AO), which converts reduced ascorbate (AsA) to nonabsorbing oxidized forms. AsA was measured without pretreatment of extracts, while total AsA was measured after conversion of DHA to AsA by incubation with dithiothreitol (DTT). AO was dissolved in 0.2M NaH_2PO_4 (pH 5.6) at 40 U ml^{-1}, divided into batches (0.2 ml) that were stored at –20°C, and freshly thawed prior to the analysis. To assay AsA, aliquots of 0.15 ml neutralized supernatant were added to UV-cuvettes (Plastibrand, Germany) containing 1.5 ml of 0.2M NaH_2PO_4 (pH 5.6) and 1.1 ml of milli-Q water. The initial A_{265} of the mixture was recorded and 30 µl of AO was added, the solution remixed, and the decrease in A_{265} value was monitored. In general, a stable value was reached within 2 to 3 min, so the absorbance change reached 5 min after the addition of AO was used to estimate the AsA concentration. To assay total ascorbate, 0.3 ml neutralized supernatant was added to 2.1 ml of 0.12M NaH_2PO_4 (pH 7.5) and 60 µl of 25 mM DTT, and solutions were incubated for 20 min at room temperature. Thereafter, the solution was assayed as described for AsA. The absorbance was converted to ascorbic acid concentration in terms of milligrams of AsA per 100 gram of fresh weight (FW) of sample.

Extraction and quantification of polyphenolic compounds

Ten micrograms of powdered freeze-dried lettuce sample was mixed with 1.5 ml of 80% methanol, and the mixture was placed in an ultrasonic bath at 20°C for 4 minutes. After centrifugation, the supernatant was used for the determination of polyphenolic compounds.

The total amount of polyphenolic compounds in lettuce was determined using Folin-Ciocalteu's reagent according to the method of Singleton and Rossi (1999). Three hundred microliters of the methanolic extract was mixed with 1.5 ml of 2M Folin-Ciocalteu reagent, and then the mixture was added to 1.20 mL of 20% Na_2CO_3. After incubation at room temperature for 30 min, the mixture was centrifuged, and the absorbance of the supernatant was measured at 735 nm. The standard curve was prepared using gallic acid

(GA). The absorbance was converted to phenolic concentration in terms of milligrams of GA equivalent (GAE) per 100 gram of fresh weight (FW) of sample.

Oxygen radical absorbance capacity (ORAC)

The extraction of the antioxidant fraction and determination of antioxidant capacity in lettuce was performed using the oxygen radical absorbance capacity (ORAC) method modified from Huang et al. (2002). Fifteen micrograms of powdered freeze-dried lettuce sample was vortexed for 1 min with 1 ml of hexane. After centrifugation the supernatant was collected, and the pellet re-extracted in 1 ml of hexane. The combined hexane extracts were evaporated to dryness at 40°C using a vacuum evaporator (Savant SPD2010 SpeedVac, Thermo Scientific, Asheville, NC, USA). The pellet remaining after the hexane extraction was dried and re-extracted with 1.5 ml of acetone/water/acidic acid mixture (70:29.5:0.5; v/v/v) to obtain the hydrophilic fraction. For the lipophilic antioxidant assay, the dried hexane extract was re-dissolved in 250 μl of acetone and then diluted with 750 μl of a 7% randomly methylated b-cyclodextrin solution (RMCD; 50% acetone/50% water, v/v; Huang et al., 2002). Any further dilution was made with the 7% RMCD solution. The 7% RMCD solution was also used as a blank and to dissolve and dilute the Trolox standards for the lipophilic assay. For the hydrophilic assay, phosphate buffer (0.075 M, pH 7.4) was used as the solvent. This phosphate buffer was also used as the blank and the solvent for the Trolox standards in the hydrophilic assay. The fluorescence intensity measurement was performed using a Safire monochromator-based microplate reader (Tecan USA, Research Triangle Park, NC) with the sample loaded on polystyrene, flat-bottom 96-well plate (Fluotrac, Greiner America, Inc.). The 20 μl of diluted sample was mixed with 100 μL fluorescein solution in a microplate and incubated at 37° C for 15 min, after which 40 μl of 2,20-Azobis (2-amidino propane) dihydrochloride (AAPH) (17.2 mg ml^{-1}) was added to each well using a multi-channel pipette. Immediately following the addition of AAPH, the plate was agitated for 5 s prior to the first reading and for 2 s before each subsequent reading. Readings were done at 2 min intervals for 40 min. Excitation and emission filter wavelengths were set at 484 nm and 520 nm, respectively. Data were expressed as μmol Trolox equivalents (TE) per gram of lettuce on a fresh dry weight (FW) basis. The ORAC values were calculated by using a linear regression equation (Y = a + bX) between concentration (Y) (μM) and the net area under the fluorescence decay curve (X). Linear regression was used in the range of 6.25–50 μM Trolox. Blank, standard and sample were analysed in triplicate to eliminate position effects.

The area under curve (AUC) was calculated as follows:

$$AUC = (0.5 + f_1/f_0 + f_2/f_0 \cdots + f_i/f_0)\ CT$$

where f_0 is the initial fluorescence reading at cycle 0, f_i is the fluorescence reading at cycle i, and CT is the cycle time in minutes. The net area under the curve was obtained by subtracting the blank value from that of a sample or standard.

Statistical analysis and curve fitting

Data were processed using the SPSS statistical package (IBM SPSS, release 19.0.0.1, 2010. SPSS Inc. and IBM Company, Chicago, IL, USA). Each experiment had three replicates per light condition during growth. In both experiments, FW, SLW, chlorophyll, carotenoids,

and polyphenolic compounds concentrations, $ORAC_{total}$, and $ORAC_{lipophilic}$ were analysed as dependent variables, using mixed analysis of variance. For initial levels of analysed characteristics we assumed fixed effect for light conditions and genotype, and random effect of the replicate. The interactions between genotype × light conditions were studied by looking at light condition differences per genotype, and at genotype differences per light condition. The effect of cold storage on OVQ was analysed per genotype and for each light condition separately. Mean separations was performed using the LSD procedure, and significance was declared at $P < 0.05$. The trends in OVQ data were fitted in Excel (Microsoft Office 2010 for Windows) with polynomial (order 2) regression lines.

Results

The levels at harvest of all parameters were measured for both lettuce genotypes grown under three light conditions, i.e. control (C), reduced light (RL), and light emitting diodes (LEDs). In two independent experiments this yielded essentially similar results. Only in the second experiment changes in the parameters during cold storage of excised discs were measured for the RL and LEDs.

Parameters measured at harvest

Structural properties

The light conditions during growth resulted in a range of specific leaf weight (SLW) (Figure 1). The SLW was usually slightly lower in RB compared to GB, irrespective of the light condition. For both lettuce genotypes, the SLW was lowest for plants grown under RL and highest for plants grown under LEDs.

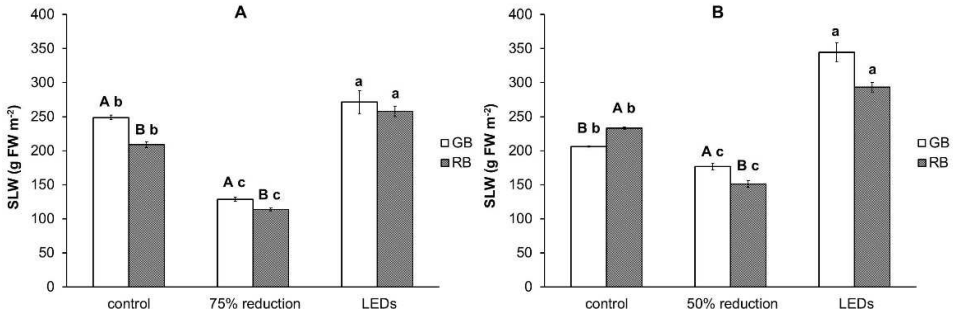

Fig. 1. Initial levels of specific leaf weight (SLW) of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light and LED lighting in experiment 1 (A) and 2 (B). Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for comparison between genotypes per light condition, and lower case for comparison between light condition per genotype (a–c), are significantly different ($P<0.05$).

Pigment concentrations

In general, the chlorophyll and carotenoids concentrations were on average 2-fold higher in RB than in GB irrespective the light condition (Figure 2). However, in experiment 2 the differences in the concentration of both compounds were not significant for the plants grown under LEDs (Figure 2b, d). In both experiments, the chlorophyll (Figure 2a, b) and carotenoids (Figure 2c, d) concentration in GB was higher in plants grown under LEDs than under the other light conditions (Figure 2a, b). In experiment 1, the chlorophyll (Figure 2a) and carotenoid (Figure 2c) concentration in RB was similar for plants grown under control and LEDs, and lowest from plants grown under RL. In experiment 2, concentration of these compounds in RB (Figure 2b, d) was similar for plants grown under all light conditions.

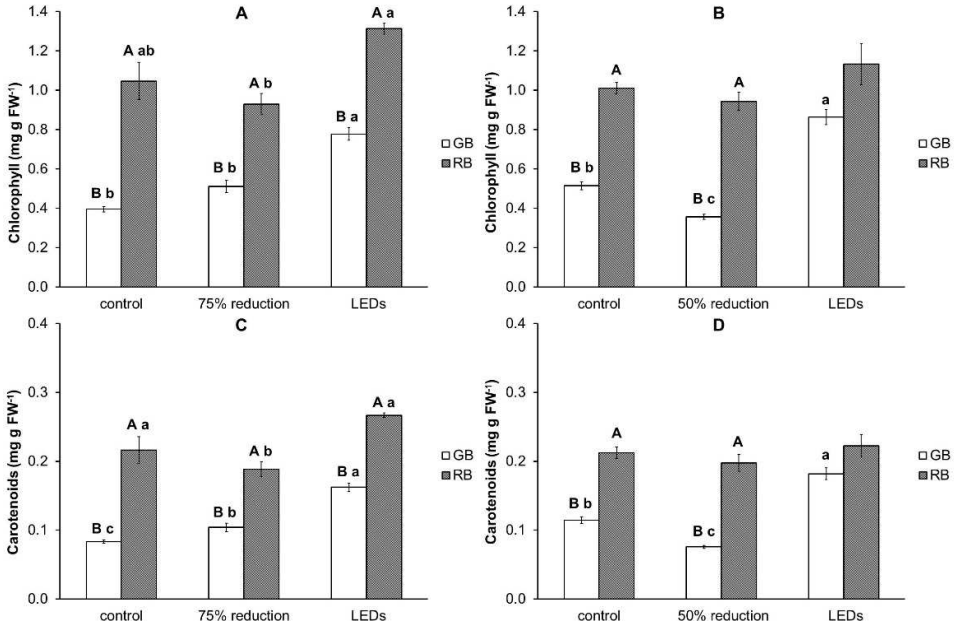

Fig. 2. Initial levels in chlorophyll and carotenoids of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light and LED lighting in experiment 1 (A, C) and 2 (B, D). Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for comparison between genotypes per light condition, and lower case for comparison between light condition per genotype (a–c), are significantly different ($P<0.05$).

Polyphenolic compounds and oxygen radical absorbance capacity (ORAC)

As was found for the chlorophyll and carotenoids concentrations, the concentrations of polyphenolic compounds, total ORAC and the lipophilic fraction of ORAC were in general higher in RB than GB (Figure 3e, f). However, in experiment 2 the differences in the lipophilic fraction of ORAC was not significant for the plants grown under LEDs (Figure 7f). For GB, in both experiments the levels of polyphenolic compounds (Figure 3a, b) and total ORAC (Figure 3c, d) were highest for plants grown under LEDs, and lowest for plants grown under RL, whereas the lipophilic fraction of ORAC (Figure 3e, f) was highest for plants grown under LEDs, but similar for plants grown

under control and RL. For RB, in experiment 1 the levels of polyphenolic compounds (Figure 3a), total ORAC (Figure 3c), and the lipophilic fraction of ORAC (Figure 3e) were lowest for plants grown under RL, and similar for plants grown under control and LEDs. In experiment 2, the levels of polyphenolic compounds (Figure 3b) and total and lipophilic ORAC (Figure d, f) were lowest for plants grown under RL, and highest for plants grown under LEDs. The total ORAC was 99% hydrophilic fraction, and only 1% lipophilic fraction. A positive linear relationship was established between polyphenolic compounds and total ORAC for data from these experiments, and similar experiments conducted also on other lettuce varieties (Figure 4).

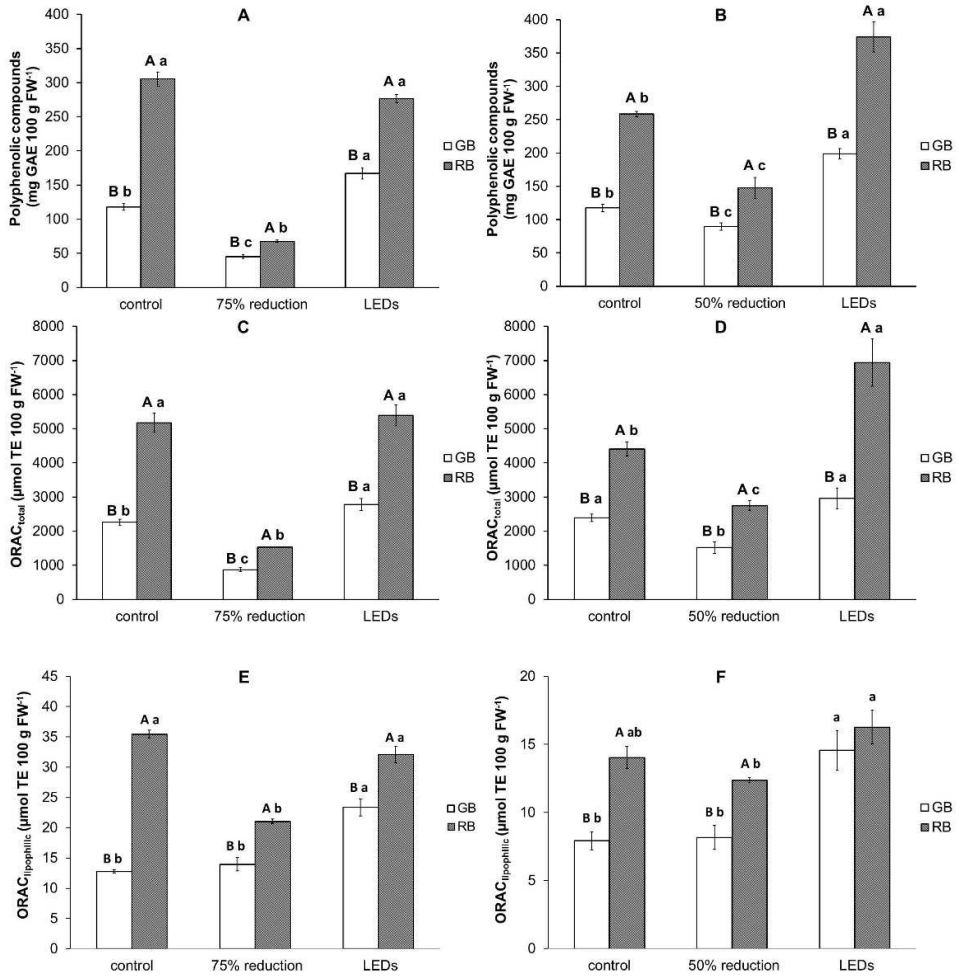

Fig. 3. Initial levels in polyphenolic compounds concentration, total oxygen radical absorbance capacity (ORAC), and lipophilic ORAC of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light and LED lighting in experiment 1 (A, C, E) and 2 (B, D, F). Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for comparison between genotypes per light condition, and lower case for comparison between light conditions per genotype (a–c), are significantly different ($P<0.05$).

Total ascorbate (AsA)

The total AsA concentration was higher in RB than in GB for plants grown under RL, and was about similar in both genotypes for plants grown under LEDs (Figure 5). The AsA concentration in GB was 8-fold higher, and in RB 2-fold higher when plants were grown under LEDs compared to RL.

Parameters measured during storage experiment

The most striking differences in initial parameters were observed between reduced light and LED treatments in experiment 1. Therefore, the changes in these parameters during dark storage of excised leaf discs in experiment 2 were studied only in plants from these light conditions.

Structural properties

There were generally no changes in SLW during storage with the exception in GB grown under LEDs (Figure 6). For plants grown under this light condition, SLW of discs increased slightly (15-20%) during storage, suggesting that the discs took up water from the humid environment.

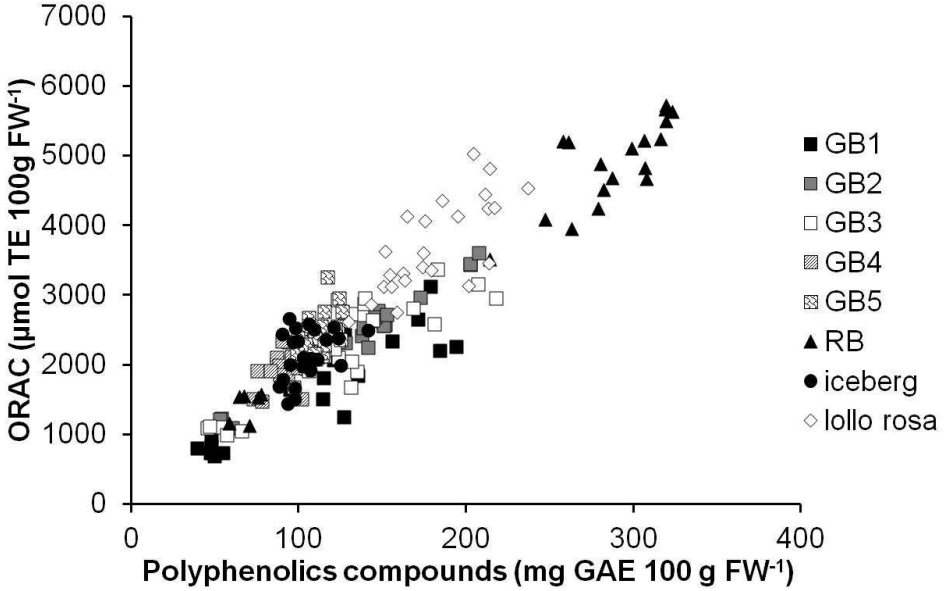

Fig. 4. Relationship between polyphenolic compounds and oxygen radical absorbance capacity (ORAC) established in different lettuce genotypes, i.e. butterhead (green, GB and red, RB), iceberg and lollo rosa.

Pigment concentrations

As for SLW, the chlorophyll and carotenoid concentrations showed little change during storage (Figure 7), except in GB that were grown under LEDs, where the chlorophyll and carotenoids concentrations decreased by about 50% during storage.

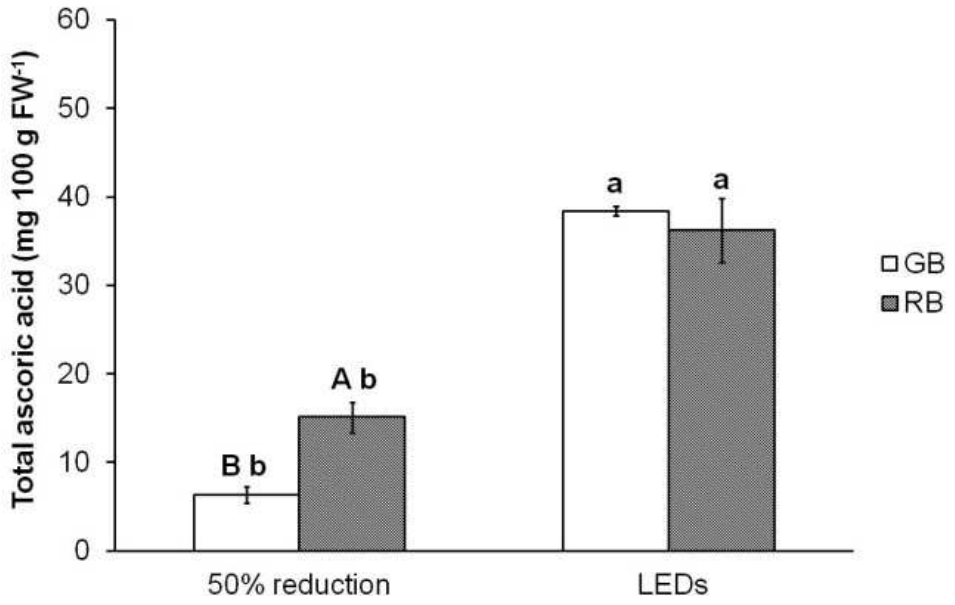

Fig. 5. Initial levels of total ascorbic acid of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light and LED lighting in experiment 2. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for comparison between genotypes per light condition, and lower case for comparison between light conditions per genotype (a–c), are significantly different (P<0.05).

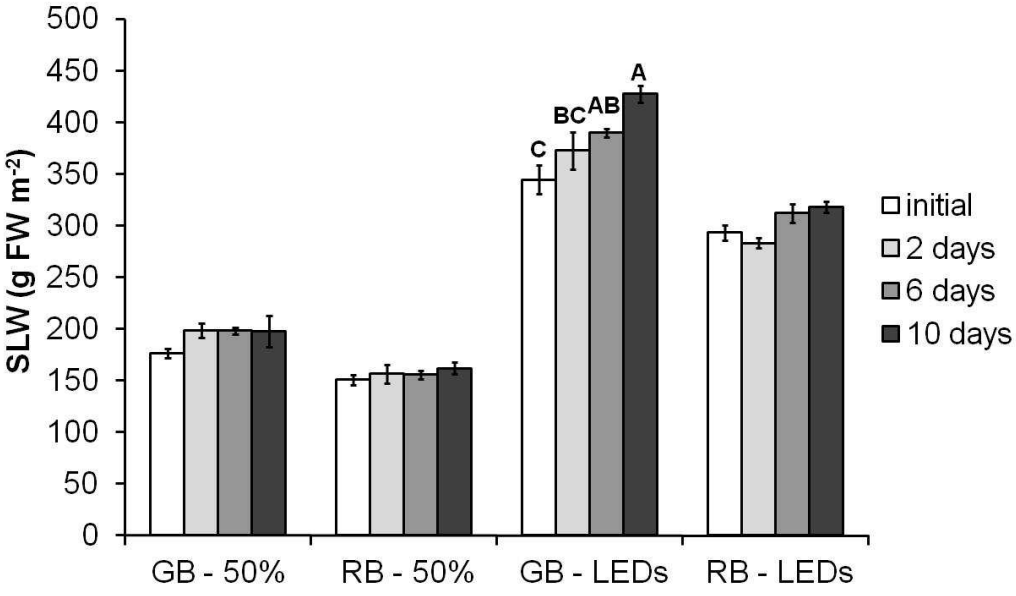

Fig. 6. Changes during cold storage in specific leaf weight (SLW) of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. reduced light and LED lighting in experiment 2. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for comparison between storage times are significantly different (P<0.05).

Polyphenolic compounds

The concentration of polyphenolic compounds decreased by 20 to 30% during storage with the exception for GB grown under LEDs, where no clear change was observed (Figure 8). The differences between treatments in the levels of polyphenolic compounds at the end storage corresponded to the differences observed in the initial levels. Based on the positive linear relationship between polyphenolic compounds and ORAC in a number of experiments (Figure 4), the same pattern of change is expected for the total ORAC.

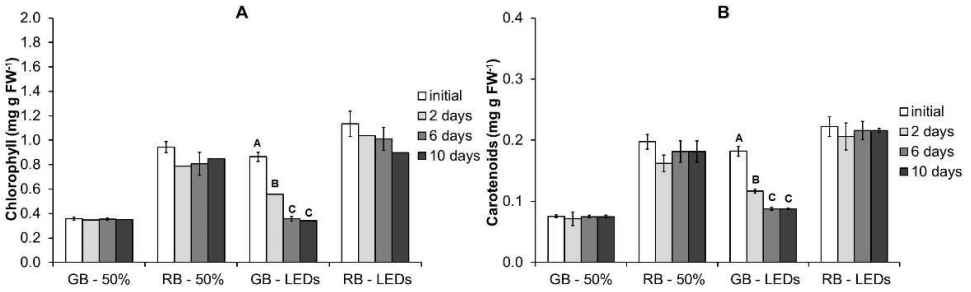

Fig. 7. Changes during cold storage in chlorophyll concentration (A) and carotenoids (B) concentration of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. reduced light and LED lighting in experiment 2. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for GB, and lower case for RB (a–c), for comparison between storage times are significantly different (P<0.05).

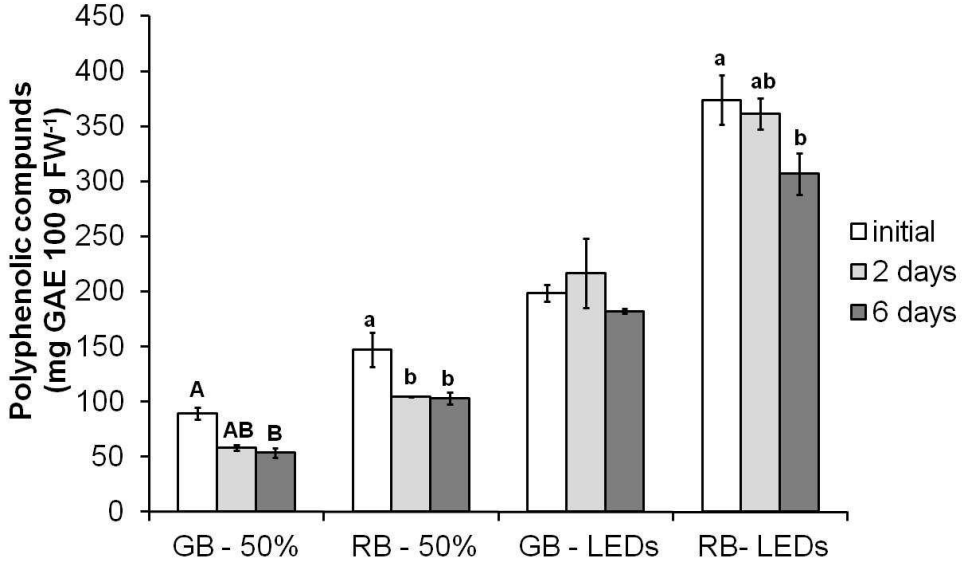

Fig. 8. Changes during cold storage in polyphenolic compounds concentration of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. reduced light and LED lighting in experiment 2. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-B) for GB, and lower case for RB (a–c), for comparison between storage times are significantly different (P<0.05).

Total ascorbate (AsA)

Without exception, a significant decrease in total AsA was found during dark storage for both genotypes grown under both growth light conditions (Figure 9). The total AsA decreased to a greater extends during storage for both genotypes grown under LEDs (80%) than for plants grown under 50% reduced light (50-60%). At the end of the storage the lowest AsA levels were reached in GB grown under 50% reduced light.

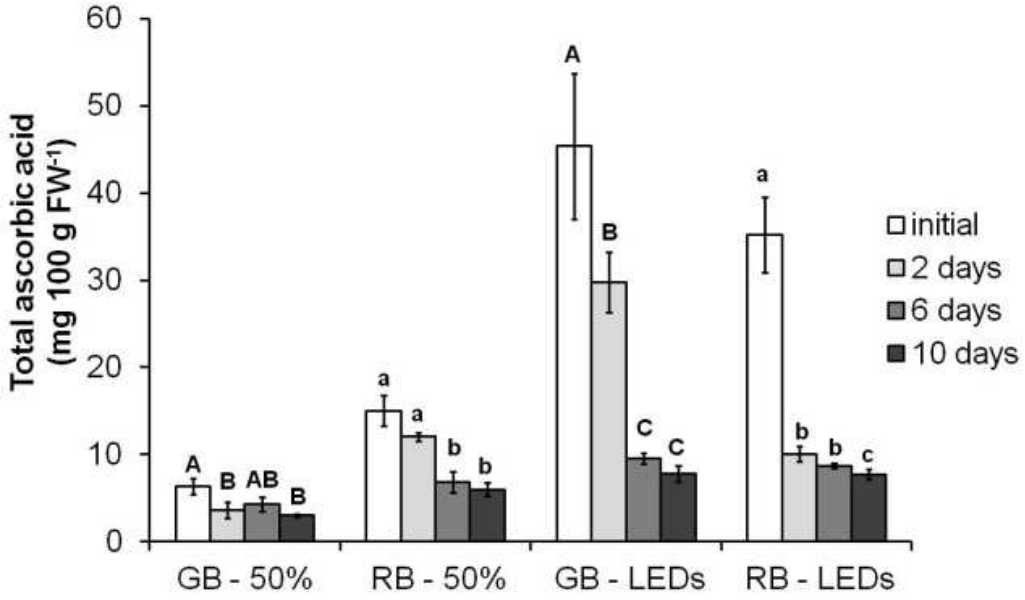

Fig. 9. Changes during cold storage in concentrations of total ascorbic acid of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. reduced light and LED lighting in experiment 2. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-C) for GB, and lower case for RB (a–c), for comparison between storage times are significantly different ($P<0.05$).

Overall visual quality (OVQ)

Overall visual quality (OVQ) of dark-stored excised discs decreased for both genotypes grown under all light conditions in both experiments (Figure 10). Compared to the control, the decline in OVQ was faster in discs excised from plants grown under reduced light. The response of the two genotypes to reduced light was similar (Figure 10b, e). Expressed in shelf-life (number of days until the OVQ score fell to 6) 75% light reduction decreased shelf-life by over 10 days and 50% light reduction decreased shelf-life by about 2 days in both genotypes. In contrast, the decline in OVQ differed between two genotypes when they were grown under LEDs (Figure 10c, f). Under this light condition the OVQ of GB showed a faster decrease, relative to the respective control, than the OVQ of RB. Expressed in shelf-life LEDs had no negative effect in RB, whereas in GB the LED treatment decreased the shelf-life by 2 (experiment 1) and 10 days (experiment 2). The quality of the dark stored intact leaves was preserved 5 to 10 days longer than the quality of excised discs, depending on the light conditions during growth (Figure 11). Compared to the control, 75% light reduction decreased shelf-life by about 14 days in both genotypes

(Figure 11b); whereas 50% light reduction during growth significantly decreased the shelf-life of GB but not of RB (Figure 11e). Compared to the control, the LED lighting during growth significantly decreased shelf-life of GB in both experiments, by 4 (experiment 1, Figure 11c) and 14 days (experiment 2, Figure 11f). The LED lighting effect on shelf-life of RB was significant only in experiment 1.

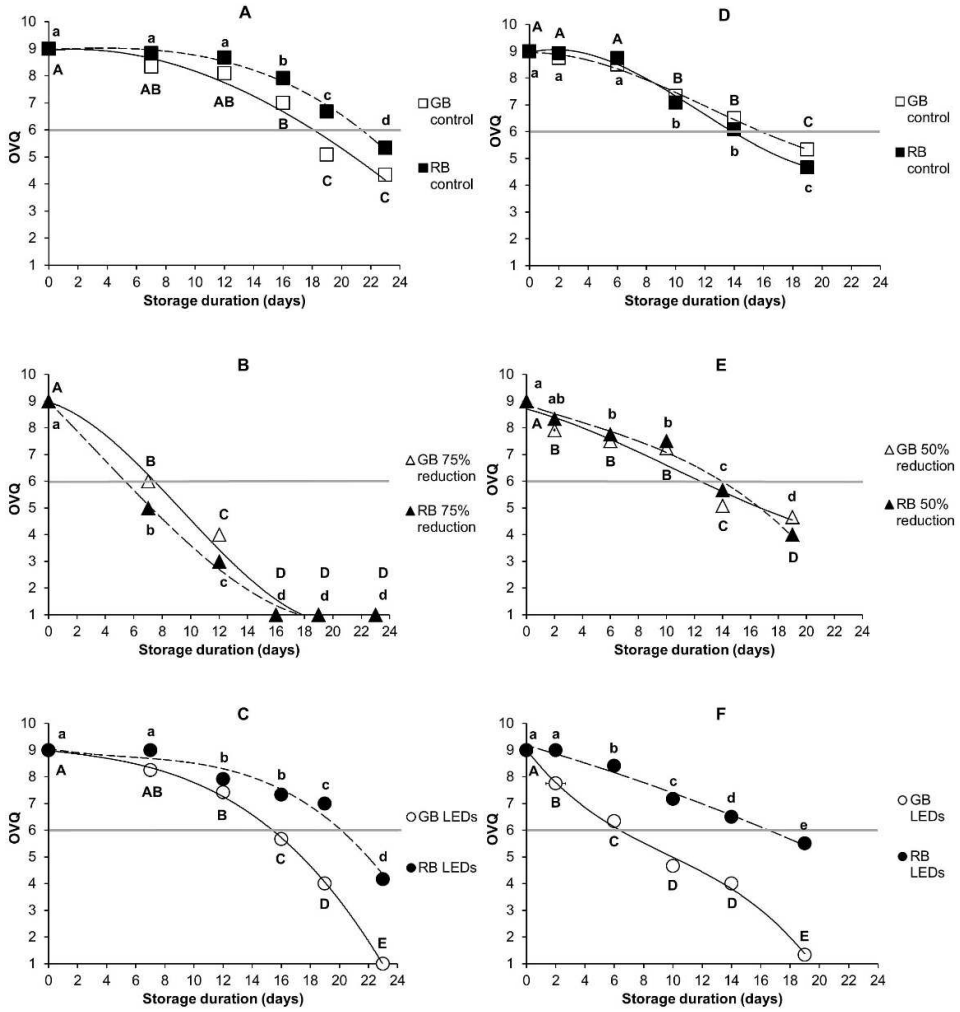

Fig. 10. Overall visual quality (OVQ) changes during storage of excised leaf discs of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light and LED lighting in experiment 1 (A, B, C) and 2 (D, E, F). Data points marked by different letters, i.e. upper case (A-E) for GB, and lower case for RB (a–e), for comparison between storage times are significantly different ($P<0.05$).

Relationship between shelf-life of excised discs and ORAC

A correlation graph between the initial levels of antioxidants (total ORAC) and the shelf-life of excised discs derived from this study shows that there is no clear correlation (Figure

12). For example, with ORAC value of 3000 μmol TE 100g FW^{-1} the shelf-life was in range from 6 to 20 days.

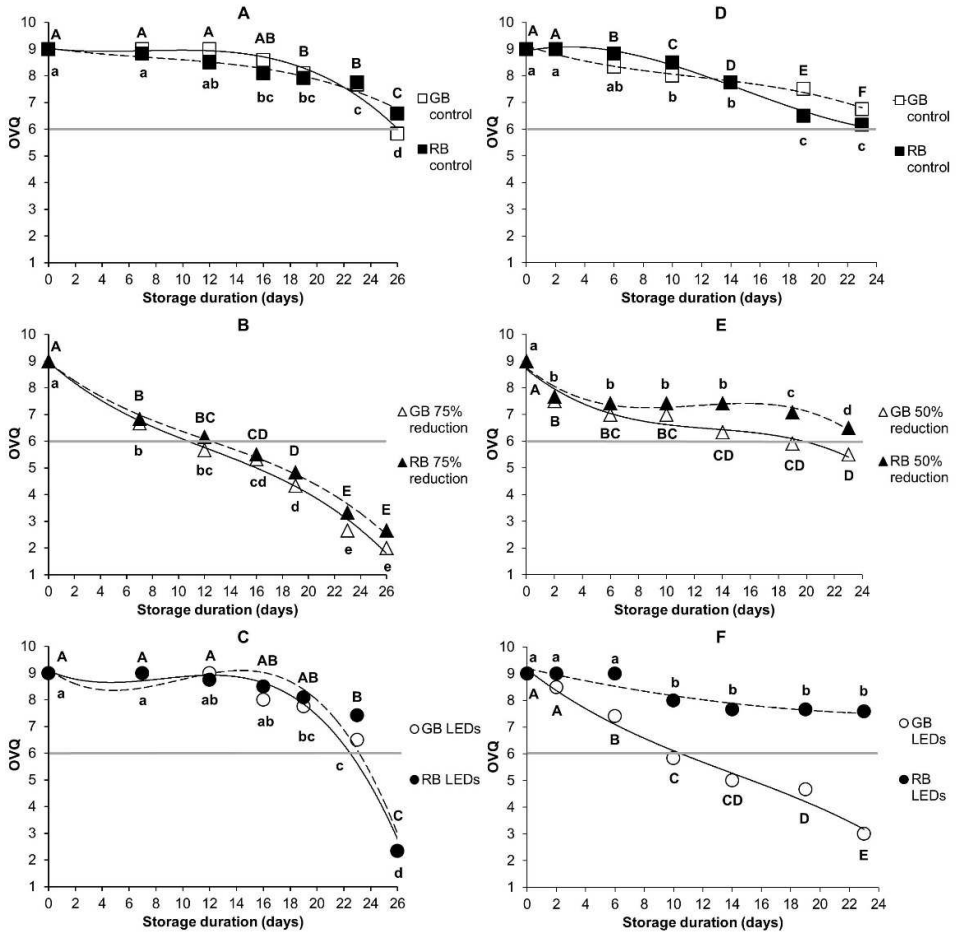

Fig. 11. Overall visual quality changes during storage of leaves of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light and LED lighting in experiment 1 (A, B, C) and 2 (D, E, F). Data points marked by different letters, i.e. upper case (A-F) for GB, and lower case for RB (a–c), for comparison between storage times are significantly different ($P<0.05$).

Discussion

A common denominator of many types of stress is accumulation of potentially toxic reactive oxygen species (ROS) within the plant system, and subsequent up-regulation of the enzymatic antioxidant activity and of the hydrophilic and lipophilic antioxidants within different compartments of the cell to detoxify ROS (McKersie and Leshem, 1994; Baker and Orlandi, 1995; Dixon and Paiva, 1995; Hodges et al., 1996, 1997a, b; Hodges and Forney, 2000; Wang et al., 2003). Therefore, it has been suggested that the stress tolerance of plants depends strongly on its antioxidant capacity (Hodges and Forney, 2000; 2003). In

this study we show that the antioxidant status does not increase the capacity of lettuce plants to withstand postharvest wounding.

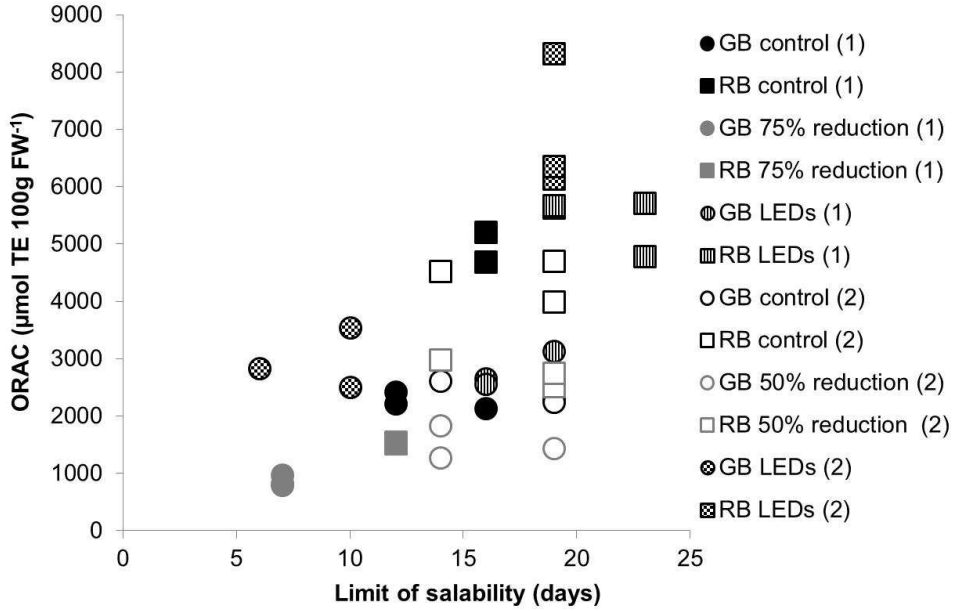

Fig. 12. Relationship between oxygen radical absorbance capacity (ORAC) of the tissue at harvest and the end of shelf-life leaves of two butterhead genotypes (green, GB and red, RB) grown under different light conditions, i.e. control, reduced light (50% and 75%) and LED lighting in experiment 1 (1) and 2 (2).

Antioxidants in growing plants

We succeeded in obtaining plant material with contrasting levels of non-enzymatic antioxidants using two different lettuce genotypes and different quantities and qualities of light during growth. In general, the concentration of carotenoids (Figure 2), polyphenolic compounds, total antioxidant capacity, and its lipophilic fraction (Figure 3) were highest for plants grown under higher light intensities (provided by Fluorescent tubes and by LEDs), compared to plants grown under 50 and 75% reduced light (RL). Also the total ascorbic acid (AsA) concentration was higher for plants grown under LED lighting than for plants grown under RL (Figure 5). These results are in close agreement with other work on the effects of light on carotenoids (Demming-Adams and Adams, 1992; Demming-Adams and Adams, 1993; Asada et al., 1994; Thayer and Bjorkman, 1990), polyphenolic compounds (Awada et al., 2001, Ebisawa et al., 2008; Kojima et al., 2010; Johkan et al., 2010), and AsA (Harris, 1975; Mozafar et al., 1994; Lee and Kader, 2000; Gatzek et al., 2002; Bartoli et al., 2006).

The effect of different light conditions during growth on the antioxidants in GB and RB appeared to be determined by difference in the concentration originally present in these genotypes. In general, the levels of carotenoids (Figure 2), polyphenolic compounds, ORAC, the lipophilic fraction of ORAC (Figure 3), and AsA (Figure 5) were higher in red butterhead (RB) than in green butterhead (GB). Only in plants grown under LED lighting in experiment 2 were the levels of carotenoids, the lipophilic fraction of ORAC, and AsA the same in both genotypes. The red tissue, which is inherently high in antioxidants

(Caldwell, 2003; Ferreres et al., 2007; Zhao et al., 2007; Liu et al., 2007; Llorah et al., 2008), in particular in polyphenolic compounds, reacted less than GB to the LED lighting with its high percentage of blue light. It is well established that plant development and physiology are strongly influenced by the light spectrum (McNellis and Deng, 1995). The spectrum of blue light near the ultraviolet region may induce similar responses to environmental signals for ultraviolet, and induce synthesis of polyphenolic compounds and overproduction of reactive oxygen species (ROS) (Ebisawa et al., 2008; Ryan et al., 2002). Only in experiment 2 did we observe an increase in polyphenolic compounds and total ORAC under LED lighting, whereas levels of carotenoids and the lipophilic fraction of ORAC were hardly affected by LED lighting. In contrast, GB, inherently low in antioxidants, reacted strongly to the LED lighting by increasing the concentration of all antioxidants measured in both experiments. Evidently, the existing pool of antioxidants in RB was sufficient to protect this tissue from oxidative processes induced by the applied light conditions during growth due to i) a sun screen effect of anthocyanins on the blue light, and ii) an active involvement of carotenoids, polyphenolics and AsA in scavenging of ROS. The opposite was true for GB, to prevent oxidative damage to the thylakoids membranes the tissue accumulated higher levels of antioxidants.

Total antioxidant capacity varied with light conditions during growth and with genotype in the same manner as the concentration of polyphenolic compounds (Figure 3). Consequently, we established a high correlation between antioxidant activity and total phenolic concentration (Figure 4), which is in agreement with previous studies (Gardner et al., 2000; Reyes et al., 2007; Johkan et al., 2010), and observation of Proteggente et al. (2002) that the polyphenolic compounds are the group of compounds contributing mainly to the antioxidant capacity of photosynthetic tissue. It's not surprising, since certain flavonoids such as for example quercetin or epicatechin have 5-fold higher antioxidant power than ascorbate and tocopherol (Rice-Evans et al., 1995).

Senescence in detached and wounded plants during storage

Stresses, such as physical detachment of leaves and wounding promote oxidative processes in the tissue, and may accelerate leaf senescence (Philosoph-Hadas et al., 1991; Bartoli et al., 1996). A link between the regulation, properties, and/or dynamics of senescence and ROS has been previously reported (Droillard et al., 1987; Hodges and Forney, 2003; Philosoph-Hadas et al., 1994; Thompson et al., 1991). Chlorophyll breakdown has been considered as one of the senescence symptoms, and is often used to estimate extend of senescence in photosynthetic tissue (Kar and Feierabend, 1984; Philosoph-Hadas et al., 1991; Meir et al., 1995). With an exception for GB grown under LED lighting, the chlorophyll concentration did not decrease during dark storage (Figure 7). These results are in agreement with Bergquist et al. (2006) and Hodges and Forney (2003) who found no decrease in chlorophyll concentration in young spinach leaves until 12 days of storage at 10°C. However, in our opinion these results do not necessarily mean that the senescence was not induced by wounding, and that the senescence related process were not responsible for the quality deterioration. The chlorophyll measurements ended before the fresh-cuts reached the end of shelf-life and the visible symptom in loss of colour developed. Besides, chlorophyll not always is a reliable indicator of senescence (Munné-Bosch and Alegre, 2004). In contrast, the rapid decrease in chlorophyll in the discs excised from GB plants grown under LED lighting clearly indicated high oxidative stress in this

tissue, and the on-going senescence. In these excised leaf discs senescence may have played a decisive role in the pronounced postharvest quality deterioration.

Antioxidants in detached and wounded plants during storage

As carotenoids are relatively stable compared to other antioxidants, e.g. ascorbic acid (Buescher et al., 1999), stable levels of carotenoids during storage of non-senescing tissue, i.e. GB grown under RL, and RB grown under both light conditions seem to be well justified (Figure 7). Changes during storage observed in carotenoids concentration that paralleled changes in chlorophyll concentration of GB previously grown under LED lighting may be explained by carotenoids being involved in the protection of thylakoid membranes during senescence. Carotenoids together with tocopherols are the most abundant groups of lipid soluble antioxidants in chloroplasts (Munné-Bosch and Folk, 2004), responsible for scavenging of ROS (Cazzonelli et al., 2011). Also other studies on leafy vegetables have mainly reported decreased or unchanged carotenoid concentration during storage (Ezell and Wilcox, 1962; Kopas-Lane and Warthesen, 1995; Pandrangi and Laborder, 2004).

Unlike the majority of previous studies, which have typically chosen just a few days of storage (Tomas-Barberan et al., 1997a, b; Kang and Saltveit, 2002; Reyes et al., 2007), we have monitored changes in polyphenolic compounds during prolonged cold storage (Figure 8). In doing so, we have revealed that polyphenolic compounds decrease during cold storage of wounded tissue. The difference between our results and results of others may be also explained by differences in types of lettuce used (Reyes et al., 2007), and by the type of tissue used, i.e. photosynthetic versus vascular (mid rib) tissue (Ferreres et al., 1997). We agree with Ferreres et al. (1997) that the wound-induced accumulation of polyphenolic compounds might have a minor contribution to the total pool of polyphenolics in the photosynthetic tissues, which is very rich in this type of metabolite.

Finally, a decrease of AsA in plants from all treatments (Figure 9) agrees with a common response in photosynthetic tissue to placement in darkness (Bartoli et al., 2006).

Antioxidants and shelf-life of detached and wounded plants

There was no clear trend between the initial levels of antioxidants and the shelf-life of excised discs. RB, being remarkably higher in antioxidants than GB had approximately the same shelf-life (Figure 2, 3, 4). Light conditions leading to higher initial antioxidants levels did not increase the shelf-life (Figure 3, 10 and 12). AsA was the only component that decreased sharply during storage of excised leaf discs from all light treatments. However, both the initial levels of AsA (Figure 4) and the rate of decrease during dark storage of the discs (Figure 7) could not explain the variation in the shelf-life.

The most striking observations were the differences in the behaviour between GB grown under LED lighting and the GB grown under other light conditions. When grown under LED lighting, initial levels of chlorophyll and carotenoids were high but severe breakdown took place during later dark storage. As green colour is an important determinant of OVQ, the shelf-life of these discs was scored much lower compared to discs from plants grown under other light conditions. The behaviour of this tissue during storage might be explained by two phenomena. Firstly, the pre-harvest stage gives tissue certain potential with respect to antioxidants and ROS (Mckersie and Leshem, 1994; Foyer et al., 1994a; Baker and Orlandi, 1995; Dixon and Paiva, 1995; Hodges et al., 1996, 1997a, b;

Hodges and Forney, 2000; Wang et al., 2003), and the detachment and wounding of the plant adds to the pool of oxidative stress. Secondly, the difference in post-harvest behaviour of wounded lettuce is a function of the present pool of oxidative stress in the tissue (Weston and Barth, 1997; Galvis-Sanchez et al., 2004; Calderon-Lopez et al., 2005). Apparently the tissue of LED light grown GB, although boosted in carotenoids (Figure 2) and AsA (Figure 5) to the similar levels as RB, was not able to cope with the postharvest oxidative stress. The increased initial levels of antioxidants in response to growth conditions (section 4.1) together with the accelerated loss of chlorophyll during storage (section 4.2) in this tissue indicate increased level of oxidative stress comparing to other treatments. In detached and wounded tissue remobilisation of metabolic components is not possible; a hyper accumulation of toxic molecules, such as free radicals may therefore occur (Wagstaff et al., 2007). Given that the level of antioxidants decreases during storage in darkness, we suggest that the ratio between ROS and antioxidants became unfavourable in LED lighting grown GB tissue and that the antioxidant system became overwhelmed and the tissue suffered degradation. In contrast, the excised discs from RB, being initially higher in polyphenolic compounds, i.e. anthocyanins, were better protected from high percentage of blue light in LED lighting (as discussed in section 4.1), and this resulted in a more beneficial oxidative status of the tissue, and therefore this tissue did not show accelerated postharvest senescence.

Conclusions

The present study shows that the levels of antioxidants at harvest, due to genetic background or caused by the light conditions during growth, do not correlate well with the storability of wounded tissue. The applied LED lighting that stimulated the production of major antioxidants caused a dramatic decrease in storability of discs excised from GB. This effect was not observed in discs from RB, presumably because of the higher initial amount of antioxidants and the less pronounced response to the LED lighting which may have caused a more favourable oxidative balance. Enhancing levels of antioxidants by applying stress may induce ROS production as well, and if the balance becomes unfavourable, the stress application will not benefit the quality retention. It is concluded that a measurement of antioxidants and total antioxidant capacity is not sufficient to predict a shelf-life of fresh-cut lettuce, as it is presumably the oxidative status of the tissue that counts, and should become a focus of future studies.

Acknowledgements

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CHAPTER 3

Plant age affects the shelf-life of fresh-cut *Lactuca sativa* L.

Abstract

To obtain high quality fresh-cut vegetables, it is necessary to start with high quality raw material. In the present study we investigated the performance of dark-stored wounded (fresh-cut) *Lactuca sativa* L. in relation to the physiological maturity at harvest. We used two related genotypes, i.e. green (GB, cv. Troubadour) and red butterhead (RB, cv. Teodore) differing in their antioxidant levels. To investigate possible differences in shelf-life between fresh-cuts prepared from plants harvested at different age, a number of physiological and nutritional parameters was determined at harvest. For both butterhead lettuce genotypes, the shelf-life of fresh-cuts prepared from younger plants was significantly longer than of the fresh-cut prepared from the more mature plants. However, no simple one to one relationship emerged between any of the measured nutritional parameters, their change during maturation and the eventual shelf-life of the fresh-cut produce. The RB contained about two times more chlorophyll, carotenoids, and polyphenolic antioxidants than the GB, whereas their shelf-life was about similar. The content of chlorophyll, carotenoids, phenolic compounds, as well as total antioxidant capacity was not affected by age of the plants for either genotype. The content of ascorbic acid (AsA) decreased with maturation for GB; it was not influenced by maturity in RB. The net photosynthesis rate and carbohydrate reserves of RB were about half of that in GB. The net photosynthesis rate was not influenced by maturity in GB, whereas it decreased with maturation in RB. A decrease in sucrose and starch, and therefore the total content of carbohydrates with aging was observed in both genotypes. This effect was more pronounced in RB than in GB. However, there was no apparent relationship between the absolute levels of the total carbohydrates and the shelf-life. Carbohydrate levels were about two times higher in GB than in RB, but the shelf-life of both genotypes was similar. Moreover, carbohydrate levels were similar for the older GB and the younger RB, whereas the shelf-life of the older GB was about 12 days shorter than that of the younger RB. The older leaves appeared to have reduced capability to cope with the stress from wounding, but the inverse relationship between the plant age and the shelf-life of fresh-cuts could not be explained by any of the measured parameters.

Witkowska, I.M., Woltering, E.J. 2013. Plant age affects the shelf-life of fresh-cut *Lactuca sativa* L. *(submitted)*

Introduction

The industry of ready-to-use fresh fruits and vegetables is constantly growing as a result of consumer awareness of the importance of a healthy diet (Steinmetz and Potter, 1991; Wiley, 1994; Parish et al., 2003) and the convenience of these products. Lettuce is an important agricultural commodity, which contains appreciable amounts of bioactive compounds (Caldwell, 2003; Cao et al., 1996; Chu et al., 2002; Vinson et al., 1998), and appears to exert a diversity of beneficial health effects (Caldwell, 2003; Llorach et al., 2004; Nicolle et al., 2004a, b). However, the quality of fresh-cut lettuce is still unpredictable and its shelf-life limited. Effective, non-invasive and non-chemical techniques for improving and maintaining quality are therefore needed.

In order to obtain high quality fresh-cut vegetables, it is necessary to start with high quality raw material (Watada et al., 1996). To this respect, genetic background (Varoquaux et al., 1996; Degl'Innocenti et al., 2007) and pre-harvest factors, such as cultivation conditions (Voipio et al., 1995; Eskins, 1996; Krizek et al., 1998; Romani et al., 2002; Kleinhenz et al., 2003; Gazula et al., 2005; Gruda et al., 2005; Garcia-Marcias et al., 2007; Tsormpatsidis et al., 2008) and plant age at harvest (Couture et al., 1993; Pandjaitan et al., 2005; Bergquist et al., 2006; Zhao et al., 2007) affect quality of fresh-cut vegetables. Among few reports on the influence of the plant age on the chemical composition and storability of leafy vegetables, only a small number has been devoted to lettuce, and its properties and quality as a fresh-cut product (Couture et al., 1993). The significance of the maturity on lettuce quality was inconsistent between these studies, possibly due to the interaction of plant age with weather conditions during different growing times. The majority of these investigations also focused only on the effect of crop maturity on the content of antioxidants at harvest. Knowledge about age-dependent differences in the shelf-life of fresh-cut lettuce, as well as the mechanisms responsible for these differences, are still lacking, and were the subject of the present study. We investigated the shelf-life of fresh-cut lettuce in relation to the age of the plant at harvest. Leaf aging in plants is accompanied by a genetically programmed senescence process (Buchanan-Wollaston et al., 2005), therefore, we studied how shelf-life of fresh-cut lettuce relates to a number of physiological processes associated with senescence, such as a decrease in photosynthesis, chlorophyll degradation, and loss of antioxidants and cellular integrity.

Materials and methods

Plant material and growth conditions

Two related genotypes of *Lactuca sativa* L. green butterhead genotype (GB) *cv.* Troubadour and red butterhead genotype (RB) *cv.* Teodore (Rijk Zwaan, The Netherlands) were used in two experiments. In experiment 1 the seeds were sown in boxes filled with vermiculite, and one week after germinating, seedlings were transplanted to a hydroponic system (Hoagland's solution, pH 5.9±0.2; EC=1.2 mS cm^{-1}) in a climate chamber located at Wageningen University, The Netherlands. Temperatures were maintained at 20°C during the day and at 15°C during the night, and the relative humidity was 70%. Two light sources, i.e. white fluorescent tubes (FL; TLD 50 W 840 HF, Philips, The Netherlands) or light emitting diodes (LEDs) were used to provide photosynthetically active radiation (PAR) at 250 μmol m^{-2} s^{-1} (12h photoperiod). The LEDs arrays consisted of 30% of red

(peak wavelength at 667 nm), and of 70% of blue (peak wavelength at 465 nm) LED modules (Greenpower LED modules HF, Philips, Eindhoven, The Netherlands). The seedlings and lettuce plants were not chemically treated before or during the experiment. 30 and 42 days after transplanting the plants were harvested in the morning, and the middle leaves were used for the storage experiment.

In experiment 2, the seeds were sown in boxes filled with vermiculite, and one week after germinating were transplanted to pots with standard pot soil in a climate chamber located at Wageningen University, The Netherlands. Temperatures were maintained at 20°C during the day and at 15°C during the night, and the relative humidity was 70%. Photosynthetically active radiation (PAR) at 250 μmol m^{-2} s^{-1} was provided by white fluorescent tubes (TLD 50 W 840 HF, Philips, The Netherlands). The seedlings and lettuce plants were not chemically treated before or during the experiment. 35, 42 and 49 days after transplanting, plants were harvested in the morning, and second whorl leaves were used for the storage experiment.

Storage experiment with excised discs, leaf pieces, and intact leaves

In experiment 1, leaf discs excised with a stainless steel cork borer (18 mm diameter) were used as mimic of commercial fresh-cut lettuce. The leaf discs were cut from the leaf lamina avoiding the inclusion of the midrib and major veins. This method of cutting was adapted to keep the same extend of wounding for all samples and treatments. Randomly selected leaf discs and intact leaves were placed in plastic Petri dishes (25×100-mm), with vents, lined with wet filter papers (Whatman, #3) to prevent desiccation. The dishes were placed in temperature controlled storage units (Elbanton, Kerkdriel, The Netherlands) at 12°C in the dark, and relative humidity of 90%.

In experiment 2, leaf pieces (4 x 4 cm) were cut from the middle part of the leaf lamina with a sharp stainless knife avoiding the inclusion of the midrib and major veins. Randomly selected leaf pieces were placed in white plastic boxes (1300×1800-mm) lined with wet filter papers (Whatman, #3) to prevent desiccation. The boxes were equipped with a transparent plastic lids in which 16 small holes (approximately 0.5 mm diameter) were punched. This allowed sufficient ventilation to prevent depletion of oxygen or accumulation of carbon dioxide or ethylene under the experimental conditions (data not shown). The boxes were placed in dark storage rooms for further storage at 12°C in the dark, and relative humidity of 90%.

In experiment 1, at every sampling time 9 leaf discs, were randomly selected from 3 different Petri dishes (replicates), weighted, and specific leaf weight on fresh weight basis (SLW) was calculated. In experiment 2, at every sampling time, 3-4 leaf pieces were randomly selected from 3 different boxes (replicates), and from each leaf piece, 2-3 discs were excised. In each experiment, per each replicate, two leaf discs were used directly for determination of pigments, two leaf discs were frozen in liquid nitrogen and stored at -80°C until analysis of AsA, and two (phenolics) or three (ORAC) leaf discs were frozen in liquid nitrogen, dried using a freeze-dryer (Modulyo, Pirani 501, Edwards, UK) and ground into fine powder with a ball-mill (Retsch, Germany).

Overall visual quality (OVQ) and shelf-life

Lettuce quality of both the discs and the leaf pieces was evaluated using overall visual quality (OVQ) ratings modified from Kader et al. (1973). The OVQ was evaluated on the

basis of leaf characteristics such as color (yellowing, browning, and brightness), wilting, and presence or absence of defects. Quality ratings were made on a scale from 9 to 1, where 9 = excellent, essentially free from defects, and 1 = extremely poor, not usable. An OVQ rating of 6 was considered the lower limit of consumer acceptance.

Determination of pigments

Pigments were extracted in dimethylformamide (DMF) in the dark at -20˚C. The absorbance of the extract was measured in the range 400–750 nm using a Cary 4000 spectrophotometer (Varian Instruments, Walnut Creek, CA, USA), and the pigment content was calculated using the equations provided by Wellburn (1994).

Determination of ascorbate (AsA)

Ascorbate (AsA) was measured following a procedure modified from Davey et al. (2003). The leaf discs that were previously stored at -80°C were ground in liquid nitrogen, and thawed on ice in the dark. These samples were mixed with 0.5 ml ice-cold 3.3% meta-phosphoric acid, and the mixture was placed in an ultrasonic bath at 0˚C (melting ice) for 10 minutes in the dark. Total AsA was determined followed the reduction of DHA to AsA by DTT. 100 µl of the extract was added to 50 µl of 5 mM DTT in 400mM Tris base, resulting in a final pH of 6–6.8. Previous experiments showed that a DTT concentration of 5 mM was sufficient for this type of plant tissue. The reaction was stopped after 15 min at room temperature by acidification with a 50 µl of 8.5% ortho-phosphoric acid. Extracts were then analyzed using HPLC for total AsA. The Dionex ICS5000 HPLC was equipped with a Omnispher 5 C18 (150x3 mm; Varian) column that was eluted with 400 µl l^{-1} H_3PO_4, 2.5 ml l^{-1} MeOH and 0.1 mM EDTA in H_2O followed by a wash step with 30% acetonitrile.

Quantification of polyphenolic compounds

Ten micrograms of powdered freeze-dried lettuce sample was mixed 1.5 ml of 80% methanol, and the mixture was placed in an ultrasonic bath at 20˚C for 4 minutes. After centrifugation, the supernatant was used for the determination of polyphenolic compounds. The total amount of polyphenolic compounds in lettuce was determined using Folin-Ciocalteu's reagent according to the method of Singleton and Rossi (1999). Three hundred microliters of the methanolic extract was mixed with 1.5 ml of 2M Folin-Ciocalteu reagent, and then the mixture was added to 1.20 ml of 20% Na_2CO_3. After incubation at room temperature for 30 min, the mixture was centrifuged, and the absorbance of the supernatant was measured at 735 nm. The standard curve was prepared using gallic acid (GA). The absorbance was converted to phenolic content in terms of milligrams of GA equivalent (GAE) per 100 gram of fresh weight (FW) of sample.

Oxygen radical absorbance capacity (ORAC)

The extraction of the antioxidant fraction and determination of antioxidant capacity in lettuce was performed using the oxygen radical absorbance capacity (ORAC) method modified from Huang et al. (2002). Fifteen micrograms of powdered freeze-dried lettuce sample was vortexed for 1 min with 1 ml of hexane. After centrifugation the supernatant was collected, and the pellet re-extracted in 1 ml of hexane. The combined hexane extracts were evaporated to dryness at 40°C using a vacuum evaporator (Savant SPD2010

SpeedVac, Thermo Scientific, Asheville, NC, USA). The pellet remaining after the hexane extraction was dried and re-extracted with 1.5 ml of acetone/water/acidic acid mixture (70:29.5:0.5; v/v/v) to obtain the hydrophilic fraction. For the lipophilic antioxidant assay, the dried hexane extract was re-dissolved in 250 µl of acetone and then diluted with 750 µl of a 7% randomly methylated b-cyclodextrin solution (RMCD; 50% acetone/50% water, v/v; Huang et al., 2002). Any further dilution was made with the 7% RMCD solution. The 7% RMCD solution was also used as a blank and to dissolve and dilute the Trolox standards for the lipophilic assay. For the hydrophilic assay, phosphate buffer (0.075 M, pH 7.4) was used as the solvent. This phosphate buffer was also used as the blank and the solvent for the Trolox standards in the hydrophilic assay. The fluorescence intensity measurement was performed using a Safire monochromator-based microplate reader (Tecan USA, Research Triangle Park, NC) with the sample loaded on polystyrene, flat-bottom 96-well plate (Fluotrac, Greiner America, Inc.). The 20 µl of diluted sample was mixed with 100 µL fluorescein solution in a microplate and incubated at 37° C for 15 min, after which 40 µl of 2,20-Azobis (2-amidino propane) dihydrochloride (AAPH) (17.2 mg ml^{-1}) was added to each well using a multi-channel pipette. Immediately following the addition of AAPH, the plate was agitated for 5 s prior to the first reading and for 2 s before each subsequent reading. Readings were done at 2 min intervals for 40 min. Excitation and emission filter wavelengths were set at 484 nm and 520 nm, respectively. Data were expressed as µmol Trolox equivalents (TE) per gram of lettuce on a fresh dry weight (FW) basis. The ORAC values were calculated by using a linear regression equation (Y = a + bX) between concentration (Y) (µM) and the net area under the fluorescence decay curve (X). Linear regression was used in the range of 6.25–50 µM Trolox. Blank, standard and sample were analyzed in triplicate to eliminate position effects.
The area under curve (AUC) was calculated as follows:

$$AUC = (0.5 + f_1/f_0 + f_2/f_0 \cdot \cdot \cdot + f_i/f_0)\ CT$$

where f0 is the initial fluorescence reading at cycle 0, fi is the fluorescence reading at cycle i, and CT is the cycle time in minutes. The net area under the curve was obtained by subtracting the blank value from that of a sample or standard.

Determination of soluble sugars and starch

Ten micrograms of powdered freeze-dried lettuce sample were extracted in 5 ml of 80% ethanol for 20 minutes at 80°C. One milliliter of supernatant was vacuum dried (Savant SPD2010 SpeedVac, Thermo Scientific, Asheville, NC, USA), the residue was re-suspended in 2 ml of 0.01 M HCl, and resulting extracts were passed through an Extract CleanTM SCX column 100 mg 1.5 ml^{-1} (Grace, Deerfield, IL, USA) activated with 5 ml 0.01 M HCl. The eluents were analyzed for soluble sugars on a Dionex ICS5000 HPLC equipped with a CarboPac1 (250x2mm) column eluted with a gradient from 16 to 45 mM NaOH. The remaining 4 ml of supernatant were discharged, the residue was centrifuged, and the pellet was washed three times with 80% ethanol before vacuum drying. The pellet was suspended in 1 mg m^{-1} thermostable α-amylase (Serva 13452) in water at 90°C, and subsequently with amyloglucosidase (Fluka 10115) in 50 mM citrate buffer with pH=4.6 at 60°C to hydrolyze starch to glucose. The obtained starch extracts were analyzed on

a Dionex ICS5000 HPLC equipped with a CarboPac1 (250x2mm) column eluted with 100 mM NaOH and 12.5 mM sodium acetate.

Net photosynthetic rate and dark respiration

Photosynthetic rates (A_{net}) of plants at harvest were measured with a portable gas analysis system (LI-6400 equipped with a leaf chamber fluorometer; Li-Cor Inc., Lincoln, NE). The leaf chamber temperature was set to 20°C, the air flow at 150 μmol s^{-1}, the CO_2 concentration during the irradiance-response measurements was 400 μmol mol^{-1} and the LED light source was set at 10% blue light. Water vapour concentration was similar to that in the ambient air (22.5 mmol mol^{-1}; RH = 70%). The A_{net} were calculated as the mean value during a 120-s window following the establishment of a stable CO_2 fixation rate. Dark respiration (R_D) was measured after 10 min of dark adaptation in the leaf chamber.

Statistical analysis and curve fitting

Data were processed using the SPSS statistical package (IBM SPSS, release 19.0.0.1, 2010. SPSS Inc. and IBM Company, Chicago, IL, USA). Each experiment had three replicates per treatment. For initial levels of analyzed characteristics we assumed fixed effect for plant maturity stage and genotype, and random effect of the replicate. The interactions between maturity stage x genotype were studied by looking at maturity stage differences per genotype, and at genotype differences per maturity stage. The effect of cold storage was analyzed per genotype, and for each treatment separately. Mean separations was performed using the LSD procedure, and significance was declared at $P < 0.05$. The trends in OVQ data were fitted in Excel (Microsoft Office 2010 for Windows) with polynomial (order 2) regression lines.

Results

Structural properties

The specific leaf weight (SLW) was similar for both genotypes (Figure 1). In general, the SLW did not greatly differ between maturity stages of plants in experiment 1 and 2. However, the SLW tended to be a little lower for younger plants, and this difference only appeared to be statistically significant comparing 30 and 42 days old plants of GB and RB grown under fluorescent light (FL) in experiment 1 (Figure 1A).

Pigment concentration, polyphenolic compounds and oxygen radical absorbance capacity (ORAC)

The red butterhead (RB) generally contained much higher amounts of pigments (Figure 2 A-D) and polyphenolic compounds (Figure 3A) than the green butterhead (GB), at all growth stages. Total ORAC value was about twice as high in RB compared to GB (Figure 3 B, C). The chlorophyll, carotenoid and polyphenolic content, as well as total antioxidant capacity ($ORAC_{total}$) and its lipophilic fraction ($ORAC_{lipophilic}$) did not differ between maturity stages of plants of both genotypes in experiment 1 and 2 (Figure 2 and 3).

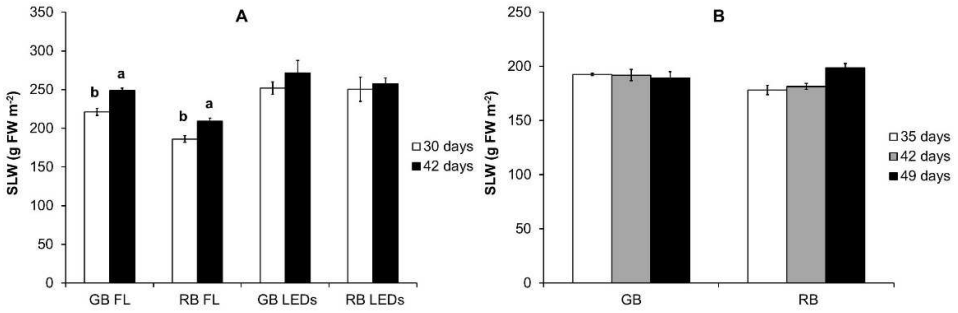

Fig. 1. Initial levels of specific leaf weight (SLW) of two butterhead genotypes (green, GB and red, RB). Plants in experiment 1 (A) were grown under different light conditions, i.e. white fluorescent tubes (FL) and 70B:30R light emitting diodes (LEDs) for 30 or 42 days. Plants in experiment 2 (B) were grown under FL for 35, 45 and 49 days. Error bars represent standard errors. Data points marked by different lower case letters for comparison between growth stages per genotype (a–b), are significantly different ($P<0.05$).

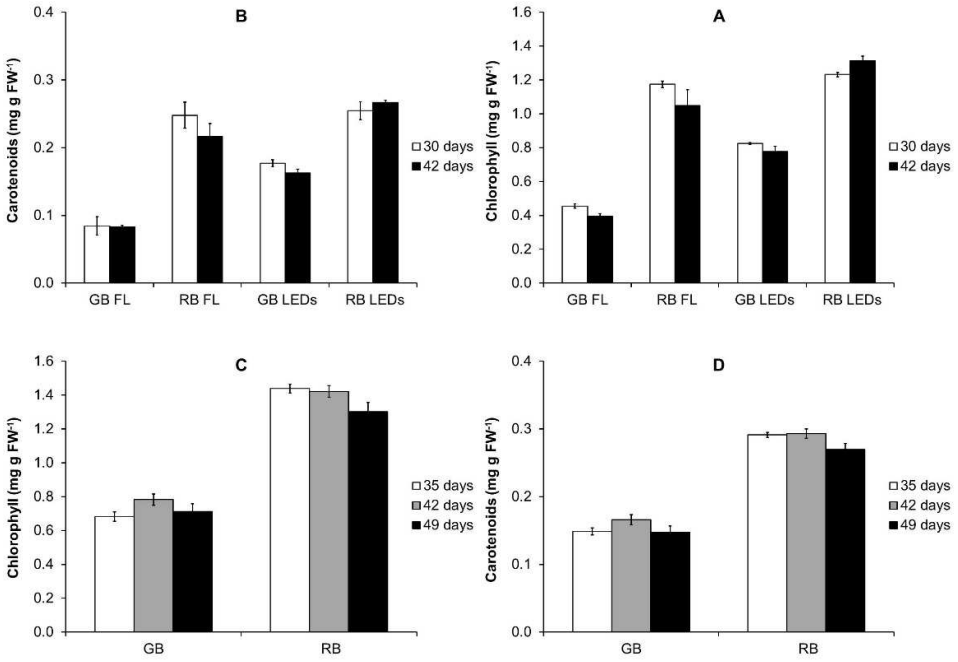

Fig. 2. Initial levels in chlorophyll and carotenoids of two butterhead genotypes (green, GB and red, RB) in two experiments. Plants in experiment 1 (A, B) were grown under different light conditions, i.e. white fluorescent tubes (FL) and 70B:30R light emitting diodes (LEDs) for 30 or 42 days. Plants in experiment 2 (C, D) were grown under FL for 35, 45 and 49 days. Error bars represent standard errors.

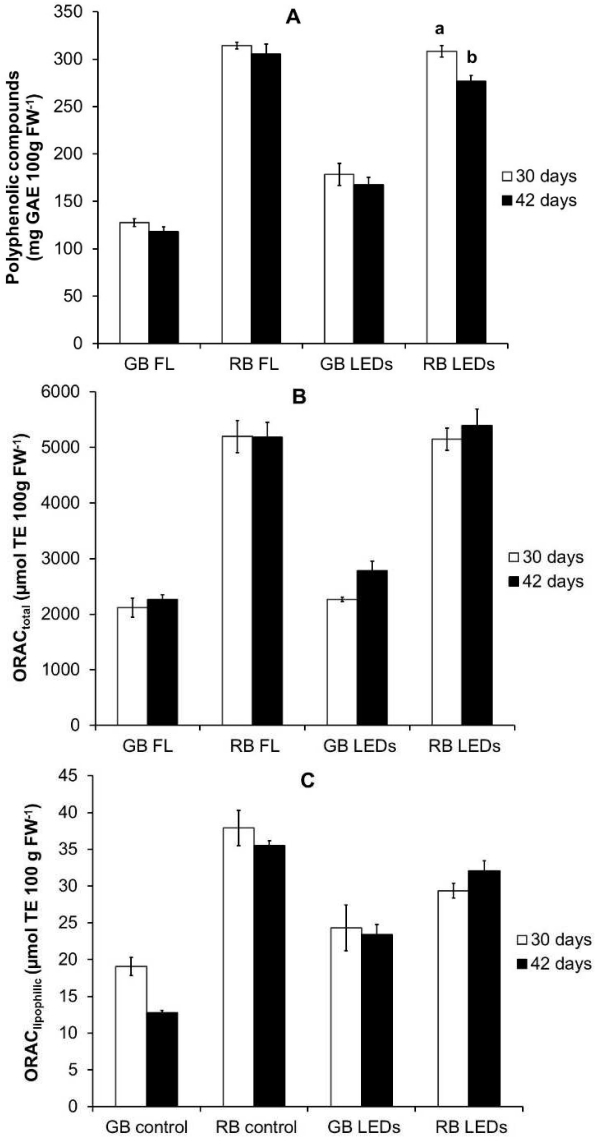

Fig. 3. Initial levels in polyphenolic compounds (A), total oxygen radical absorbance capacity (ORAC, B), and lipophilic ORAC (C) of two butterhead genotypes (green, GB and red, RB). Plants were grown under different light conditions, i.e. white fluorescent tubes (FL) and 70B:30R light emitting diodes (LEDs) for 30 or 42 days in experiment 1. Error bars represent standard errors. Data points marked by different lower case letters for comparison between growth stages per genotype (a–b), are significantly different ($P<0.05$).

Total ascorbate (AsA)

The content of total ascorbic acid (AsA) was about the same in both genotypes (Figure 4). The content of total AsA decreased with an increasing plant maturity in GB, whereas a less pronounced (but statistically not significant) trend was observed in RB (Figure 4).

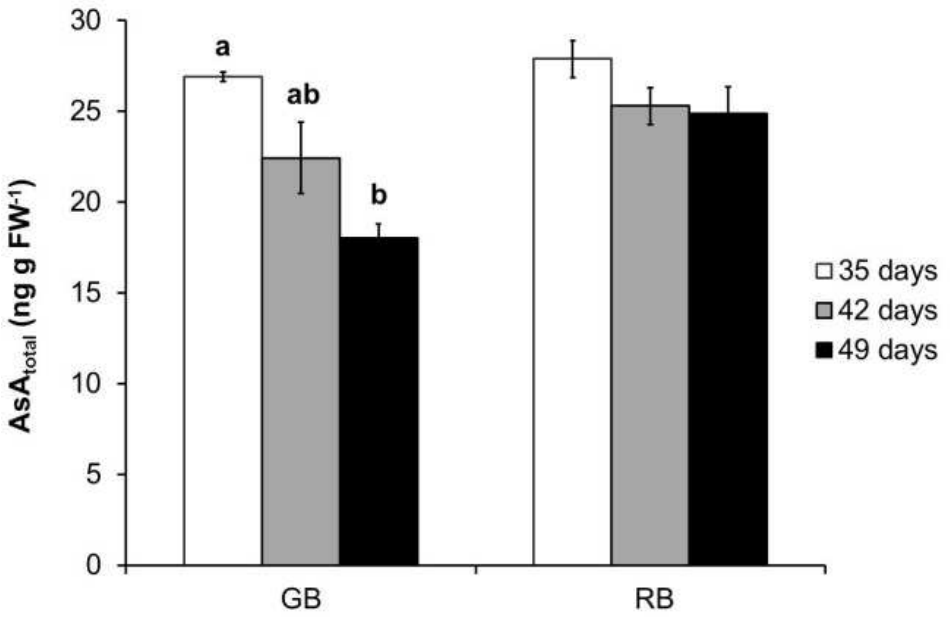

Fig. 4. Initial levels in total ascorbic acid (AsA) of two butterhead genotypes (green, GB and red, RB) grown for 35, 45 and 49 days in experiment 2. Error bars represent standard errors. Data points marked by different lower case letters for comparison between growth stages per genotype (a–b), are significantly different ($P<0.05$).

Soluble sugars and starch

In all growth stages RB had a lower sucrose (Figure 5A), starch (Figure 5D) and total soluble sugar (Figure 5E) content than GB. Total carbohydrate content in RB was on average less than half of that in GB (Figure 5F). In RB all individual sugars and starch showed a clear decrease with increasing age of the plant (Figure A-D). In GB only a consistent decrease in sucrose (Figure 5A) and total carbohydrate (Figure 5F) was observed with increasing age of the plant, whereas no consistent decrease in other soluble sugars was observed (Figure 5 B, C, E). The starch decreased only between 35 and 42 days of growth (Figure 5D). In RB total carbohydrates decreased more than 50% between 35 and 49 days of growth (Figure 5F); at 49 days the amount of carbohydrate in GB was comparable to the level in RB at 35 days.

Net photosynthesis and dark respiration

In all growth stages, net photosynthesis rate (Figure 6A) was about 40-50% lower in RB compared to GB, and the dark respiration rates (Figure 6B) of RB and GB were similar. Net photosynthesis and the ratio between net photosynthesis and dark respiration (Figure 5C) did not change with maturity of plants in GB. In contrast, a significant decrease in both parameters was observed with the increased maturity for RB, indicating that the plants of RB became photosynthetically less efficient with age.

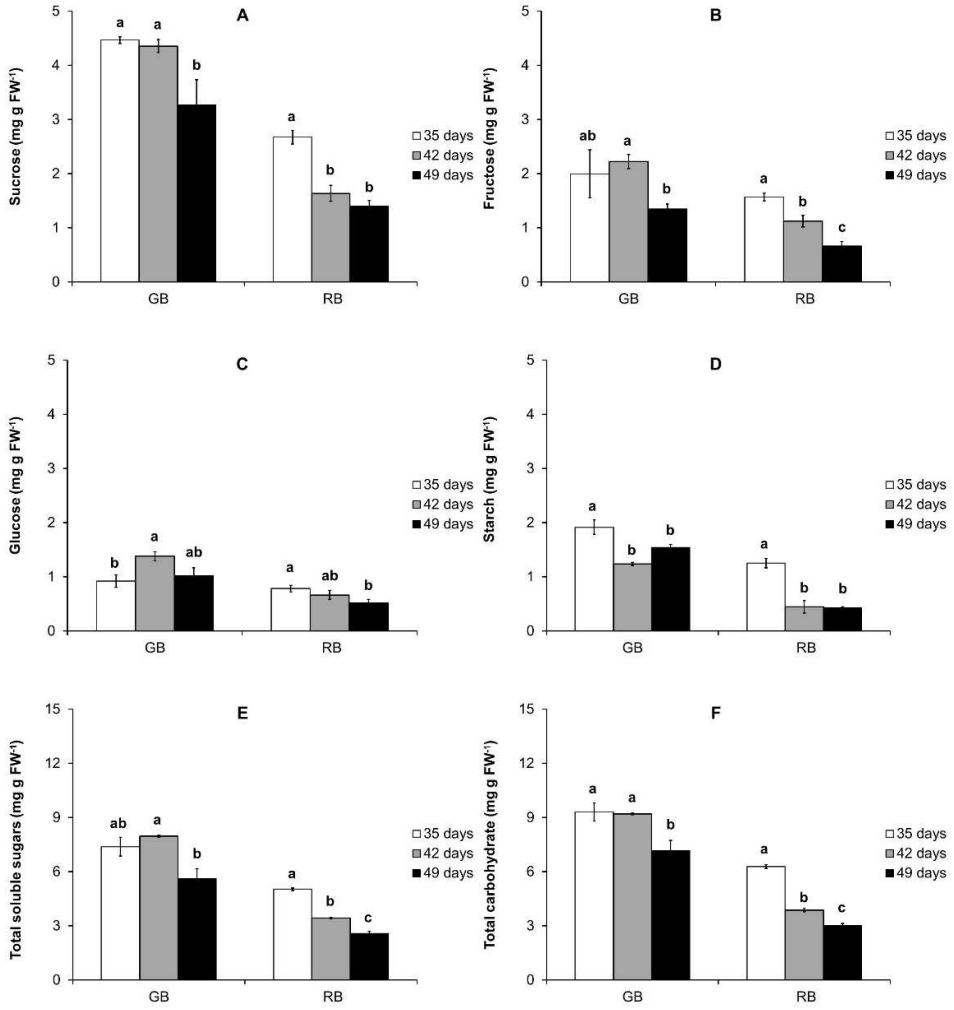

Fig. 5. Initial levels in sucrose (A), fructose (B), glucose (C), starch (D), sum of three soluble sugars (E), and total carbohydrate (F) content of two butterhead genotypes (green, GB and red, RB) in experiment 2. Plants were grown for 35, 45 and 49 days. Error bars represent standard errors. Data points marked by different lower case letters for comparison between growth stages per genotype (a–c), are significantly different ($P<0.05$).

Overall visual quality (OVQ)

The shelf-life of discs and leaf pieces of both genotypes was approximately similar, and in both genotypes a comparable decrease in shelf-life was observed with increasing plant age (Table 1, Figure 7 and 8).

In experiment 1, the shelf-life of discs excised from older plants was shorter than that of discs from younger plants. This difference was more pronounced in GB (6-8 days depending on the light source) than in RB (3- 4 days). The shelf-life of the intact leaves from plants of different maturity stages was not different. Leaves of both genotypes grown under FL and LEDs irrespectively of the age had a shelf-life of 25-27 days (data not shown).

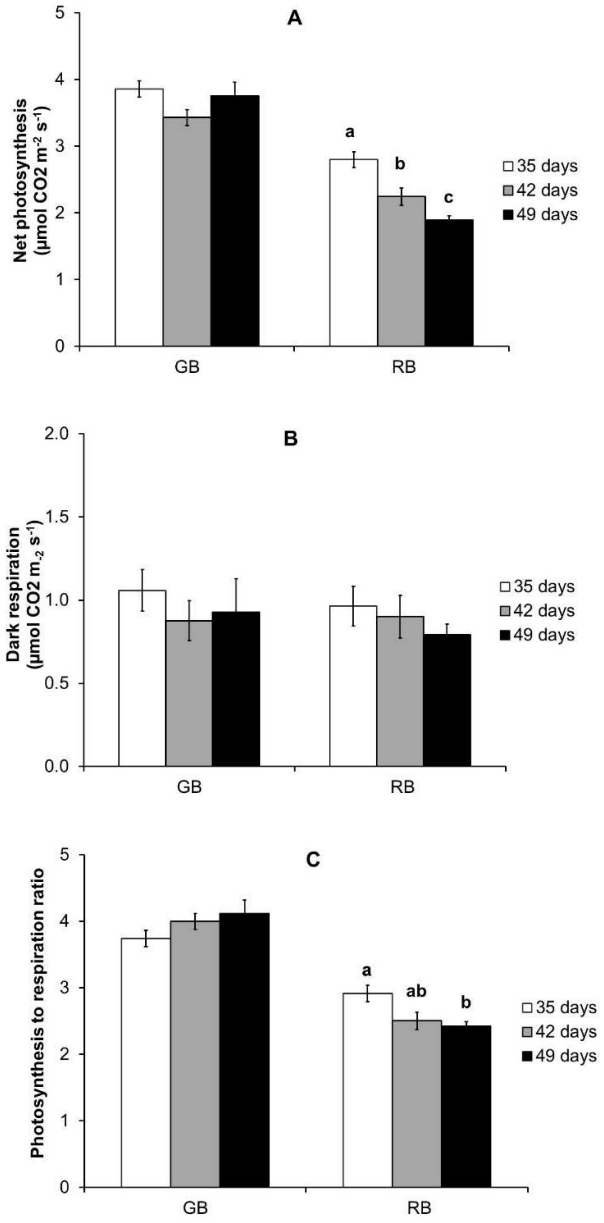

Fig. 6. Initial levels in net photosynthesis (A), dark respiration (B) and ratio of photosynthesis to dark respiration (C) of two butterhead genotypes (green, GB and red, RB) in experiment 2. Plants were grown for 35, 45 and 49 days. Error bars represent standard errors. Data points marked by different lower case letters for comparison between growth stages per genotype (a–c), are significantly different ($P<0.05$).

In experiment 2, for both genotypes grown under FL the difference in the shelf-life between leaf pieces prepared from 35 days and 42 days old plants was 4 days; the difference between leaf pieces from 42 days and 49 days old plants was 6 days.

Table 1. Shelf-life of leaf discs (experiment 1) and pieces (experiment 2) excised from plants of different maturity stages.

		Experiment 1		Experiment 2		
Genotype	Light source	30 days	42 days	35 days	42 days	49 days
GB	FL	24	18	14	10	4
RB	FL	24	21	16	12	6
GB	LEDs	24	16	-	-	-
RB	LEDs	24	20	-	-	-

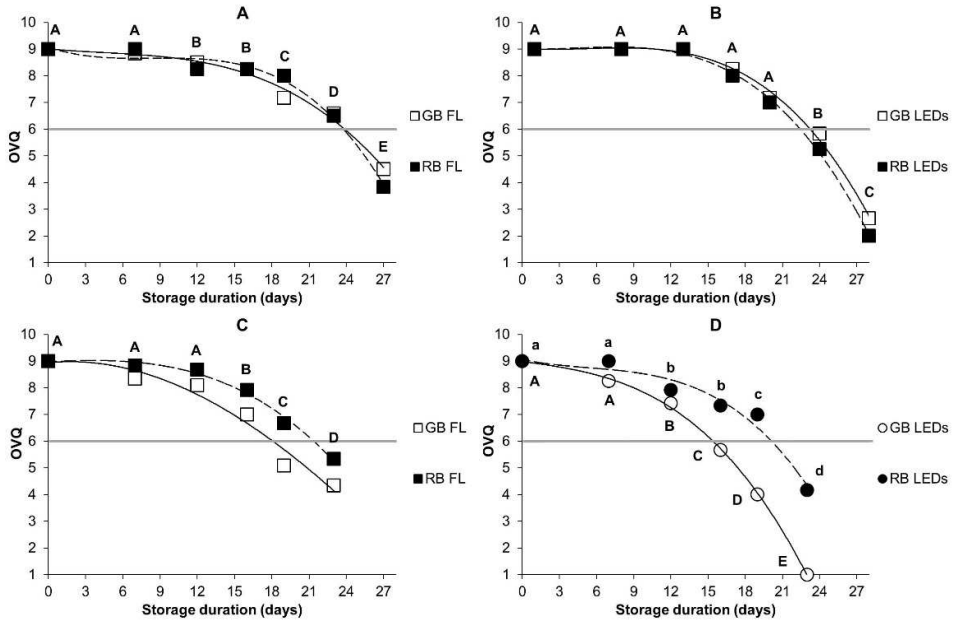

Fig. 7. Overall visual quality changes during storage of fresh-cuts of two butterhead genotypes (green, GB and red, RB) in experiment 1. Plants were grown under different light conditions, i.e. white fluorescent tubes (FL) and 70B:30R light emitting diodes (LEDs) for 30 (A, B) or 42 (C, D) days. Data points marked by different letters, i.e. upper case (A-E) for GB, and lower case for RB (a–e), for comparison between storage times are significantly different ($P<0.05$).

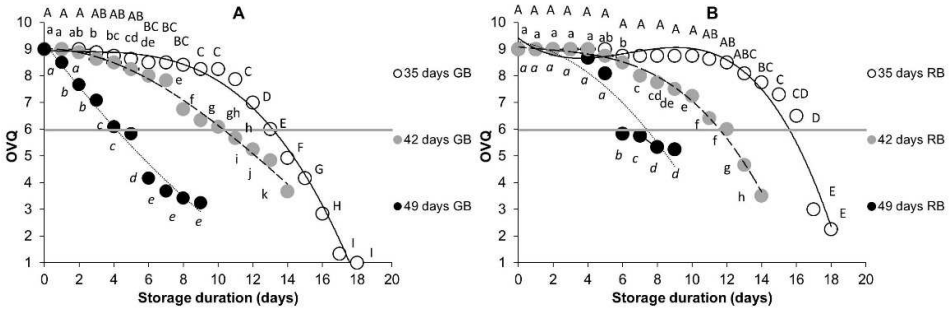

Fig. 8. Overall visual quality changes during storage of fresh-cuts of two butterhead genotypes (green, GB (A) and red, RB (B)) in experiment 2. Plants were grown under white fluorescent tubes (FL) for 35, 45 and 49 days. Data points marked by different letters, i.e. upper case (A-E) for GB, and lower case for RB (a–e), for comparison between storage times are significantly different ($P<0.05$).

Discussion

The maturity of lettuce appeared to be a critical factor determining the potential shelf-life of wounded tissue (fresh-cut) (Figure 7 and 8). In two independent experiments, it was clearly shown that with an increased plant maturity the shelf-life of fresh-cut lettuce decreases. We used atmospheric conditions to avoid any interference of the changing gaseous composition in the package with the processes under study. The observed effects of plant maturity might, in commercial packaging, might be masked by the effects of a modified atmosphere that often leads to anaerobic conditions during storage.

Age-dependent shelf-life of fresh-cut lettuce

Fresh-cut lettuce prepared from younger plants (e.g. 35 days of growth) had a longer shelf-life than when prepared from older plants (e.g. 49 days of growth). It should be noted that for both younger and older plants leaves from a similar position in the rosette were used for fresh-cut preparation; the leaves of older plants were therefore more mature, more developed than the leaves from younger plants. These results are in agreement with results of Couture et al. (1993), the only report on the age-dependent shelf-life of fresh-cut lettuce. Our study additionally showed that this phenomenon is independent of the genotype (GB vs. RB) and conditions during growth, i.e. light source (FL vs. LEDs). Therefore, to obtain a high quality fresh-cut product, it is advisable to harvest lettuce at earlier developmental stages.

Developmental leaf senescence at harvest

Initial phase of senescence

At harvest, plants of all ages were healthy, and visible signs of senescence were absent. We hypothesized that older plants may internally already show symptoms of senescence, e.g. chlorophyll degradation (Noodén et al., 1997) that relate to the lesser resistance to wound-induced deterioration. However, Buchanan-Wollaston et al. (2005) has already suggested that the senescence is well underway by the time the leaf shows visible signs of

senescence. Therefore, the retention of chlorophyll in older plants may not always mean that they are not senescing. Munné-Bosch and Algre (2004) reported that the chlorophyll degradation is an unreliable indicator for senescence.

Other initial measurable symptoms of senescence, i.e. a decreased photosynthesis and increased respiration (Munné-Bosch and Alegre, 2004; Yoshida, 2003; Noodén et al., 1997) showed a genotype-dependent behaviour with advancing maturity in our experiments. The net photosynthesis rate and dark respiration were similar for different plant ages in GB, whereas the net photosynthesis rate significantly decreased in the aging tissue of RB. The latter might be due to the increased red pigmentation of RB with age allowing less light to penetrate the leaf. Due to the decrease in carbon fixation in aging RB, the concentration of fructose, glucose, sucrose and starch all decreased with plants age. In GB, sugar levels were more stable, however, as in RB sucrose and starch decreased with age.

Destructive phase of senescence

We hypothesize that processes occurring during the later stages of developmental leaf senescence, such as lipid degradation, loss of antioxidants and loss of cellular integrity (Munné-Bosch and Alegre, 2004; Yoshida, 2003; Noodén et al., 1997) were generally absent in aging tissues of both lettuce genotypes. Cell membrane disruption due to lipid peroxidation has been reported to correlate with chlorophyll loss (Zhuang et al., 1994, 1997). As the chlorophyll content did not decrease with the age of the plant for either genotype we assume there the lipid peroxidation was also not present. In all plant ages, sugars were still available; therefore, it is rather unlikely that lipids were used as alternative energy source.

The observed stable levels of carotenoids, phenolics and total antioxidant capacity with plant maturation are in disagreement to a number of other studies (Couture et al., 1993; Pandjaitan et al., 2005; Bergquist et al., 2006; Zhao et al., 2007). For example, Bergquist et al. (2006) found an increase of carotenoids with age of spinach leaves; Couture et al. (1993) found lower levels of phenolic compounds in over-mature than in mature leaves of crisphead lettuce, whereas Pandjaitan et al. (2005) reported higher levels of phenolic compounds, flavonoids and ORAC value in mid-mature than in immature and mature leaves of spinach. In addition, Zhao et al. (2007) reported higher ORAC values in mature than in baby size spinach leaves, whereas no difference was reported in red leaf and romaine lettuce of different ages. The discrepancies in the effects of plant maturity on above mentioned characteristics between these studies might be explained by the interaction of plant age with weather conditions during different growing periods. It is likely that if the "maturity x weather condition" interaction is removed by growing plants under constant conditions, the plant age effect becomes more consistent.

In contrast to carotenoids and phenolic antioxidants that were stable during plant maturation in both genotypes, AsA levels in GB, but not (or to a lesser degree), in RB showed a clear decrease with maturation. This might have been due to developmental shifts in antioxidant requirements of both genotypes. The lack of age-dependent decrease in AsA in the red tissue, which is inherently high in antioxidants (Caldwell, 2003; Ferreres et al., 2007; Zhao et al., 2007; Liu et al., 2007; Llorah et al., 2008), might have been due to the involvement of other than AsA antioxidants, i.e. carotenoids and anthocyanins in counteracting age-driven oxidative stress during growth. Carotenoids are well known for scavenging of ROS (Cazzonelli et al., 2011), whereas polyphenolic compounds has been

suggested to act as a backup to the primary AsA-dependent detoxification system (Sakihama, 2002), although their role in detoxifying ROS is often not sufficiently recognized. Polyphenolic compounds are often ascribed only to the epidermis cells, whereas it has been shown that they are also located in mesophyll cells where they act as antioxidants (Agati et al., 2009, Neill et al., 2002).

Relationship between nutritional content and the (maturity-related) shelf-life

In general, no simple one to one relationship emerged between any of the measured nutritional parameters, their change during maturation and the eventual shelf-life of the fresh-cut produce. This is in contrast to results presented by a number of researchers in last years. For instance, it has been shown that high antioxidant content decreases the rate of senescence (Meir et al., 1995; Hodges and Forney, 2003). Following this rationale, Bergquist et al. (2006) looked at levels of ascorbic acid (AsA) in spinach as affected by plant age, and showed that the content of AsA in the tissue can be used to predict the shelf-life, since AsA is supposed to protect against oxidative stress and, therefore, to reduce the rate of senescence. Our results contradict these models. Firstly, the levels of chlorophyll, carotenoids and phenolic compounds, as well as antioxidant capacity were about two times higher in RB than in GB, whereas the shelf-life of fresh-cut from both genotypes was similar. Secondly, in both genotypes these levels were not influenced by the maturation of the head, whereas the shelf-life was. Thirdly, a significant decrease in AsA was observed in GB with the maturation of the head, possibly explaining the shortened shelf-life of fresh-cuts from older plants. However, no, or a much smaller decrease in AsA was observed in RB heads, yet there was still decrease in shelf-life occurred with age. Together this shows that content of AsA, carotenoids, phenolic compounds, and well as total antioxidant activity at harvest have no relation to either the maturation of the head or the fresh-cut shelf-life.

Soluble sugars and starch are the primary energy reserves in the tissue. The levels of these energy reserves as well as the rate of their loss during storage have been suggested to define the storability of the product (Kays and Paull, 2004). However, this relationship did not appear to be that simple in our study. The levels of soluble sugars and the total carbohydrates (soluble sugars + starch) were about two times higher in GB than in RB, whereas the shelf-life of both genotypes was similar. Carbohydrate levels in the older GB were similar to those in the younger RB, whereas the shelf-life of the older GB was about 12 days shorter than that of the younger RB. However, the decrease in the levels of sucrose and starch, and therefore the level of the total carbohydrate with age correlated with the maturation dependent decrease in the shelf-life in each individual genotype. It appears that the level of the carbohydrate reserves in the tissue required for good quality retention can be genotype dependent, and may be linked to the reserves of other respiratory substrates, e.g. lipids, proteins and organic acids in the tissue. Under conditions of severe depletion of primary respiratory reserves, these secondary respiratory substrates can be utilized (Kays and Paull, 2004).

It has also been suggested that the quality retention is worse in young tissue owning to its high metabolic activity and respiration rates at harvest (Burton, 1982). In our study, also this concept appeared to be invalid. For RB the shelf-life of wounded tissue was higher in young tissue with higher levels of net photosynthesis, but with the same respiration rate, as the older tissue. For GB, the age-dependent decreases in the shelf-life of

wounded tissue appeared to be independent on both, the photosynthesis and respiration rates.

Conclusions

The inverse relationship between plant age and the shelf-life of fresh-cuts could not be explained by the initial levels of antioxidants or carbohydrates. No clear signs of on-going senescence were observed in the older plants, and the shelf-life of excised intact leaves was not affected by plant age. The older leaves apparently have lesser capability to cope with the stress from wounding.

Acknowledgements

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CHAPTER 4

Low light levels during storage prolong the shelf-life of fresh-cut *Lactuca sativa* L.

Abstract

The postharvest longevity of plant tissue is influenced by the senescence processes that occur during storage. Post-harvest treatments preserving energy reserves in the tissue, and levels of antioxidants have been suggested to extend the storability of the fresh-cut product. Light drives photosynthesis, and photosynthesis results in *de novo* synthesis of primary energy reserves, i.e. carbohydrates in the tissue. Therefore, in the present study we investigated the performance of fresh-cut photosynthetic tissue of *Lactuca sativa* L. during storage in darkness, and under low light conditions. Continuous illumination of fresh-cuts at intensities below (8 μmol m^{-2} s^{-1}) and above (30 μmol m^{-2} s^{-1}) light compensation point (LCP) effectively increased the shelf-life of this product, and its visual and nutritional quality. Storage of fresh-cuts in darkness caused a rapid depletion of chlorophyll and carotenoids, a depletion of ascorbic acid (AsA), and an increase in electrolyte leakage. Under light, AsA levels were preserved, carbohydrate accumulation occurred, and the increase in electrolyte leakage was delayed. Chlorophyll and carotenoids breakdown, however, was not prevented by the light. The similar chlorophyll and carotenoids breakdown in all treatments, indicates that also lipids and proteins were broken down to a similar extent in the dark and in the light. The accumulation of carbohydrates, even under light below the LCP, indicates non-photosynthetic sugar production. It is hypothesized that, under low light levels, the break down products of non-carbohydrate substrates (proteins, lipids) may be recycled into sugars (gluconeogenesis). The increased sugar levels might give an increase in respiratory activity, and consequently improve ATP levels, which might facilitate maintenance processes, and delay cell death in wounded tissue. In addition, the soluble sugars may act as ROS scavengers, and may preserve membrane properties. The maintenance of AsA in light due to either a coupling between photosynthetic electron transport and AsA biosynthesis or increased AsA production, may benefit tissue in two ways, i.e. by reducing the browning of cut edges and by preventing ROS-associated membrane lipid peroxidation. Together the above described mechanisms delay senescence processes, and therefore quality deterioration of fresh-cut photosynthetic tissue.

Witkowska, I.M., Harbinson J., Woltering, E.J. 2013. Low light levels during storage of fresh-cut *Lactuca sativa* L. prolong the shelf-life *(submitted)*

Introduction

The shelf-life of fresh-cut leafy vegetables when determined by means of visual appearance is generally short due to severe wounding of the plant material and its subsequent storage in darkness. In processing, the wounding is perceived by the plant and subsequently a signal is generated, which leads to changes in physiological and biochemical processes. Consequently, a number of wound-induced symptoms develop, s uch as discoloration (Ke and Saltveit, 1988, 1989; Peiser et al., 1998; Tay and Perera, 2004; Martín-Diana et al., 2005a, 2005b), increased respiration and ethylene production (Peiser et al., 1998; Fan and Matthies, 2000; Saltveit, 2004; Tay ad Perera, 2004; Martínez-Romero, 2007), loss of flavor and texture (Tay and Perera, 2004; Martín-Diana et al., 2005a, 2005b, 2006), loss of weight (Ihl et al., 2003; Agüero et al., 2008), decline in levels of antioxidants such as ascorbate (Ihl et al., 2003, Murata et al., 2004; Degl'Innocenti et al., 2007) and/or carotenoids (Martín-Diana et al., 2005), development of off-odours (Beaulieu, 2006), and membrane disintegration (Hodges et al., 2000). Most of these wounding-associated symptoms in fresh-cut lettuce leaves may also occur as a consequence of detachment of the lettuce head from the roots followed by dark storage, factors which promote senescence (Wangermann, 1965; Trippi and Thimann, 1983; Biswal and Biswal, 1984; Hodges and Forney, 2003; Buchanan-Wollaston, 1997). Senescence symptoms, such as chlorophyll degradation and membrane disintegration, have been identified as important factors in the postharvest longevity of plant tissue (Noodén et al., 1997).

In darkness, the absence of photosynthesis and, as a consequence, the rapidly declining levels of carbohydrates (Buchanan-Wollaston et al., 2005) have been proposed to be the main cause of dark-induced senescence (Gan and Amasino, 1997). Consequently, light has been suggested as a possible factor delaying senescence during postharvest storage (Hosoda et al., 2000; Ranwala and Miller, 2000). Light drives photosynthesis, and photosynthesis results in *de novo* synthesis of carbohydrates. These carbohydrates are respiration substrates, and therefore the energy reserves in the tissue. To date the effect of light on the quality of vegetable products has been contradictory. In some cases a negative effect of light on the quality retention in vegetables has been reported, for example in *Brasscia oleraceae* L. var. *italica* (Kasim and Kasim 2007; Olarte et al., 2009), cabbage *Brassica oleracea* var. (Perrin et al., 1982), *Brassica oleracea* L. var. *botrytis* L. (Sanz et al., 2007; Olarte et al., 2009), *Beta vulgaris* L. var. *vulgaris* (Sanz et al., 2008), *Lepidium sativum* L. (Zhan et al., 2009), *Allium porrum* L. (Ayala et al., 2009), *Lactuca sativa* L. (Martínez-Sánchez et al., 2011), and *Asparagus officinalis* L. (Sanz et al., 2009). Other authors have found a positive effect of light on the postharvest quality retention in vegetables such as *B. oleraceae* var. *gemmifera* (Kasim and Kasim, 2007), *Brassica oleracea* var. *alboglabra* (Noichinda et al., 2007), *Brassica rapa* L. subsp. *sylvestris* (Barbieri et al., 2009), and *Spinacia oleracea* L. (Toledo et al., 2003; Lester et al., 2010). These discrepancies in the effect of light can be explained by differences in the type of vegetable used (e.g. leaf, inflorescence, root or stalk), the type of tissue used (i.e. intact vs. wounded), tissue coloration (red or green), the duration of storage, the intensity and spectrum of the applied light and the type of packaging used. An important factor in the interpretation of the light effects on product quality is the possible interaction of the light with the atmosphere within the packages when modified atmosphere is applied.

The delaying effect of light on the senescence has been attributed to its effects on the retention of important antioxidants, such as carotenoids (Noichinda et al., 2007; Lester et al., 2010), phenolics (Zhan et al.., 2009), and ascorbic acid (AsA, Noichinda et al., 2007; Zhan et al., 2009). Of these, preserving AsA by applying light during storage of fresh-cut vegetables may be the most useful as AsA may be involved in inhibition of browning of cut edges (Walker et al., 1995; Alscher et al., 1997) and it may play a role in delaying senescence through the inhibition of H_2O_2 accumulation (Borraccino et al., 1994; Hodges and Forney, 2000, Hodges et al., 2001; Jimenez et al., 1998).

Retail outlets currently display fresh-cut vegetables in light-transmissible packages, which are exposed to 12 to 24 h per day of continuous artificial, low-intensity lighting. Therefore, the exposure of fresh-cut produce to light is unavoidable in parts of the supply chain. So far only a few studies have examined the effect of light on leafy vegetables (Toledo et al., 2003; Noichinda et al., 2007; Sanz et al., 2008; Barbieri et al., 2009; Zhan et al., 2009; Lester et al., 2010). The effect of light on the quality of fresh-cut vegetables has received even less attention (Sanz et al., 2009; Zhan et al., 2009; Martínez-Sánchez et al., 2011) and, surprisingly, only one study was devoted to lettuce (Martínez-Sánchez, et al., 2011). Given the general lack of knowledge on the effects of light on the storability of fresh-cut vegetables and the contradictory results that have been reported, we investigated the effect of light during storage of fresh-cut lettuce. The model plant used for the experiment was butterhead lettuce (*Lactuca sativa* L.), which is an important leafy vegetable available over the whole year worldwide. Four genotypes of this vegetable were examined. To ensure that the changes in quality and related senescence symptoms were solely due to the effects of the applied light during storage, we took care that the possible indirect effects on the composition of the storage atmosphere caused by photosynthetic activities were prevented by conducting the experiment in ventilated boxes under ambient atmospheric conditions.

Materials and methods

Plant material and growth conditions

In experiment 1, four morphologically related genotypes of *Lactuca sativa* L. were used, green butterhead genotype (GB) *cv.* Troubadour 1 (GB 1), *cv.* Troubadour 2 (GB 2), *cv.* Troubadour 3 (GB 3), and red butterhead genotype (RB) *cv.* Teodore (Rijk Zwaan BV, The Netherlands). The plants, at the commercial maturity stage, were received from Rijk Zwaan. In experiment 2, two morphologically related genotypes of *Lactuca sativa* L., green butterhead genotype (GB) *cv.* Troubadour 1 (GB 1), and red butterhead genotype (RB) *cv.* Teodore (Rijk Zwaan, The Netherlands) were used. The plants were grown in a climate chamber located at Wageningen University, The Netherlands. The seeds were sown in boxes filled with vermiculite, and one week after germinating seedlings were transplanted to a hydroponic system (Hoagland's solution, pH 5.9±0.2; EC=1.2 mS cm^{-1}). Temperatures were maintained at 20°C during the day and at 15°C during the night, and a relative humidity was 70%. White fluorescent tubes (FL; TLD 50 W 840 HF, Philips, The Netherlands) were used to provide photosynthetically active radiation (PAR) at 250 μmol m^{-2} s^{-1} (12h photoperiod). The seedlings and lettuce plants were not chemically treated before or during the experiment. In experiment 3, mature heads of unknown genotype of green butterhead (GB) were purchased from local supermarket (Albert Hein, Bennekom,

The Netherlands). In each experiment, lettuce heads used for storage experiments were selected for uniformity in their size and color, and for the absence of defects.

Storage experiment with leaf pieces and intact leaves

In all experiments, leaf pieces (4 x 4 cm) were cut from the middle part of the leaf with a sharp stainless steel knife avoiding the inclusion of midrib and major veins. Randomly selected leaf pieces were placed as a single layer in white plastic boxes (1300×1800-mm) lined with wetted filter paper (Whatman, #3) to prevent desiccation. The boxes were equipped with a transparent plastic lid in which 16 small holes (approximately 1 mm diameter) were made. This allowed sufficient ventilation to prevent any depletion of oxygen or accumulation of carbon dioxide or ethylene under the experimental conditions (data not shown). The boxes were placed in storage rooms at 12°C in the dark, and relative humidity of 90%.

The samples were kept under three storage conditions i) light below light compensation point (LCP) at 8 μmol m^{-2} s^{-1}, ii) light above LCP at 30 μmol m^{-2} s^{-1} iii) and darkness. White fluorescent tubes (FL; TLD 36 W 830 HF, Philips, The Netherlands) were used to provide continuous photosynthetically active radiation (PAR) at 8 μmol m^{-2} s^{-1} for light below LCP, and 30 μmol m^{-2} s^{-1} for light above LCP.

In experiment 3, at every sampling time 3-4 leaf pieces were randomly selected from 3 different boxes (replicates), and from each leaf piece, 3-4 discs were excised, weighted, and specific leaf weight on fresh weight basis (SLW) was calculated. Per each replicate, two leaf discs were used directly for determination of pigments, five leaf discs were used for the electrolyte leakage, two leaf discs were directly frozen in liquid nitrogen and stored at -80°C until analysis of AsA, and two leaf discs (sugars) were directly frozen in liquid nitrogen, dried using a freeze-dryer (Modulyo, Pirani 501, Edwards, UK) and ground into fine powder with a ball-mill (Retsch, Germany).

Light compensation point, net photosynthetic rate and dark respiration

The LCP was measured using a portable gas analysis system (LI-6400 equipped with a leaf chamber fluorometer; Li-Cor Inc., Lincoln, NE). During all measurements, CO_2 concentration in the leaf chamber was 400 μmol mol^{-1}, the air flow was 250 μmol s^{-1}, the leaf chamber temperature was 22°C, the humidity was approximately 80%, and the percentage blue light in the leaf chamber was set at 10%. The light compensation point was estimated by measuring net photosynthesis in the range of PAR from 0 to 50 μmol s^{-1} m^{-2}, in steps of approximately 5 μmol s^{-1} m^{-2}. At each irradiance level the rate of photosynthesis was calculated as the mean of the last 40 s after steady-state gas exchange was reached.

Photosynthetic rates (A_{net}) of plants at harvest were measured with a portable gas analysis system (LI-6400 equipped with a leaf chamber fluorometer; Li-Cor Inc., Lincoln, NE). The leaf chamber temperature was set at 20°C, the air flow at 150 μmol s^{-1}, the CO_2 concentration during the irradiance-response measurements at 400 μmol mol^{-1} and the LED light source was set at 10% blue light. Water vapor concentration was similar to that in the ambient air (22.5 mmol mol^{-1}; RH = 70%). The A_{net} were calculated as the mean value during a 120-s window following the establishment of a stable CO_2 fixation rate. Dark respiration (R_D) was measured after 10 min of dark adaptation in the leaf chamber.

The light response curves were measured for leaf pieces stored under each condition. After clamping a leaf piece in the leaf chamber, it was dark-adapted for 120 sec

and dark respiration (R_d) was measured. Then, after a 120 sec adaptation period the net photosynthesis (A_{net}) was measured under the same light intensities, i.e. 8 and 30 μmol m^{-2} s^{-1} as used for storage.

Overall visual quality (OVQ) and shelf-life

Lettuce quality of the leaf pieces was evaluated using overall visual quality (OVQ) ratings modified from Kader et al. (1973). The OVQ was evaluated on the basis of leaf characteristics such as color (yellowing, browning, and brightness), wilting, and presence or absence of defects. Quality ratings were made on a scale from 9 to 1, where 9 = excellent, essentially free from defects, and 1 = extremely poor, not usable. An OVQ rating of 6 was considered the lower limit of consumer acceptance.

Determination of pigments

Pigments were extracted in dimethylformamide (DMF) in the dark at -20°C. The absorbance of the extract was measured in the range 400–750 nm using a Cary 4000 spectrophotometer (Varian Instruments, Walnut Creek, CA, USA), and the pigment content was calculated using the equations provided by Wellburn (1994).

Determination of ascorbate (AsA)

Ascorbate was measured following a procedure modified from Davey et al. (2003). Leaf discs that were previously stored at -80°C were ground in liquid nitrogen, and thawed on ice in the dark. These samples were mixed with 0.5 ml ice-cold 3.3% meta-phosphoric acid, and the mixture was placed in an ultrasonic bath at 0°C (melting ice) for 10 minutes in the dark. Total ascorbate was determined followed the reduction of DHA to AsA by DTT. 100 μl of the extract was added to 50 μl of 5 mM DTT in 400mM Tris base, resulting in a final pH of 6–6.8. Previous experiments showed that a DTT concentration of 5 mM was sufficient for this type of plant tissue. The reaction was stopped after 15 min at room temperature by acidification with a 50 μl of 8.5% ortho-phosphoric acid. Extracts were then analyzed using HPLC for total AsA. The Dionex ICS5000 HPLC was equipped with a Omnispher 5 C18 (150x3 mm; Varian) column that was eluted with 400 μl l^{-1} H_3PO_4, 2.5 ml l^{-1} MeOH and 0.1 mM EDTA in H_2O followed by a wash step with 30% acetonitrile.

Determination of soluble sugars

Ten micrograms of powdered freeze-dried lettuce sample was extracted in 5 ml of 80% ethanol for 20 minutes at 80°C. One milliliter of supernatant was vacuum dried (Savant SPD2010 SpeedVac, Thermo Scientific, Asheville, NC, USA), the residue was re-suspended in 2 ml of 0.01 M HCl, and resulting extracts were passed through an Extract CleanTM SCX column 100 mg 1.5 ml^{-1} (Grace, Deerfield, IL, USA) activated with 5 ml 0.01 M HCl. The eluents were analyzed for soluble sugars on a Dionex ICS5000 HPLC equipped with a CarboPac1 (250x2mm) column and eluted with a gradient from 16 to 45 mM NaOH. The remaining 4 ml of supernatant were discharged, the residue was centrifuged, and the pellet was washed three times with 80% ethanol before vacuum drying. The pellet was resuspended in 1 mg m^{-1} thermostable α-amylase (Serva 13452) in water at 90°C, and subsequently with amyloglucosidase (Fluka 10115) in 50 mM citrate buffer with pH=4.6 at 60°C to hydrolyze starch to glucose. The obtained starch extracts were analyzed on

a Dionex ICS5000 HPLC equipped with a CarboPac1 (250x2mm) column eluted with 100 mM NaOH and 12.5 mM sodium acetate.

Electrolyte leakage

Tissue electrolyte leakage was measured following a procedure modified from Saltveit (2002). The leaf discs were washed in milli-Q water for 10 min, then blotted dry and submerged in 0.2 M Mannitol solution. The electrolytes leaching into solution were measured using a conductivity meter (340i WTW, Weilheim, Germany) after 2h at ambient temperature. The total electrolyte content of the samples was determined by freezing the samples at -20°C for 24h, and subsequently thawing them. Electrolyte leakage measured after 2h of leaching was expressed as a percentage of the total electrolyte leakage.

Statistical analysis and curve fitting

The experiment with two storage conditions, i.e. darkness and light below LCP was conducted once; the experiment with darkness and light below and above LCP was repeated two times with similar results; this paper reports the results from representative experiments. Data were processed using the SPSS statistical package (IBM SPSS, release 19.0.0.1, 2010. SPSS Inc. and IBM Company, Chicago, IL, USA). Each experiment had three 3 boxes per treatment. For changes in characteristics analyzed during storage we assumed a fixed effect for storage condition and storage time, and a random effect of the replicate. The interactions between storage condition and storage time were studied by looking at storage condition per storage time, and storage time per storage condition. Mean separations was performed using the LSD procedure, and significance was declared at $P < 0.05$. The trends in OVQ data were fitted in Excel (Microsoft Office 2010 for Windows) with polynomial (order 2) regression lines.

Results

For all analysed characteristics there were significant interactions between storage condition, i.e. darkness vs. light and storage duration, implying that each storage condition resulted in different dynamics of analysed characteristics during storage.

Overall visual quality (OVQ)

In all experiments and for all 4 genotypes, the OVQ of dark stored fresh-cuts declined more rapidly than that of light stored fresh-cuts (Figures 1, 2 and 3). In all experiments, the decline in OVQ followed a down-concave pattern (Figures 1, 2 and 3). In the first experiment (Figure 1), the loss of OVQ in light was minimal until day 28 for all green (GB), and for the red (RB) butterhead genotypes. A rapid loss of OVQ in dark stored fresh-cuts occurred around day 16 (GB 1, and GB 2) and day 19 (GB 3 and RB). In experiment 2 (Figure 2), the saleability limit of fresh-cuts prepared from GB was reached on day 7 in dark storage, day 12 when stored in light below the light compensation point (LCP), and on day 17 when stored in light above the LCP. In contrast, there were no significant differences in shelf-life for RB when stored under these storage conditions. In experiment 3, the saleability limit of fresh-cuts prepared from GB was reached on day 4 in darkness, and on day 7 during storage in light below and above the LCP (Figure 3).

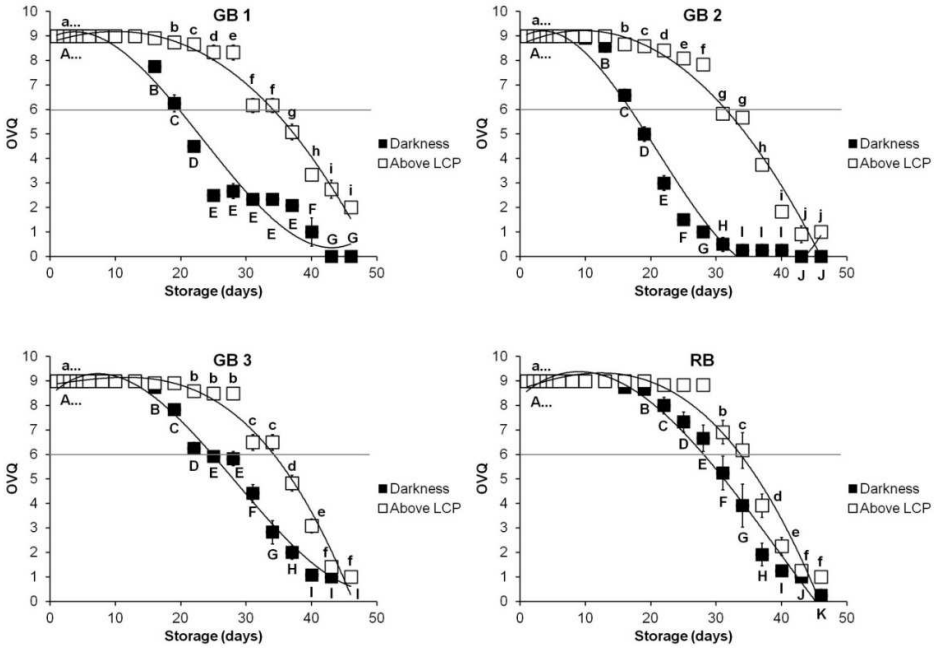

Fig. 1. Overall visual quality of leaf pieces of four butterhead genotypes (green: GB1, GB2, GB3, GB and red: RB) during storage at 12°C in darkness or light above the light compensation point (LCP) (30 μmol m^{-2} s^{-1}) in experiment 1. The horizontal line at OVQ = 6 represents the limit of saleability. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-J) for darkness, and lower case (a-j) for light above the LCP are significantly different between storage times (P<0.05).

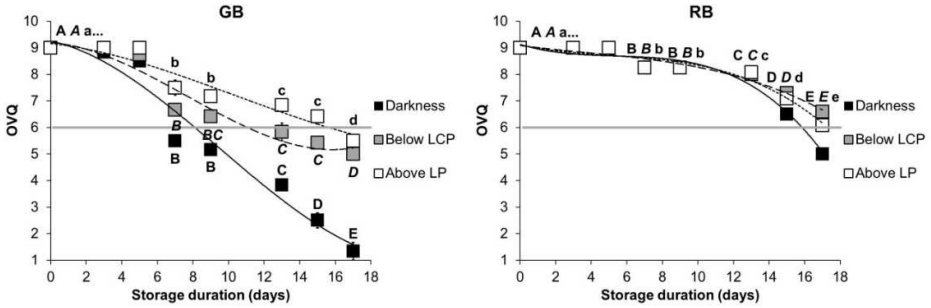

Fig. 2. Overall visual quality of leaf pieces of two butterhead genotypes (green, GB and red, RB) during storage at 12°C in darkness, light below the light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and above LCP (30 μmol m^{-2} s^{-1}) in experiment 2. The horizontal line at OVQ = 6 represents the limit of saleability. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-E) for darkness, upper case in italic (A-E) for light below the LCP, and lower case (a-f) for light above the LCP are significantly different between storage times (P<0.05).

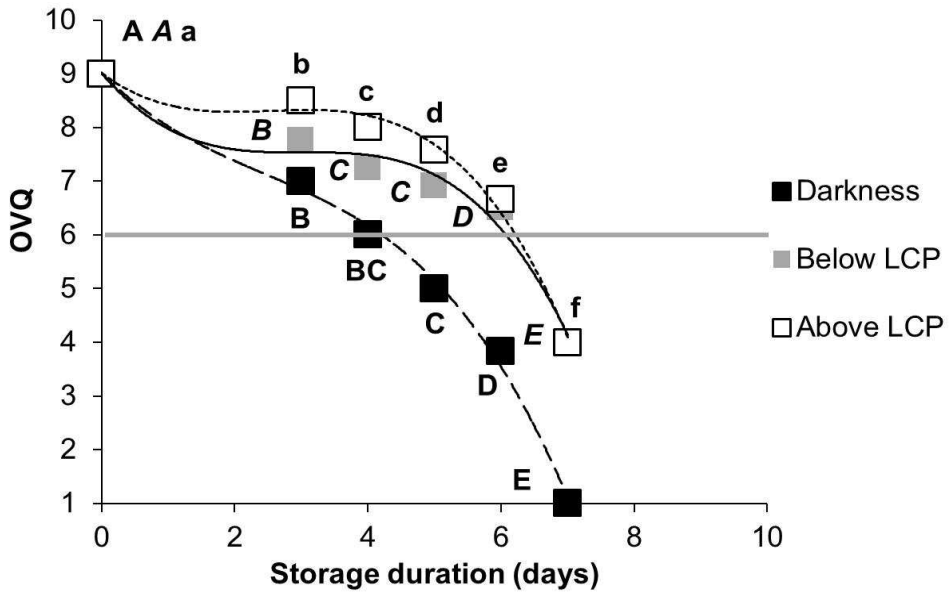

Fig. 3. Overall visual quality (OVQ) of leaf pieces of green butterhead (unknown variety purchased at local supermarket) genotype during storage at 12°C in darkness, light below the light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and above the LCP (30 μmol m^{-2} s^{-1}) in experiment 3. The horizontal line at OVQ = 6 represents the limit of saleability. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-E) for darkness, upper case in italic (A-E) for light below the LCP, and lower case (a-f) for light above the LCP are significantly different between storage times ($P<0.05$).

Pigment concentrations

The levels of chlorophyll and carotenoids decreased in fresh-cuts stored under all tested light conditions (dark, 8 and 30 μmol m^{-2} s^{-1}); the greatest loss of pigments for all treatments occurred between days 3 and 4 (Figure 4). Fresh-cuts stored under either light condition (below and above the LCP) reached lower final levels of pigments than fresh-cuts stored in darkness. Although fresh-cuts stored in darkness retained more chlorophyll and carotenoids until the end of storage period, these fresh-cuts approached the limit of saleability, as determined by an OVQ assessment, sooner than the fresh-cuts stored under either light condition.

Total ascorbate (AsA)

The content of total ascorbic acid (AsA) behaved differently under each storage condition (Figure 5). Under storage in darkness the loss of AsA occurred immediately, and it decreased progressively until the end of storage, when it had fallen to about 5% of the initial level. Under storage in light below LCP, the levels of AsA first slightly increased, to decrease thereafter until the end of the storage, when it had fallen to about 60% of the initial level. Under storage in light above LCP, the levels of AsA did not decrease during storage, instead the AsA levels slightly increased and remained on this level until the end of the storage.

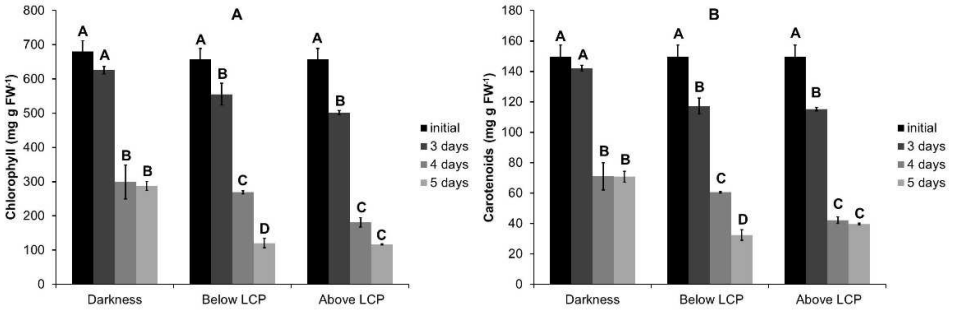

Fig. 4. Changes in chlorophyll (A) and carotenoids (B) content of leaf pieces of green butterhead (unknown variety purchased at local supermarket) during storage at 12°C in darkness, light the below light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and above the LCP (30 μmol m^{-2} s^{-1}) in experiment 3. Error bars represent standard errors. Data points marked by different upper case letter (A-D) are significantly different between storage times (P<0.05).

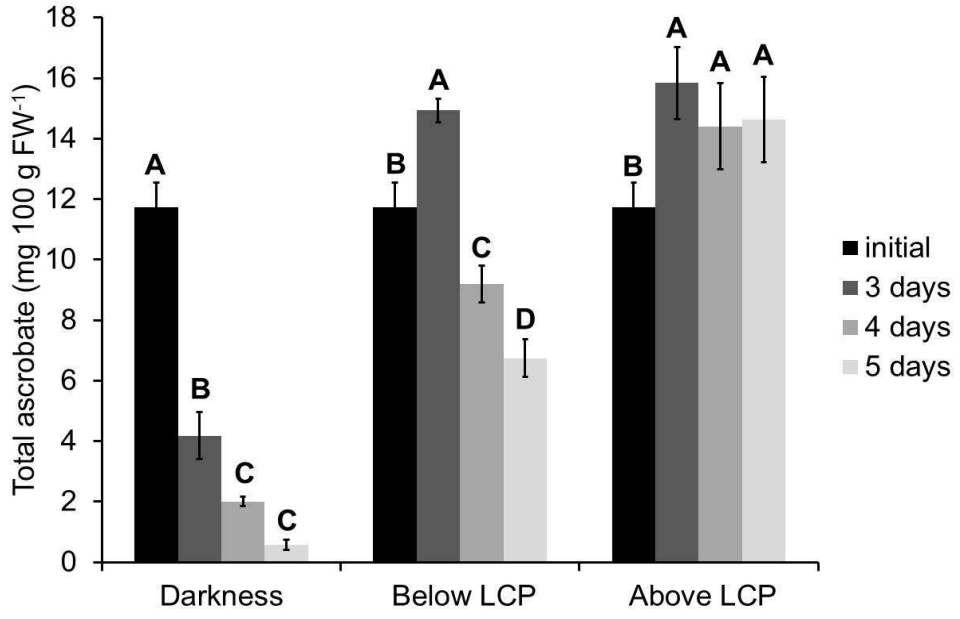

Fig. 5. Changes in total ascorbate of leaf pieces of green butterhead (unknown variety purchased at local supermarket) during cold storage in darkness, light below the light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and above the LCP (30 μmol m^{-2} s^{-1}) in experiment 3. Error bars represent standard errors. Data points marked by different upper case letter (A-D) are significantly different between storage times (P<0.05).

Soluble sugars and starch

The content of sucrose, glucose, fructose and starch showed a different pattern during storage in darkness compared to either of the light storage conditions (Figure 6). In general, during storage in darkness, the levels of all soluble sugars and starch remained

close to the initial levels. In contrast, during storage in light, the levels of soluble sugars and starch increased. The increases in sugars and starch were somewhat more pronounced and the end levels were higher in fresh-cuts that were stored in light intensity above the LCP than in the fresh-cuts stored in light intensity below the LCP.

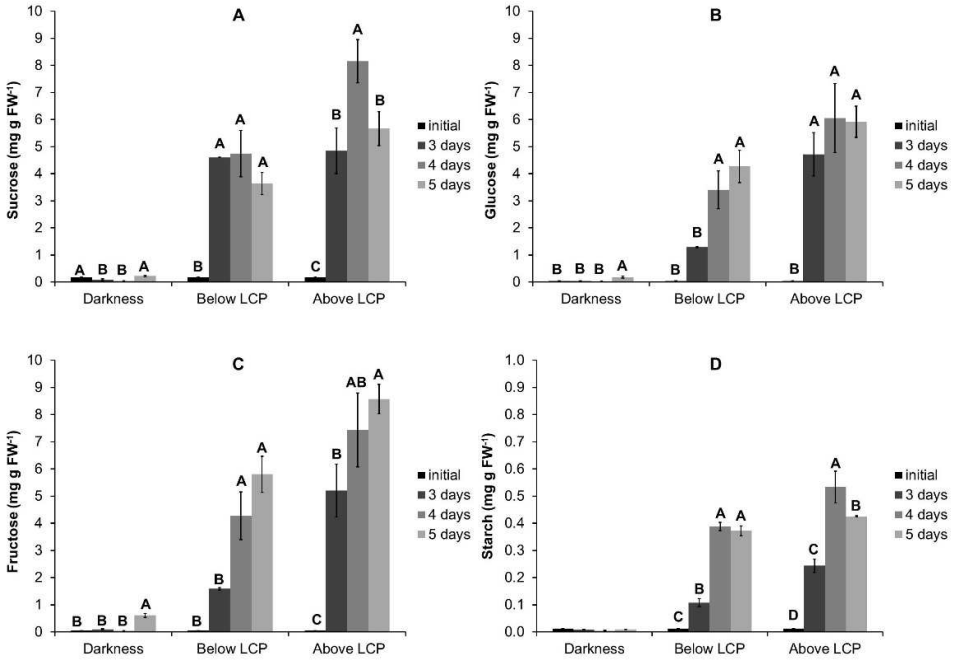

Fig. 6. Changes in glucose (A), fructose (B), sucrose (C) and starch (D) of green butterhead (unknown variety purchased at local supermarket) during cold storage in darkness, light below the light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and above the LCP (30 μmol m^{-2} s^{-1}) in experiment 3. Error bars represent standard errors. Data points marked by different upper case letter (A-D) are significantly different between storage times ($P<0.05$).

Net photosynthesis and dark respiration

Dark respiration of fresh-cuts stored in darkness did not change during the storage period, whereas dark respiration increased considerably in fresh-cuts stored in either of the light conditions (Figure 7A). The net photosynthesis (measured at 8 μmol m^{-2} s^{-1}) did not change during storage in dark stored fresh-cuts, but it decreased in fresh-cuts stored under both light conditions (Fig. 7b). The net photosynthesis (measured at 30 μmol m^{-2} s^{-1}) decreased for fresh-cuts stored in both light treatments and in dark conditions (Fig 7b). From the light response curves, it appeared that the quantum yield of fresh-cuts did not change under either of the storage conditions (data not shown). The decrease in the net photosynthesis rates during storage under light below and above LCP was entirely due to the increase in dark respiration.

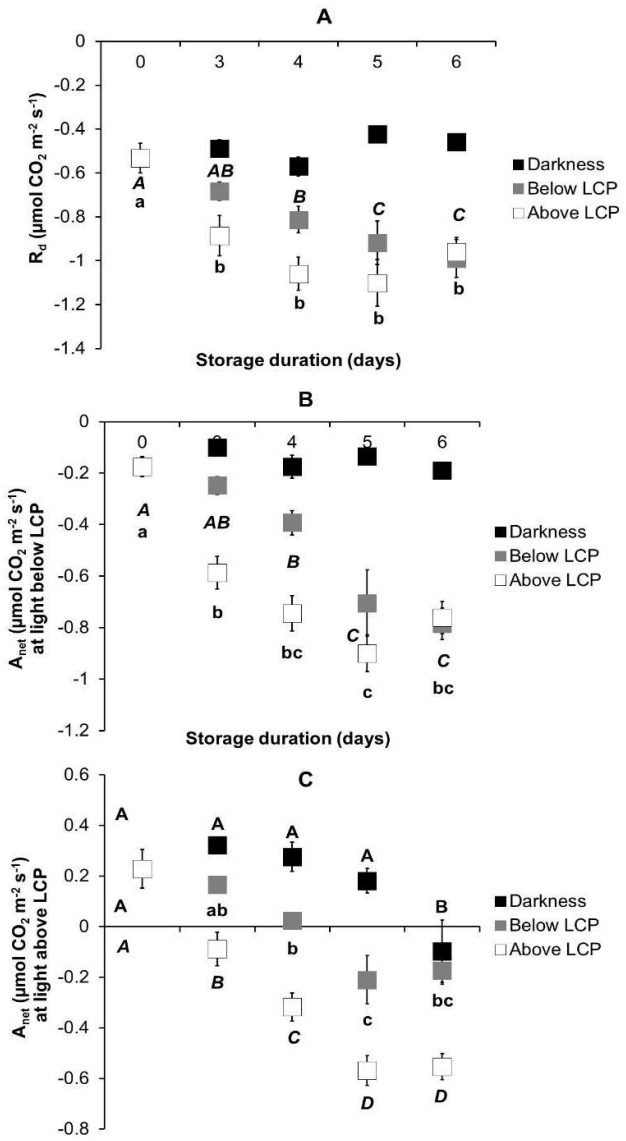

Fig. 7. Dark respiration (R_d; A) and net photosynthesis (A_{net}; B) measured at light below (8 µmol m^{-2} s^{-1}) and light above (C; 30 µmol m^{-2} s^{-1}) light compensation point (LCP) of green butterhead (unknown variety purchased at local supermarket) during storage at 12°C in darkness, light below and above the LCP in experiment 3. Data points marked by different letters, i.e. upper case (A-C) for darkness, upper case in italic (A-D) for light below the LCP, and lower case (a-c) for light above the LCP are significantly different between storage times ($P<0.05$).

Electrolyte leakage

In general, the electrolyte leakage increased during storage in fresh-cuts stored in darkness (Figures 8 and 9). In the fresh-cuts stored under either light below or above LCP little changes in electrolyte leakage were observed (Figure 8). Only in the experiment done with lettuce sourced from the supermarket (experiment 3), did an increase in electrolyte leakage

occurred for fresh-cuts stored in light below LCP occur, though this was less pronounced than in the dark-stored fresh-cuts (Figure 9).

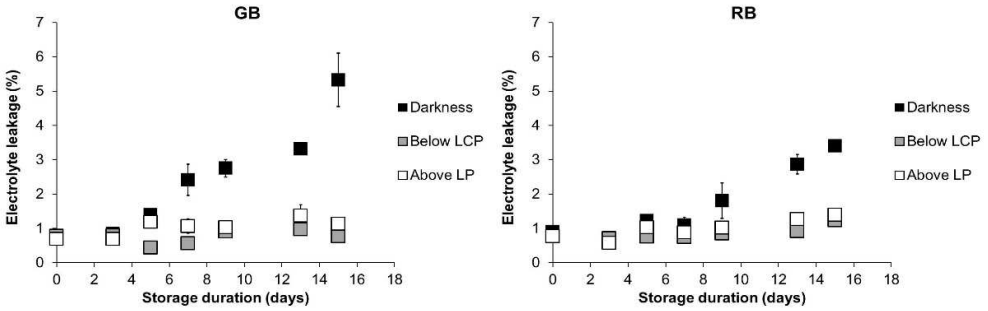

Fig. 8. Changes in electrolyte leakage of two butterhead genotypes (green, GB and red, RB) during storage at 12°C in darkness, light below the light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and above the LCP (30 μmol m^{-2} s^{-1}) in experiment 2. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-C) for darkness, upper case in italic (A-D) for light below the LCP, and lower case (a-c) for light above the LCP are significantly different between storage times ($P<0.05$).

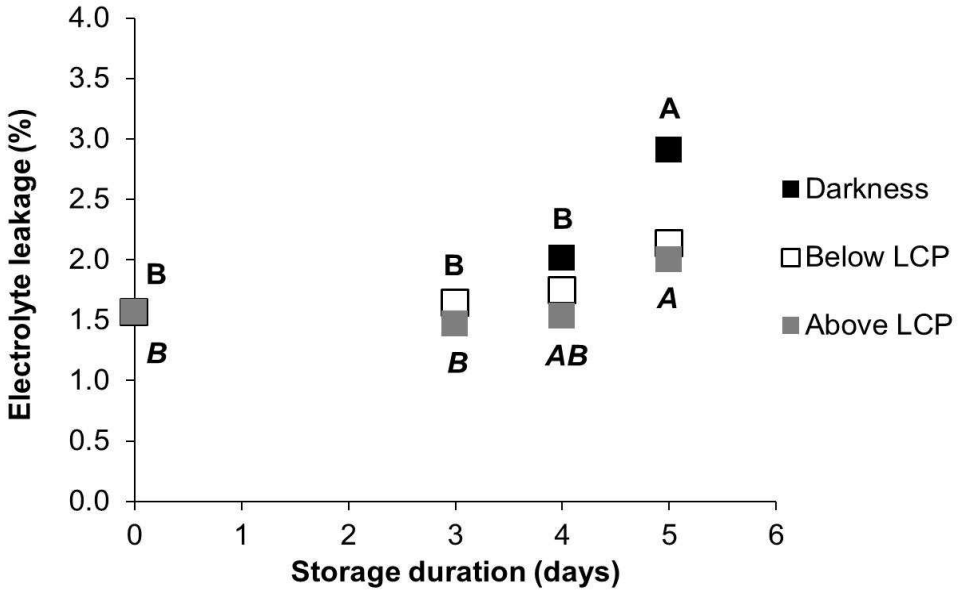

Fig. 9. Changes in electrolyte leakage of green butterhead (unknown variety purchased at local supermarket) during cold storage in darkness, light below the light compensation point (LCP) (8 μmol m^{-2} s^{-1}), and the above LCP (30 μmol m^{-2} s^{-1}) in experiment 3. Error bars represent standard errors. Data points marked by different letters, i.e. upper case (A-C) for darkness, and upper case in italic (A-D) for light below the LCP are significantly different between storage times ($P<0.05$).

Discussion

Overall visual quality

Overall visual quality has been used commonly as a sensory index closely associated with consumer acceptability (Kader, 1957; López-Gálvez et al., 1996, 1997; Gil et al, 1998; Fan et al., 2003; Aguero et al., 2008). Also in this research, it appeared to be a useful tool for comparing effects of different treatments on the end of shelf-life of fresh-cut lettuce, i.e. when the limit of saleability was reached. There were quite some inter-experimental differences in the number of days until the fresh-cut product reached the saleability limit, i.e. 15 – 35 days in experiment 1 (Figure 1), 8-16 days in experiment 2 (Figure 2), and 4-6 days in experiment 3 (Figure 3). This was related to i) different storage temperatures 4°C (exp. 1) and 12°C (exp. 2 and 3), ii) differences in the developmental stage of plants (relatively young plants in exp. 1, and more mature plants in exp. 2 and 3), and iii) the origin of the plant material (own laboratory grown plants in exp. 1 and 2, and supermarket distribution centre in exp. 3). Despite these differences in plant material and the associated shelf-life, the effects of light versus dark storage on fresh-cut lettuce quality were consistent. Continuous illumination of fresh-cut lettuce at intensities below and above the light compensation point (LCP) effectively increased the storability of this product, by for example reducing browning of cut edges. The lesser effect of light on the quality retention of RB might be explained by a lack of visible symptoms of edge browning in this genotype, being a significant contributor to OVQ scores. In addition, the lesser light perception of this anthocyanin rich tissue, which is also reflected in the general lower photosynthesis activity of this tissue may have reduced the effects of low light intensities on the quality retention. The improved storability of the fresh-cuts under light when compared to darkness coincided with increased levels of soluble sugars, starch and ascorbic acid (AsA) in light treated fresh-cuts. Clearly, light during storage improved both, the visual and nutritional quality of fresh-cut lettuce.

Dark induced senescence during postharvest storage

The postharvest longevity of plant tissue is influenced by the senescence processes that occur during storage (Noodén et al., 1997; Rosenwasser et al., 2006, 2011). Senescence leads to metabolic changes such as loss of chlorophyll, proteolysis, and changes in nucleic acids, photosynthesis and respiration. However, it is the absence of photosynthesis and, as a consequence, the rapidly declining levels of carbohydrates (Buchanan-Wollaston et al., 2005) that have been proposed to be the main cause of dark induced senescence (Gan and Amasino, 1997). The observed senescence changes in fresh-cut lettuce are discussed below.

Loss of chlorophyll, and changes in photosynthesis and respiration

In this study, the decrease in chlorophyll levels was slightly higher in light-stored than in dark-stored fresh-cuts (Figure 4). This result is both supported (Noichinda et al., 2007) and contradicted (Barbieri et al., 2009) by earlier studies on intact leaves of other species of leafy vegetables. Our results might be explained by i) an application of insufficient light intensity during storage to prevent chlorophyll breakdown, ii) chlorophyll being not always a reliable indicator of senescence (Munné-Bosch and Alegre, 2004). The photosynthetic efficiency of the fresh-cuts under all storage conditions was maintained (Figure 7). However, the net photosynthetic rate was lower for the fresh-cuts stored under

light as a result of an increase in respiratory activity. Despite the negative net photosynthetic rate in light treated fresh-cuts, there was a significant accumulation of sugars and starch (Figure 6).

Changes in carbohydrate levels

The increased levels of soluble sugars and starch in the tissue (Figure 6) are in agreement with Noichinda et al. (2007), the only work reporting the increased levels of fructose and glucose during light storage of a leafy vegetable. Based on their results these authors concluded that the photosynthetic processes still continued under the low light intensity (21 μmol m^{-2} s^{-1}) during storage. Other authors (Toledo et al., 2003; Lester et al., 2010) have also suggested this possibility, but so far the association between increased levels of sugars during storage in light and actual photosynthetic rates has not been reported. Furthermore, in the investigations on the effect of light on storability of various vegetables (Toledo et al,. 2003; Kasim and Kasim 2007; Noichinda et al., 2007; Sanz et al., 2007, 2008, 2009; Ayala et al., 2009; Olarte et al., 2009; Zhan et al., 2009; Lester et al., 2010; Martínez-Sánchez et al., 2011) the light intensities used ranged between 4 and 45 μmol m^{-2} s^{-1}, which is below or about equal to the LCP of these vegetables, and might have been insufficient for a positive photosynthesis rates. In addition, in these studies the limited exchange of gases through the packaging material during storage led to unfavourable treatment-dependent changes in the atmosphere, which may have masked the effects of light *per se*.

This is the first report which combines photosynthetic data measured concurrently with the levels of soluble sugars, starch and AsA in the tissue of a leafy vegetable during cold storage in light and darkness under ambient atmosphere. Storage in darkness caused a rapid depletion of chlorophyll and carotenoids (Figure 4), a depletion of AsA (Figure 5), and an increase in electrolyte leakage (Figure 8 and 9). Storage in light did not prevent chlorophyll and carotenoids breakdown (Figure 4), but preserved AsA (Figure 5), led to the accumulation of carbohydrates (Figure 6), and delayed the increase in electrolyte leakage (Figure 8 and 9). In darkness, the absence of photosynthesis and, as a consequence, severe depletion of primary respiratory reserves results in utilization of secondary respiratory substrates, e.g. lipids, proteins and organic acids, as an energy source (Kays and Paull, 2004; Buchanan-Wollaston et al., 2005). The degradation of lipids, which are the main constituents of membranes, may lead to membrane disintegration and increased ion leakiness (Thompson et al., 1998). This may explain the increase in the electrolyte leakage that was mainly observed in fresh-cuts stored in darkness (Figure 8 and 9). These results are in agreement with Martínez-Sánchez (2011) who found higher electrolyte leakage in darkness than in light after 10 days of storage of fresh-cut Romaine lettuce. Since the cell membrane disruption has been reported to correlate with chlorophyll loss (Zhuang et al., 1994, 1997) and chlorophyll and proteins are not a fundamentally different measure (Peterson et al., 1973; Telek and Graham, 1983; Gan 2007), we speculate that in parallel to chlorophyll, other proteins were also degraded and used as an alternative energy source under all storage conditions. The rapid decrease of AsA in dark stored fresh-cuts may have also compromised the membrane stability, as AsA may be required as an antioxidant involved in the prevention of ROS-associated membrane lipid peroxidation (Borraccino et al., 1994; Hodges and Forney, 2000, Hodges et al., 2001; Jimenez et al., 1998). In contrast to what was observed in darkness, sugars accumulated in the light (Figure 4), and this accumulation appeared to be dependent on the intensity of light. In irradiances higher

than the LCP, the positive photosynthetic rates during the first two days of storage may have resulted in the accumulation of sugars. However, these initial positive photosynthetic rates cannot explain the sustained level of sugars in the longer term. Also the increase in the sugar content of lettuce stored at irradiances lower than the LPC cannot be simply explained by photosynthesis. It was observed that just as in darkness, lipids and proteins (as indicated by the similar chlorophyll breakdown in all treatments) were broken down at low light levels. The low levels of light may trigger the recycling of non-carbohydrate breakdown products into sugars (gluconeogenesis) and the increased levels of sugars may in turn stimulate respiration. The increased respiratory activity may protect tissue from a depletion of ATP, which facilitates maintenance processes, and prevent tissue to enter the cell death process (Azad et al., 2008) (Figure 10). In the dark, no accumulation of sugars, and no increased respiration was observed. Apparently, the breakdown products were not recycled into carbohydrates in the absence of light, and may turn into toxic molecules. The low light levels apparently also preserve the levels of AsA, and this may be related to the coupling between photosynthetic electron transport and AsA biosynthesis or may be due to the higher sugar levels that in itself may stimulate AsA biosynthesis (Figure 10).

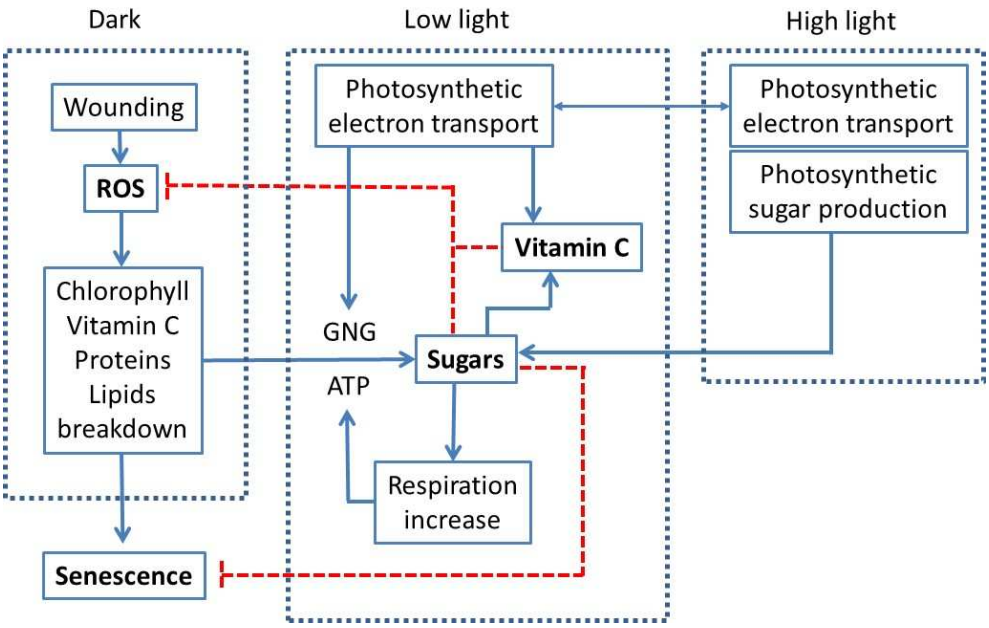

Fig. 10. Proposed mechanism of action of dark and light storage in combination with wounding on some physiological parameters in fresh-cuts produce. The arrows indicate an influence of one factor on another factor, and broken lines indicate inhibition of one factor on another.

Consequences of increased levels of sugars and starch on the shelf-life

In this study, the extended storability of the fresh-cut lettuce during light storage can be explained by the increased levels of soluble sugars and starch in the tissue. The importance for storability of fresh produce of a high level on sugars as an energy source has been already suggested by several authors e.g. Lipton (1987), Klieber et al. (2002) and Clarkson et al. (2005), since the reduced levels of carbohydrates lead to severe deterioration of the

tissue in several ways. Firstly, due to the stress resulting from the starvation of the tissue the production of reactive oxygen species (ROS) increases (Couée et al., 2006). Hydrogen peroxide and products of lipid peroxidation have been reported to increase during dark-induced senescence of watercress and parsley leaves (Philosoph-Hadas et al., 1994), wheat and rye leaves (Kar and Feierabend, 1984), and *Pelargonium* leaves (Rosenvasser et al., 2006). Secondly, the restrictive levels of the substrate (sugar) for the synthesis of AsA (Smirnoff and Pallanca, 1996), decrease levels of this important antioxidant (Walker, 1995; Alscher, 1997; Degl'Innocenti et al., 2005). The maintenance of AsA during light storage in this study may have benefited the tissue in two ways. Since AsA inhibits browning by reducing enzymatically formed o-quinones to their precursor's diphenols (Walker et al., 1995; Alscher et al., 1997; Degl'Innocenti et al., 2007), it reduced the browning of cut edges. Browning of cut edges significantly contributed to a decrease in the OVQ scores (data not shown). In addition, AsA may contribute to reduced or delayed senescence, such as lipid peroxidation, protein breakdown, and as a consequence disintegration of membranes, since AsA has been suggested to play a direct role in delaying senescence through the inhibition of H_2O_2 accumulation (Borraccino et al., 1994; Hodges and Forney, 2000, Hodges et al., 2001; Jimenez et al., 1998). Lastly, the soluble sugars might act as ROS scavengers, may increase membrane stability and may act as an osmolyticum increasing cell turgescence (Bolouri-Moghaddam et al., 2010).

Conclusions

We found that the exposure of excised tissue to low light levels during storage extends its post-harvest life. This extended storability of the fresh-cut lettuce might be explained by the increased levels of soluble sugars, starch and AsA in the tissue. Under all experimental conditions lipids and proteins (as indicated by the similar chlorophyll breakdown in all treatments) were broken down. However only in light, the break down products may be recycled into sugars (gluconeogenesis). The increased sugar levels might give an increase in respiratory activity, and consequently improve ATP levels, which facilitate maintenance processes, and delay cell death in wounded tissue. In addition, the soluble sugars might act as ROS scavengers, may increase membrane stability and may act as an osmolyticum increasing cell turgescence (Bolouri-Moghaddam et al., 2010). The maintenance of AsA in light was due to either coupling between photosynthetic electron transport and AsA biosynthesis, or through the increased sugar level providing substrate for its synthesis. The high AsA levels benefited tissue in two ways, by reducing the browning of cut edges, and preventing ROS-associated membrane lipid peroxidation. The above described mechanisms together delay senescence processes, and therefore quality deterioration of fresh-cut photosynthetic tissue (Figure 10).

Acknowledgements

This research was supported by The Greenery B.V., the Netherlands and by Rijk Zwaan B.V., the Netherlands. The assistance of Jospeh Sserwanga and Triantafyllos Xenos with data collection, help and advice with chemical analysis of Arjen van de Peppel and Niovi Christodoulou, as well as with Li-cor measurements of Wim van Ieperen and Aaron Velez Ramirez is greatly appreciated.

CHAPTER 5

Storage prior to processing of intact heads limits the shelf-life of fresh-cut *Lactuca sativa* L.

Abstract

Before processing, the raw produce is usually transported and stored for some period of time under a variety of conditions. During storage physiology and composition of the harvested produce continues to change. Up to date, the impact of storage prior to processing on the quality of the fresh-cut product has not been quantified, and became the aim of this study. We investigated the effects of different duration of storage prior to processing (3 or 5 days at 12° C) and different lighting conditions (dark vs. light) on the quality of both the intact heads and on the fresh-cut produce made from these heads. The experiments were performed with four related genotypes of *Lactuca sativa* L. and the effect of storage prior to processing of heads with and without their root system was investigated. Depending on the experiment we evaluated the changes in visual quality, the level of energy reserves in the tissue, and some selected senescence markers, i.e. chlorophyll content and electrolyte leakage. The intact heads had still good appearance even after 17 days of storage. However, a decline in the soluble sugars, a decrease in chlorophyll, and an increases in electrolyte leakage was observed. These changes in physiological parameters of the raw product had a great impact on the storability of the fresh-cuts made from these heads. In general, the longer the time between harvest and processing, the shorter was the shelf-life of fresh-cuts. The storage prior to processing of intact heads of green butterhead in light resulted in a slightly better quality retention of fresh-cuts compared to fresh-cuts prepared from heads stored in darkness. The lack of a visible effect of light on quality retention of red butterhead might be explained by a lack of visible symptoms of edge browning in this genotype, being a significant contributor to OVQ scores. Besides, the lesser light perception of this anthocyanin rich tissue may also have impeded the effect on quality retention. Storage of rooted heads did not alleviate the negative effect of prior storage on the quality of fresh-cuts.

Witkowska, I.M., Woltering, E.J. 2013. Storage prior to processing of intact heads limits the shelf-life of fresh-cut *Lactuca sativa* L. (*submitted)*

Introduction

The quality of fresh-cut product depends greatly on the initial quality i.e. the quality of the raw product used for processing (Watada et al., 1996). A high quality product can be achieved by optimizing pre-harvest factors, such as genetics (Varoquaux et al., 1996; Degl'Innocenti et al., 2007), environmental factors (Voipio and Autio, 1995; Eskins, 1996; Krizek et al., 1998; Romani et al., 2002; Kleinhenz et al., 2003; Gruda et al., 2005; Gazula et al., 2005; Garcia-Marcias et al., 2007; Tsormpatsidis et al., 2008; Chapter 2), and the developmental stage (maturity) of the plants at harvest (Couture et al., 1993; Pandjaitan et al., 2005; Bergquist et al., 2006; Zhao et al., 2007; Chapter 3). In addition, high quality can be achieved by optimizing post-harvest conditions such as temperature, humidity, atmospheric conditions and light during storage prior to processing (Hodges and Toivonen, 2008). In general the aim is to control post-harvest changes in order to manage product quality in such a way that it is as close as possible to its conditions at harvest.

Product processing is seldom done by the producer, but mainly by specialized regional or national processors. Therefore, the time between harvest and processing depends on the distance between the producer and the processor, on the current demand, and on possible speculative storage of the products. Storage of the raw product after harvest is a common practice, during which the physiology and composition of the harvested tissue continues to change.

The impact of storage prior to processing on the quality of processed fresh-cut leafy vegetables has not been quantified. The aim of this study was to investigate the effect of time between harvest and processing on the quality of both the raw and processed product. Except for assessing the visual quality of stored heads, we also evaluated the changes in the content of soluble sugars. It has previously been suggested that the storability of the product might be linked to the primary energy reserves of the tissue (Kays and Paull, 2004; Chapter 4). In addition to soluble sugars, we measured changes in selected senescence markers, (i.e. chlorophyll content and electrolyte leakage) that may be associated with prolonged dark storage (Wangermann, 1965; Trippi and Thimann, 1983; Biswal and Biswal, 1984; Buchanan-Wollaston, 1997; Hodges and Forney, 2003). Our results show that storage prior to processing of the raw produce has a significant impact on the shelf-life of the fresh-cuts made from these heads.

Materials and Methods

Plant material and growth conditions

In experiment 1, four morphologically related genotypes of *Lactuca sativa* L., green butterhead (GB) *cv.* Troubadour 1 (GB 1), *cv.* Troubadour 2 (GB 2), *cv.* Troubadour 3 (GB 3), and red butterhead (RB) *cv.* Teodore (Rijk Zwaan BV, The Netherlands) were used. The plants, at the commercial maturity stage, were received from Rijk Zwaan. Two genotypes, i.e. GB 1 and RB were used for the storage experiment of intact heads, whereas the other two genotypes, i.e. GB 2 and GB 3 were used for storage of fresh-cuts. In experiment 2, three morphologically related genotypes of *Lactuca sativa* L., green butterhead (GB) *cv.* Troubadour 1 (GB 1), *cv.* Troubadour 3 (GB 3), and red butterhead (RB) *cv.* Teodore (Rijk Zwaan, The Netherlands) were used. The plants were grown in a climate chamber located at Wageningen University, The Netherlands. The seeds were sown in boxes filled with

vermiculite, and one week after germinating were transplanted to a hydroponic system (Hoagland's solution, pH 5.9±0.2; EC=1.2 mS cm^{-1}). Temperatures were maintained at 20°C during the day and at 15°C during the night, and the relative humidity was 70%. White fluorescent tubes (FL; TLD 50 W 840 HF, Philips, The Netherlands) were used to provide photosynthetically active radiation (PAR) at 250 μmol m^{-2} s^{-1} (12h photoperiod). The seedlings and lettuce plants were not chemically treated before or during the experiment. 42 days after transplanting, plants were harvested in the morning, and were used for the storage experiment. In experiment 3, two morphologically related genotypes of *Lactuca sativa* L., green butterhead (GB) *cv.* Troubadour 1 (GB 1), and red butterhead (RB) *cv.* Teodore (Rijk Zwaan, The Netherlands) were used. The plants were grown under the same conditions, harvested at the same age, and the leaves used as in experiment 2. In each experiment, lettuce heads used for storage or processing experiments were selected for uniformity in their size and color, and for the absence of defects.

Storage experiments with intact heads and fresh-cuts

In experiment 1, for the storage of intact heads, 6 lettuce heads of each genotype were divided in two batches for storage i) with roots, and ii) without roots in darkness at 12°C. For storage of fresh-cuts, 9 lettuce heads of each genotype were divided in three batches for processing i) immediately after harvest, ii) after 3 days of prior dark storage at 12°C, and iii) after 5 days of prior dark storage at 12°C. In experiment 2, 15 lettuce heads of each genotype were divided in three batches for processing i) directly after harvest, ii) after 5 days of prior dark storage at 12°C of heads with roots, and iii) after 5 days of prior dark storage at 12°C of heads without roots. In experiment 3, 15 lettuce heads of each genotype were divided in three batches for processing i) directly after harvest, ii) after 5 days of prior dark storage at 12°C, and iii) after 5 days of prior light storage at 12°C. In all experiments, the heads with trimmed roots were packed in perforated plastic foil to prevent desiccation, whereas the heads with roots were stored in pots with soil, and watered when needed. We used both systems, as a reinsurance that the desiccation of heads during storage did not occur. In addition, half of the samples were kept under 30 μmol m^{-2} s^{-1} light, being a level that is well above the light compensation point (LCP) (Chapter 4). White fluorescent tubes (FL; TLD 36 W 830 HF, Philips, The Netherlands) were used to provide continuous photosynthetically active radiation (PAR).

In all experiments, leaf pieces (4 x 4 cm) were cut from the middle part of the leaf lamina with a sharp stainless steel knife, avoiding the inclusion of the midrib and major veins. Randomly selected leaf pieces were placed as a single layer in white plastic boxes (1300×1800-mm) lined with wet filter papers (Whatman, #3) to prevent desiccation. The boxes were equipped with transparent plastic lids with 16 small holes (approximately 0.5 mm diameter). This allowed sufficient ventilation to prevent depletion of oxygen or accumulation of carbon dioxide or ethylene under the experimental conditions (data not shown). The boxes were placed in dark storage rooms for further storage at 12°C in the dark, and a relative humidity of 90%.

At every sampling time intact leaves or leaf pieces, were randomly selected from three different boxes (replicates). Per replicate, from selected leaves or leaf pieces 9 leaf discs, 1.8 cm in diameter, were excised, weighed, and specific leaf weight on fresh weight basis (SLW) was calculated. Two leaf discs were used directly for determination of

pigments, five leaf discs were used for the electrolyte leakage, and two leaf discs (sugar) were directly frozen in liquid nitrogen, dried using a freeze-dryer (Modulyo, Pirani 501, Edwards, UK) and ground into fine powder with a ball-mill (Retsch, Germany).

Overall visual quality (OVQ) and shelf-life

Lettuce quality of both the discs and the leaf pieces was evaluated using overall visual quality (OVQ) ratings modified from Kader et al. (1973). The OVQ was evaluated on the basis of leaf characteristics such as color (yellowing, browning, and brightness), wilting, and presence or absence of defects. Quality ratings were made on a scale from 9 to 1, where 9 = excellent, essentially free from defects, and 1 = extremely poor, not usable. An OVQ rating of 6 was considered the lower limit of consumer acceptance.

Determination of pigments

Pigments were extracted in dimethylformamide (DMF) in the dark at -20˚C. The absorbance of the extract was measured in the range 400–750 nm using a Cary 4000 spectrophotometer (Varian Instruments, Walnut Creek, CA, USA), and the pigment content was calculated using the equations provided by Wellburn (1994).

Determination of soluble sugars

Ten micrograms of powdered freeze-dried lettuce sample was extracted in 5 ml of 80% ethanol for 20 minutes at 80°C. One milliliter of supernatant was vacuum dried (Savant SPD2010 SpeedVac, Thermo Scientific, Asheville, NC, USA), the residue was re-suspended in 2 ml of 0.01 M HCl, and resulting extracts were passed through an Extract CleanTM SCX column (Grace, Deerfield, IL, USA) activated with 5 ml 0.01 M HCl. The eluents were analyzed for soluble sugars on a Dionex ICS5000 HPLC equipped with a CarboPac1 (250x2mm) column, and eluted with a gradient from 16 to 45 mM NaOH. The levels of sucrose appeared to be below detection limit, and only the levels of fructose and glucose are presented.

Electrolyte leakage

Tissue electrolyte leakage was measured following a modified procedure from Saltveit (2002). The leaf discs were washed in milli-Q water for 10 min, then blotted dry and submerged in 0.2 M Mannitol solution. The electrolytes leaching into solution were measured using a conductivity meter (340i WTW, Weilheim, Germany) after 2h at ambient temperature. The total electrolyte content of the samples was determined by freezing the samples at -20°C for 24h, and subsequently thawing them. Electrolyte leakage measured after 2h of leaching was expressed as a percentage of the total electrolyte leakage.

Statistical analysis and curve fitting

Data were processed using the SPSS statistical package (IBM SPSS, release 19.0.0.1, 2010. Data were processed using the SPSS statistical package (IBM SPSS, release 19.0.0.1, 2010. SPSS Inc. and IBM Company, Chicago, IC, USA). Each experiment had three replicates per treatment. Each genotype was analyzed separately. For changes in analyzed characteristics during storage we assumed fixed effect for storage duration (all experiments), time of processing (experiment 1 and 2), and storage condition (experiment 3), and random effect of the replicate. The interactions between time of processing and storage duration were

studied by looking at time of processing per storage duration, and storage duration per time of processing. The interactions between storage condition and storage duration were studied by looking at storage condition per storage duration, and storage duration per storage condition. Mean separations was performed using the LSD procedure, and significance was declared at $P < 0.05$. The trends in OVQ data were fitted in Excel (Microsoft Office 2010 for Windows) with polynomial (order 2) regression lines.

Results

Quality changes in intact heads with and without roots during storage

In experiment 1, the overall visual quality (OVQ) of intact heads of both genotypes, i.e. green 1 (GB 1) and red (RB) butterhead did not decline significantly during storage at 12°C in darkness, and the limit of saleability was not reached even after 17 days (Figure 1). This very slow and minor decrease in OVQ was similar for heads of both genotypes stored with and without roots. However, the decline in OVQ was slightly quicker for RB than GB 1.

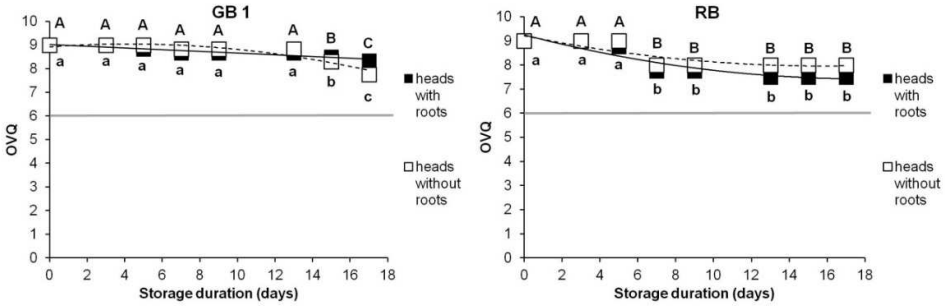

Fig. 1. Overall visual quality (OVQ) of heads with or without roots of two butterhead genotypes (green 1, GB 1 and red, RB) during storage at 12°C in darkness in experiment 1. Error bars represent standard errors. Data points marked by different upper case letters (A-C) for heads with roots, and lower case letters (a-b) for heads without roots are significantly different between storage times ($P<0.05$).

The content of chlorophyll, carotenoids, glucose and fructose declined during storage (Figure 2 and 3). In both genotypes, the decline in all compounds was similar for heads stored with and without roots. The decline in the chlorophyll and carotenoids was more pronounced in RB than in GB 1. After 15 days of storage, the content of both compounds had decreased by about 45% in the RB, whereas the decline was only 15% in the GB 1. In contrast, the decline in the glucose content was greater in GB 1 than in RB. After 15 days of storage, the content of glucose had decreased by about 95% in GB 1, whereas the decline was only about 60% in RB. The decline in the fructose content was similar for both genotypes, and only about 10% remained in the tissue after 15 days of storage.

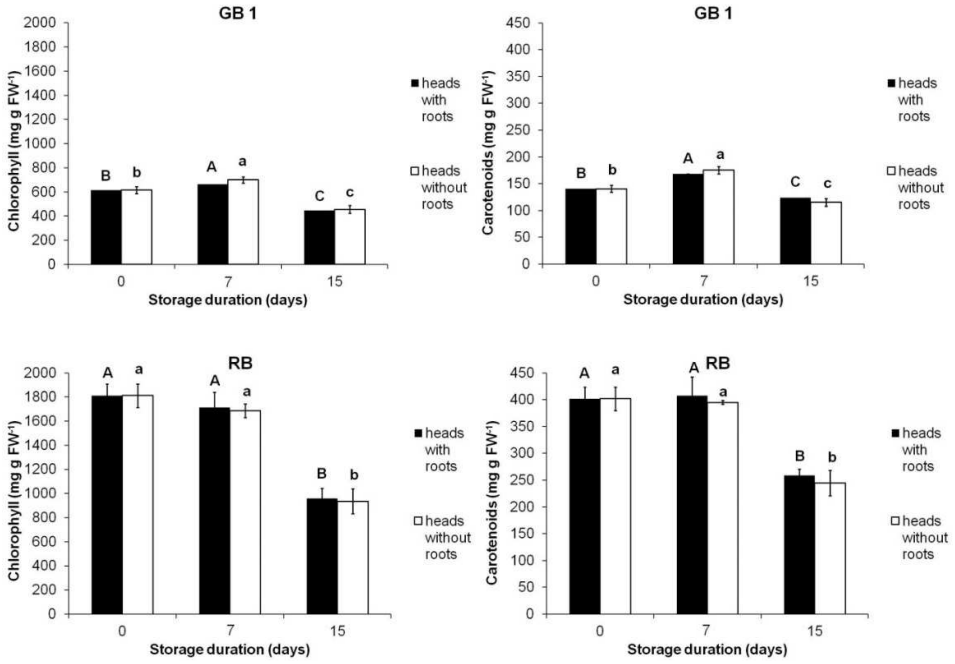

Fig. 2. Changes in chlorophyll (left) and carotenoids (right) content of heads with or without roots of two butterhead genotypes (green 1, GB 1 and red, RB) during cold storage in darkness in experiment 1. Error bars represent standard errors. Data points marked by different upper case letters (A-C) for heads with roots and lower case letters (a-c) for heads without roots are significantly different between storage times ($P<0.05$).

The electrolyte leakage (EL) of discs increased to the same extent for heads stored with and without roots. The increase in EL during storage was more pronounced in GB 1 than in RB (Figure 4).

Quality of fresh-cuts processed from intact heads immediately after harvest or after prior storage in darkness

In both experiments, the shelf-life of the fresh cut product was maximal when the heads were processed immediately after harvest. Storage prior to processing of the intact heads for 3 to 5 days prior to processing significantly affected the shelf-life of the fresh-cuts (Figure 5 and 6). In experiment 1 (Figure 5), the effect of storage prior to processing on the shelf-life of the fresh-cuts was dependent on the genotype. The effect was more pronounced in GB 3 than in GB 2. For GB 2, the shelf-life was about 4 days shorter when heads had been stored 5 days prior to processing, whereas the storage prior to processing for 3 days did not greatly affect the shelf-life of fresh-cuts. For GB 3, the shelf-life of the fresh-cuts decreased from 11 to 5-6 days when heads were stored for 3 or 5 days prior to processing (Figure 5). In experiment 2 (Figure 6), the shelf-life of the fresh-cuts of all genotypes was also greatly affected by the storage prior to processing. In all genotypes the shelf-life was reduced by 7 or more days following 5 days of prior storage of the heads. Storage of heads with or without roots had no effect on the quality loss.

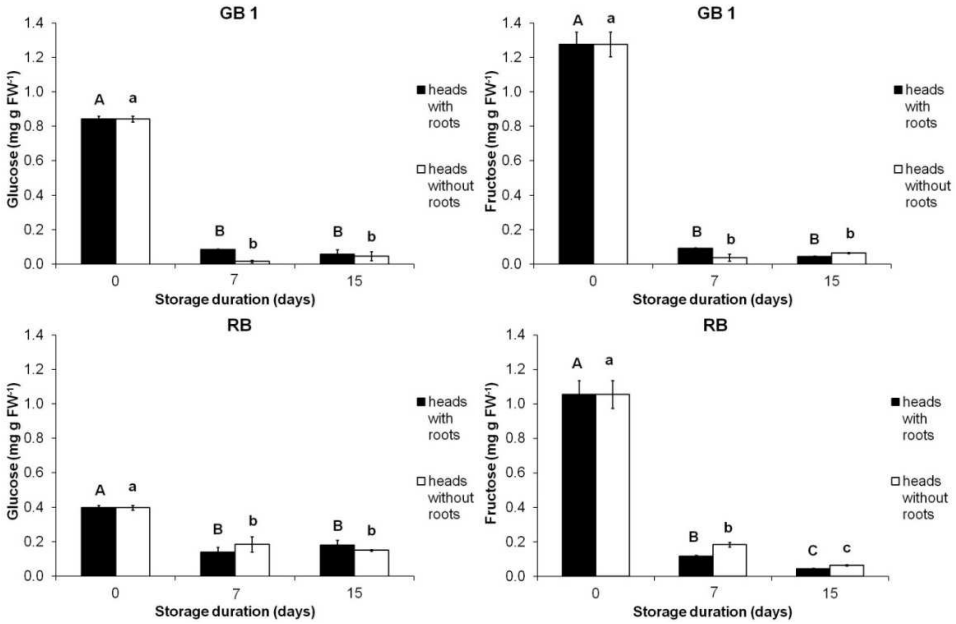

Fig. 3. Changes in glucose (left) and fructose (right) content of heads with or without roots of two butterhead genotypes (green 1, GB 1 and red, RB) during cold storage in darkness in experiment 1. Error bars represent standard errors. Data points marked by different upper case letters (A-C) for heads with roots and lower case letters (a-c) for heads without roots are significantly different between storage times ($P<0.05$).

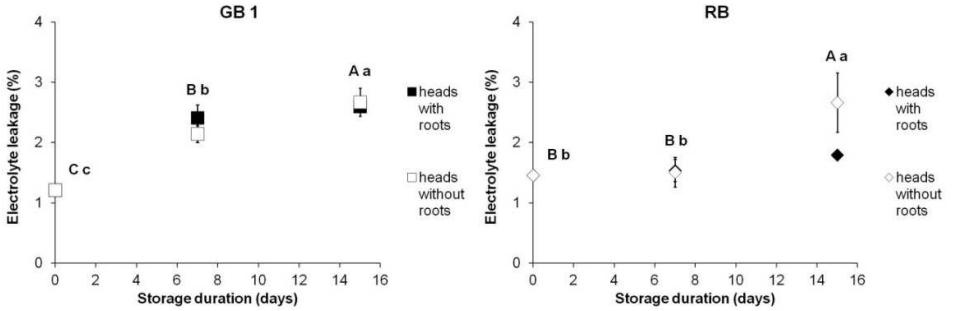

Fig. 4. Changes in electrolyte leakage of discs from heads with or without roots of two butterhead genotypes (green 1, GB 1 and red, RB) during cold storage in darkness in experiment 1. Error bars represent standard errors. Data points marked by different upper case letters (A-C) for heads with roots or lower case letters (a-c) for heads without roots are significantly different between storage times ($P<0.05$).

The content of chlorophyll and carotenoids in fresh-cuts for all treatments in experiment 2 (Figure 7) showed an overall decrease between day 0 and day 6. The decrease in both parameters was similar for fresh-cuts processed immediately after harvest or after 5 days of storage. Results were similar for heads stored with and without roots,

and the three genotypes showed essentially similar behaviour. The decline in chlorophyll and carotenoids was similar for GB 1 and GB 3 (about 15% decline), whereas the decline was more pronounced for RB (about 30% decline). The changes in EL of fresh-cuts during storage appeared to depend on the genotype (Figure 8). For GB 1 fresh-cuts no clear effect of storage of heads prior to processing was apparent, whereas GB 3 and RB fresh-cuts from stored heads reached higher EL levels sooner than the fresh-cuts processed immediately after harvest.

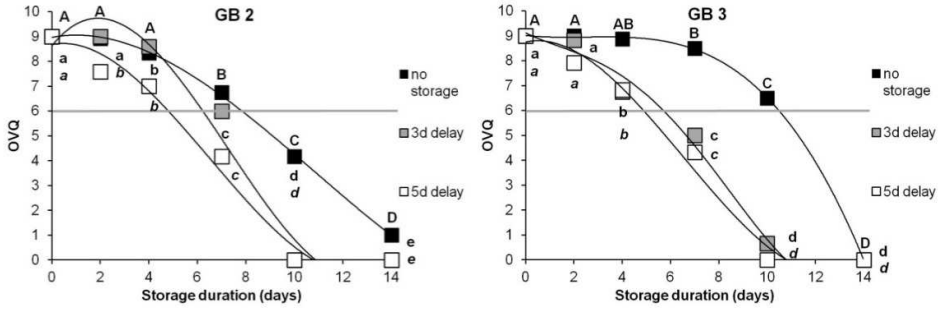

Fig. 5. Overall visual quality (OVQ) of fresh-cuts of two butterhead genotypes (green 2, GB and green 3, GB 3) processed immediately after harvest or after 3 or 5 days of prior storage (delay) of intact heads without roots at 12°C in darkness in experiment 1. Error bars represent standard errors. Data points marked by different upper case letters (A-D) for processing immediately after harvest, lower case letters for processing after 3 days of prior storage (a-e), and lower-italic case letters (i-e) for processing after 5 days of prior storage (a-e) of heads without roots are significantly different between storage times ($P<0.05$).

Quality of fresh-cuts processed from intact heads immediately after harvest or after prior storage in light

The shelf-life of the fresh-cuts was greatest when the heads were processed immediately after harvest. The storage prior to processing of the intact heads significantly reduced the shelf-life of the fresh-cuts of both genotypes under both storage conditions (dark and light). The effect of light during storage appeared to depend on genotype (Figure 9). For GB 1, the shelf-life was about 2 days longer when the heads had been stored for 5 days in light rather than in darkness prior to processing. However, for RB the shelf-life diminished to the same extend with the storage prior to processing in darkness as in light. The content of chlorophyll and carotenoids decreased in all fresh-cuts, i.e. processed immediately after harvest or after prior storage in dark and in light (Figure 10). There was no advantage of processing done immediately after harvest for the retention of pigments. The decrease in both parameters appeared to be genotype dependent. In GB, the loss of chlorophyll and carotenoids in fresh-cuts was slightly reduced when fresh-cuts were made from heads prior stored in light compared to heads stored in dark. In RB there was no apparent difference in the decrease of pigments in fresh cuts made from heads either prior stored in light or in dark.

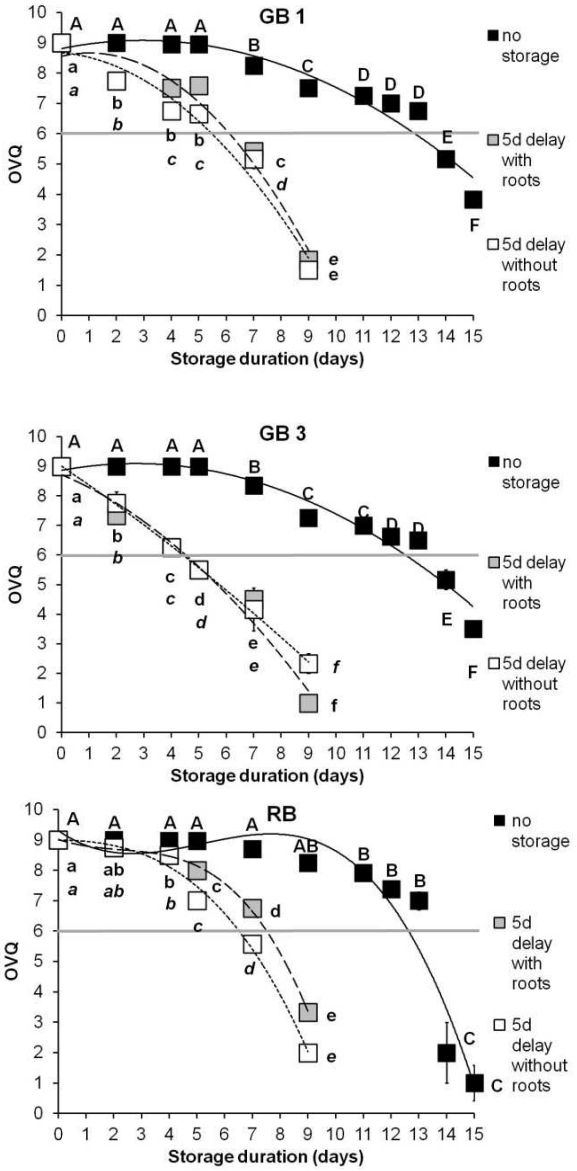

Fig. 6. Overall visual quality (OVQ) of fresh-cuts of three butterhead genotypes (green 1, GB 1; green 3, GB 3; and red, RB) processed immediately or after harvest or after 5 days of prior storage (delay) of intact heads with or without roots at 12°C in darkness in experiment 2. Error bars represent standard errors. Data points marked by different upper case letters (A-F) for processing immediately after harvest, lower case letters (a-f) for processing after 5 days of prior storage of heads with roots, and lower-italic case letters (a-f) for processing after 5 days of prior storage of heads without roots are significantly different between storage times ($P<0.05$).

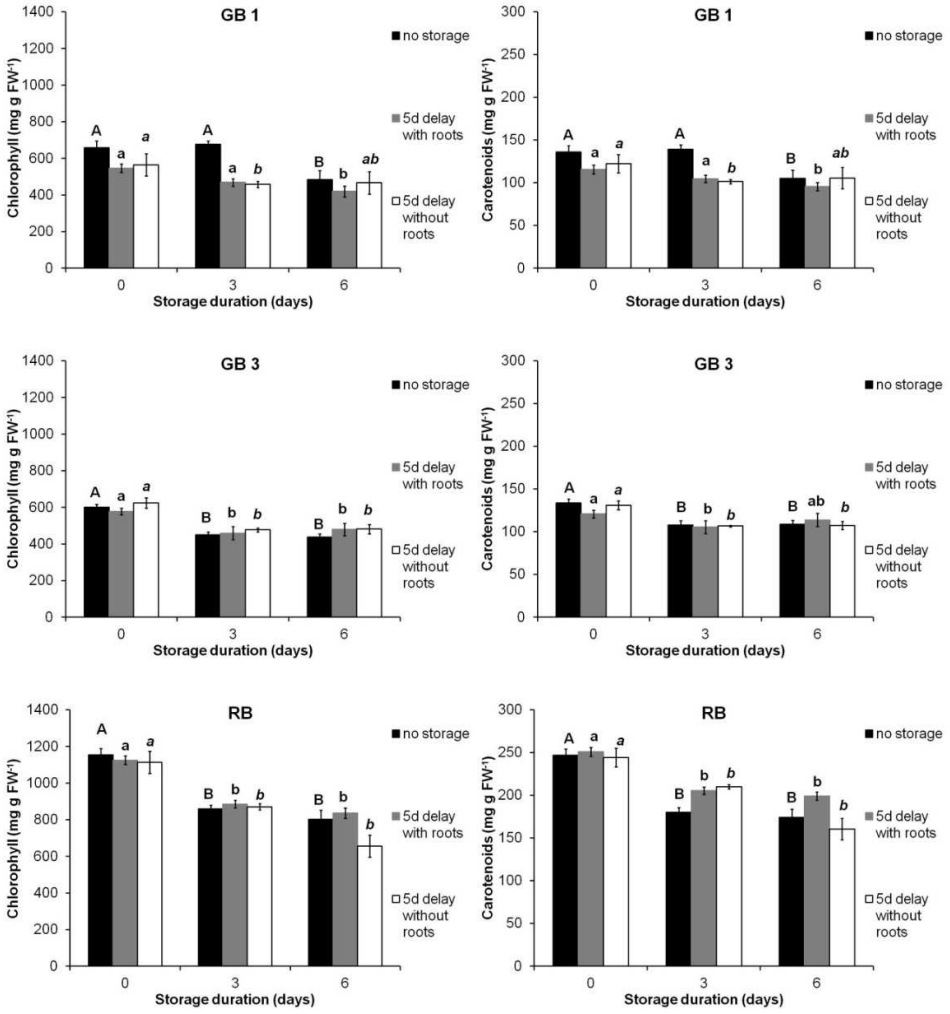

Fig. 7. Changes in chlorophyll (left column) and carotenoids (right column) content of three butterhead genotypes (green 1, GB 1; green 3, GB 3; and red, RB) processed immediately after harvest or after 5 days of prior storage (delay) of intact heads with or without roots at 12°C in darkness in experiment 2. Error bars represent standard errors. Data points marked by different upper case letters (A-B) for processing immediately after harvest, lower case letters (a-b) for processing after 5 days of prior storage of heads with roots, and lower-italic case letters (a-b) for processing after 5 days of prior storage of heads without roots are significantly different between storage times ($P<0.05$).

As in the previous experiments, the changes in EL in fresh-cuts during storage appeared to depend on the genotype (Figure 11). As in experiment 2, in GB 1 fresh-cuts no clear effect of prior storage of heads on EL was apparent. In RB fresh-cuts from prior stored heads in darkness reached the higher EL levels sooner than fresh-cuts processed immediately. The prior storage of RB heads in light had no effect on EL.

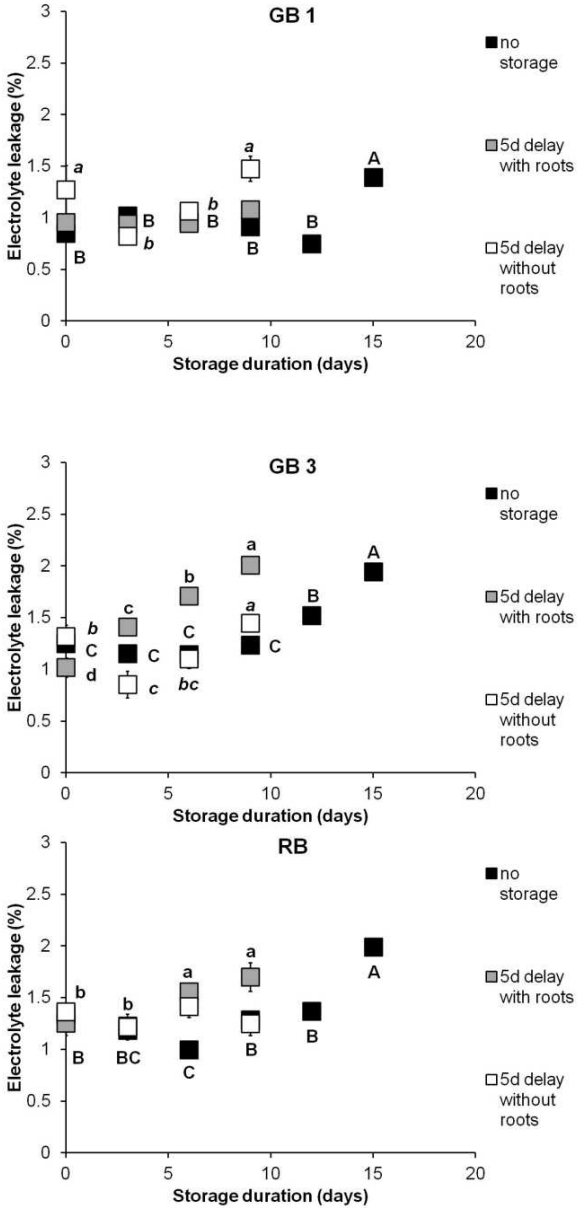

Fig. 8. Changes in electrolyte leakage of fresh-cuts of three butterhead genotypes (green 1, GB 1; green 3, GB 3; and red, RB) processed immediately after harvest or after 5 days of prior storage (delay) of intact heads with or without roots at 12°C in darkness in experiment 2. Error bars represent standard errors. Data points marked by different upper case letters (A-C) for processing immediately after harvest, lower case letters (a-b) for processing after 5 days of prior storage of heads with roots, and lower-italic case letters (a-c) for processing after 5 days of prior storage of heads without roots are significantly different between storage times ($P<0.05$).

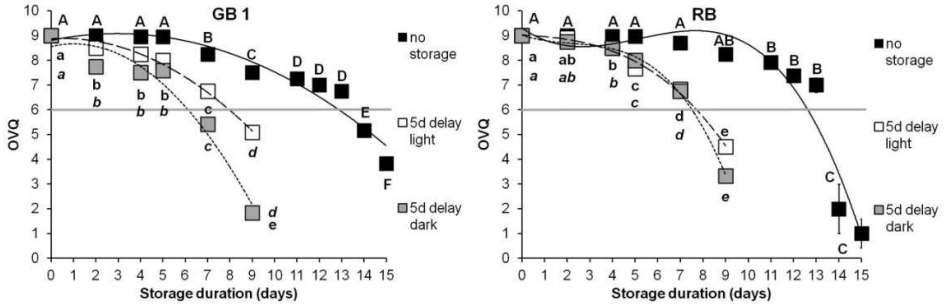

Fig. 9. Overall visual quality (OVQ) of fresh-cuts of two butterhead genotypes (green 1, GB 1, green 1, red, RB) processed immediately after harvest or after 5 days of prior storage (delay) of intact heads in light or darkness at 12°C in experiment 3. Error bars represent standard errors. Data points marked by different upper case letters (A-D) for processing immediately after harvest, lower case letters for processing after 5 days of prior storage in light (a-e), and lower-italic case letters (i-e) for processing after 5 days of prior storage in darkness (a-e) are significantly different between storage times (P<0.05).

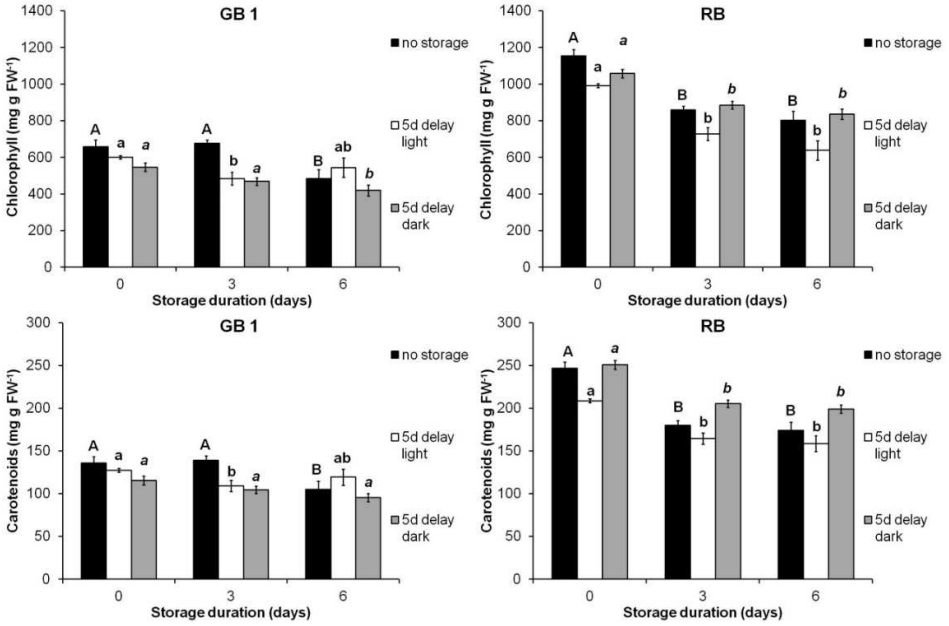

Fig. 10. Changes in chlorophyll (top row) and carotenoids (bottom row) content of two butterhead genotypes (green 1, GB 1, and red, RB) processed immediately after harvest or after 5 days of prior storage (delay) of intact heads in light or darkness at 12°C in experiment 3. Error bars represent standard errors. Data points marked by different upper case letters (A-D) for processing immediately after harvest, lower case letters for processing after 5 days of prior storage in light (a-e), and lower-italic case letters (i-e) for processing after 5 days of prior storage in darkness (a-e) are significantly different between storage times (P<0.05).

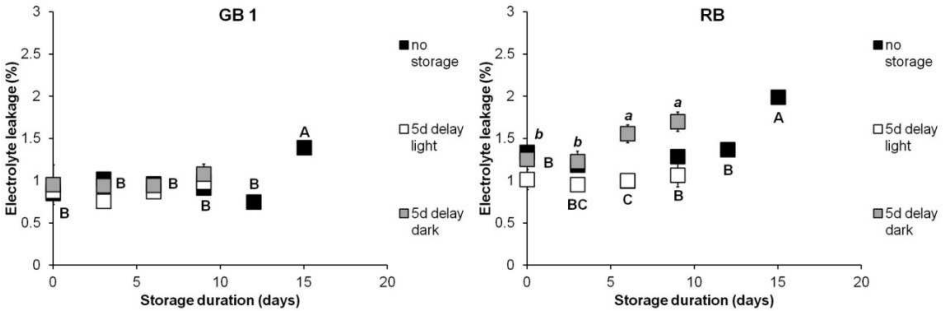

Fig. 11. Changes in electrolyte leakage of fresh-cuts of two butterhead genotypes (green 1, GB 1, and red, RB) processed immediately after harvest or after 5 days of prior storage (delay) of intact heads in light or darkness at 12°C in experiment 3. Error bars represent standard errors. Data points marked by different upper case letters (A-D) for processing immediately after harvest, lower case letters for processing after 5 days of prior storage in light (a-e), and lower-italic case letters (i-e) for processing after 5 days of prior storage in darkness (a-e) are significantly different between storage times ($P<0.05$).

Discussion

Although intact heads still had a good appearance even after 17 days of storage, a decline in the energy reserves, i.e. soluble sugars, and occurrence of selected senescence markers was observed. No problems due to desiccation of the tissue was observed during storage of heads as indicated by similar results obtained for heads stored with and without roots. These changes in physiological parameters of the raw product showed a correlation with the storability of the fresh-cuts, and this correlation depended on the time between harvest and processing.

Dark storage of fresh-cut produce induces senescence related processes, which severely reduce the postharvest longevity of the tissue. First, metabolic changes such as loss of chlorophyll, proteolysis, changes in nucleic acids, and loss of membrane integrity are stimulated (Trippi and Thimann, 1983; Biswal and Biswal, 1984; Hodges and Forney, 2003; Buchanan-Wollaston et al., 2005). In our experiments with lettuce, the presence of these senescence related processes was confirmed by the decrease in chlorophyll and the increase in EL observed during storage of intact heads. Secondly, and most importantly the carbon balance in the leaf tissue is radically changed after harvest, since the supply of carbohydrates stops, whereas the demand increases (Kays and Paull, 2004). In our experiments, this negative shift in carbon balance led to the quick depletion of the reserves, as seen by a rapid and prominent decrease in the levels of glucose and fructose during dark storage of intact heads. The levels of soluble sugars in the intact heads with and without roots were measured at harvest, and after 7 and 15 days of storage. Thus, we do not have data on the content of glucose and fructose after shorter storage times. We assume that these levels were in between the initial and final levels, showing progressive decrease with time, which correlated with the shorter shelf-life of fresh-cuts prepared after 5 days than after 3 days of storage of heads.

The reduced levels of sugars in raw produce after storage may contribute to the accelerated decay of the fresh-cuts. For example, due to the stress resulting from the

starvation of the tissue the production of reactive oxygen species (ROS) may increase, resulting in increased tissue deterioration (Couée et a., 2006; Philosoph-Hadas et al., 1994; Kar and Feierabend, 1984; Rosenvasser et al., 2006). To test the hypothesis about the importance of sugars, we investigated in experiment 3 the effect of storage prior to processing of intact heads in light on the shelf-life of the fresh-cuts. Light has been suggested (Hosoda et al., 2000; Ranwala and Miller, 2000), and shown (Chapter 4) to be a factor delaying senescence, i.e. quality deterioration during storage of fresh-cuts due to maintenance and/or accumulation of primary energy reserves (sugars). The positive influence of light during storage may further be linked to recycling of proteins and lipid breakdown products into sugars (gluconeogenesis), an increase in respiratory activity, and maintenance of AsAThe increase in respiratory activity may improve ATP levels, which facilitate maintenance processes, and delay cell death in wounded tissue (Chapter 4). In addition, the soluble sugars might act as ROS scavengers themselves, may protect the membranes from degradation and may provide osmotic pressure and enhanced crispness of the tissue (Bolouri-Moghaddam et al., 2010). The maintenance of AsA may reduce the browning of cut edges, and prevent ROS-associated membrane lipid peroxidation (Chapter 4). Indeed, the storage prior to processing storage of intact heads of GB in light resulted in slightly better quality retention of fresh-cuts compared to fresh-cuts prepared from heads stored in darkness. The lack of a visible light effect on quality retention of RB might be explained by a lack of visible symptoms of edge browning in this genotype, being a significant contributor to OVQ scores. The lesser light perception of this anthocyanin rich tissue may have also play a role.

In summary, although the visual quality of intact heads does not change during storage prior to processing, several important physiological processes take place, e.g. depletion of sugars reserves and increases in ion leakage. These processes severely affect the quality retention of fresh-cuts made from these heads. Therefore, processing immediately after harvest is advised. Storage with roots attached did not alleviate these changes, whereas the use of light during storage prior to processing partly eased the detrimental effects of prior storage of heads on quality of fresh-cuts.

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CHAPTER 6

General Discussion

The quality of fresh-cut fruits and vegetables is often offset by a rapid deterioration, due to severe physical stress, such as peeling, cutting, slicing, shredding, trimming, coring, and removal of the protective epidermal cells. Due to this wounding, the already short shelf-life of these harvested products is even more reduced in the market place. In addition, consumers have become more critical on the use of synthetic and chemical additives to preserve quality attributes of fresh-cut produce. Developing new, effective, non-invasive and non-chemical techniques for improving and maintaining quality in fresh-cut produce are the timely questions of the industry.

The work reported in this thesis adds to the increasing knowledge and understanding on how pre- and postharvest factors affect the storability of fresh-cut leafy vegetables. This work focused in particular on factors that up to date have not been systematically investigated such as:

- environment and genetic dependent antioxidant levels (Chapter 1),
- plant age at harvest (Chapter 2), and light during post-processing storage (Chapter 4), or
- duration and conditions of storage prior to processing (Chapter 3).

The factors under investigation are discussed in the light of their effects on the shelf-life of fresh-cut tissue as related to a number of physiological processes associated with senescence, such as a decrease in photosynthesis, chlorophyll degradation, and loss of antioxidants and cellular integrity. The mechanisms behind some of the findings are currently not fully understood, and would be interesting targets for future research.

4.1 Pre-harvest factors with potential to improve postharvest performance of fresh-cut leafy vegetables

In order to obtain a high quality fresh-cut product it is necessary to start with high quality raw material (Watada et al., 1996). We clearly show that some of the pre-harvest factors we investigated, i.e. growth conditions (light quality and quantity) and genotype did not have a clear effect on the postharvest storability of the fresh-cut product. Some other pre-harvest factors that we investigated, such as different levels of nitrogen, and wind stress (not discussed in this thesis) also did not appreciably affect the quality of the fresh cut produce. The only pre-harvest factor that had a clear and a commercially applicable effect on fresh-cut performance was the physiological age of the plants.

4.1.1 Growth conditions and genotypes

The differences in light conditions during growth produced plant material with contrasting concentrations of the most abundant antioxidants in photosynthetic tissue, i.e. carotenoids, polyphenolic compounds, and ascorbic acid. In general, the tissue of green butterhead (GB) contained less antioxidants (Chapter 2 and 3) and more soluble sugars (Chapter 5) than red butterhead (RB). Irrespective of differences in their chemical composition, for plants grown under the same condition (Chapter 2) and/or of the same physiological maturity (Chapter 3) the postharvest storability was about the same for both genotypes, even under growth limiting conditions, i.e. 75% reduced irradiance. This observation was also confirmed in unwounded tissue (intact leaves, Chapter 2), and when plants were grown under other "environmental" treatments, i.e. under wind stress, and with a varying strength of EC solutions (data not discussed in this thesis). The only exception from this rule was when plants were grown under light emitting diodes (LEDs) with high proportion of blue light (70%) in the spectrum. It is well established that plant development and physiology are strongly influenced by the light spectrum (McNellis and Deng, 1995). The spectrum of blue light near the ultraviolet region may induce similar responses as ultraviolet light, which is stressful for the plant, and which may induce the synthesis of polyphenolic compounds and overproduction of reactive oxygen species (ROS) (Ebisawa et al., 2008; Ryan et al., 2002). The "blue-light exposed" genotype GB (with lower intrinsic levels of polyphenolic compounds and presumably less light-shield), reacted strongly to the unfavourable growth conditions, i.e. by up-regulating the synthesis of phenolic compounds (when compared to light from fluorescent tubes (FL)). The shelf-life of fresh-cuts was much less compared to fresh-cuts made from plants grown under FL. The blue light was less detrimental for the shelf-life in RB possibly because the shielding effects of secondary metabolites. We hypothesize that the blue light stress, apart from increasing the levels of phenolic compounds possibly also evoked an increase in ROS. Therefore, the higher oxidative status of this tissue at harvest, together with the oxidative stress upon wounding, overwhelmed the antioxidant system and the tissue suffered a quicker degradation than the "blue-light protected" genotype, RB (discussed in more detail in section 4.3). A similar stress-dependent reduction of the shelf-life was observed when plants where grown under severely reduced irradiance. The fresh-cuts produced from plants grown under this reduced light condition had 50% reduced storability than plants grown under high light conditions. It is worth pointing out that the similar storability of both genotypes under non-limiting conditions, measured by changes in visual quality, was caused by different properties of the tissue. While the shelf-life of the GB was reduced due to the loss of green colour and the browning of cut edges, for the RB the reduction in the shelf-life was mainly caused by the change from red-green to brown colour. It appears that for butterhead lettuce, the browning of the cut edges is not always the main determinant of the storability.

Overall, our findings on the effect of growth conditions and genotype confirm that applying stress to enhance the antioxidants levels in the plant might be a successful commercial strategy to produce nutritionally rich plants (Voipio and Autio, 1995; Eskins, 1996; Krizek et al., 1998; Romani et al., 2002; Kleinhenz et al., 2003; Gruda et al., 2005; Gazula et al., 2005; Garcia-Marcias et al., 2007; Tsormpatsidis et al., 2008). Such a strategy is only viable if:

i) the choice of applied stress is considered in the light of the intrinsic properties, i.e. stress resistance of the plant, and
ii) the levels of applied stress do not exceed the potential of the tissue to withstand this stress.

The increased antioxidant potential of the tissue, apart from its presumed health promoting effects, in itself does not improve the shelf-life of the fresh cut product. A similar shelf-life was observed in both the green and red genotypes independently of growth conditions and plant age. Therefore, breeding strategies to produce nutritionally rich plants do contribute to goals of the fresh-cut industry.

4.1.2 Physiological maturity

Growing plants under constant environmental conditions helped us uncover that the physiological maturity at harvest is a critical factor in determining the storability of the fresh-cut photosynthetic tissue (Chapter 2). The plant age apparently affects its potential to withstand tissue wounding (preparation of fresh-cut). In unwounded tissue, plant age did not affect the shelf-life (data not shown). Besides, the significance of the plant age in determining the storability of fresh-cuts appeared to be independent of growth conditions and genotype.

The outcome of this work is that the age-dependent decrease in the storability did not show simple, one to one relationship with any of the measured senescence indicators. As plants aged, the contents of chlorophyll and antioxidants did not significantly decrease, and the electrolyte leakage did not increase, the shelf-life, however, of the fresh-cut did decrease. Also photosynthesis, as a marker of senescence showed no decrease during leaf ageing in GB. In older RB there was a slight decrease in photosynthesis, but this may have been caused by the increased pigmentation, hindering light interception, and not by senescence. The ascorbic acid (AsA) varied with the increased age of plants, but only for GB, thus also AsA did not appear to be a parameter explaining the age dependent decreased in the quality retention of fresh-cut lettuce. The only characteristics that consistently varied with age for both genotypes were sucrose and starch, but for example the levels of both were higher in GB than RB, while the shelf-life of both genotypes was about the same. Later results (not shown in this thesis) indeed showed that hydrogen peroxide levels, as measured by 3,3'-Diaminobenzidine (DAB) staining, were much higher in wounded tissue from older leaves than from younger leaves. This indicates that the oxidative status in wounded tissue from young leaves is more favourable than in wounded tissue from older leaves. The exact reasons for this may be a higher antioxidant activity (enzymatic and non-enzymatic) or a lesser oxidant activity in response to wounding.

Although the mechanisms behind the effect of physiological maturation on no withstanding wounding is not fully understood, our results clearly show that it is important to harvest plants at earlier developmental stages. The consequent reduction in commercial yield can be compensated by higher number of harvests per growing season or through denser planting. However, an important point that remains to be elucidated is how the knowledge about the plant age dependent suitability of plant material for the fresh-cut industry can be used when plants are being grown under changing environmental conditions, i.e. field and greenhouse cultivations.

4.2 Post-harvest factors with potential to improve postharvest performance of fresh-cut leafy vegetables

Starting at the beginning of the supply chain with the high quality raw material is not sufficient to preserve the quality retention of the fresh-cut leaf vegetables. Equally important is to i) keep the quality of raw product as close as possible to its condition at harvest, ii) optimise processing conditions to reduce injury, and iii) optimize storage conditions to reduce physiological and biochemical processes of the wounded tissue. In this study, we focused on issues that have not received considerable attention yet, i.e. the duration of storage prior to processing, and the application of low light intensity during both, storage prior to and after processing.

The storage and transport of the raw material between harvest and processing, as well as storage prior to processing are common practices. The conditions of these steps in the supply chain vary with respect to temperature, relative humidity, composition of the atmosphere, and exposure of the plant material to dark or light conditions. Most of these factors have been extensively investigated, and therefore their effects on the quality retention of fresh-cut product, as well as the practical recommendations for the best practice are known. However, both the qualitative effects of the time between harvest and processing, and the effects of unavoidable exposure to light in the supply chain have not been systematically investigated up to date.

We are the first to show that the low light levels during storage of the raw product and, especially during storage of the fresh-cut photosynthetic tissue, greatly extends its quality retention. The very positive effect of light was seen in a situation that differs from most commercial applications. In our experiments, the lettuce was not stored under extreme low oxygen concentration as in commercial modified atmosphere packages, but instead under normal atmospheric conditions. In commercial packages, this positive effect of light may not be observed due to the inherently short shelf-life caused by fermentative processes. Under our experimental conditions, light levels that were well below the light compensation point were able to increase sugar and starch levels in the tissue, and to preserve ascorbic acid levels. We anticipate that these metabolites preserved the quality. It is at present not know how the tissue can accumulate carbohydrates under conditions where there is no net photosynthesis. It is hypothesized that breakdown products of e.g. chlorophyll may be converted into sugars (gluconeogenesis), but this possibility needs further investigation.

Our findings not only recommend the exposure of the plant material to the light in the post-harvest part of the chain, but also suggest further research on the subject. For instance, the effect of the interchangeable exposure to light/dark in the supply chain should be investigated. It might emerge that the unavoidable changes in the conditions might compromise the positive effect of light on the quality retention of the fresh-cut product. Also, a possibility of using different light colours should be explored. For instance, the red light has been suggested to delay, while far red to promote senescence (Thimann et al., 1977). Lastly, but equally important, will be to search for technological solutions to better manage the gaseous atmosphere within the commercial packages to facilitate the use of light to enhance quality of the product.

4.3 Senescence related processes and quality deterioration of fresh-cuts

The postharvest longevity of plant tissue has been suggested to be influenced by the senescence processes that occur during storage (Noodén et al., 1997; Rosenwasser et al., 2006, 2011). In this thesis, some of the senescence processes were investigated as a cause for the quality retention of fresh-cut photosynthetic tissue. The outcome of the study results in the model as described at the end of the chapter.

The senescence processes in fresh-cut product are due to stress associated with the detachment (harvest), wounding (processing), and dark storage of the plant. Due to harvesting and placing in the darkness, the plant loses its ability to photosynthetically fix carbon, resulting in depletion of sugar levels (Chapter 4). Due to the stress resulting from the starvation of the tissue the production of reactive oxygen species (ROS) increases (Couée et al., 2006) resulting in senescence (Buchanan-Wollaston et al., 2005) (Chapter 2 and 4). The stress due to wounding further accelerate senescence by promoting additional oxidative processes in the plant (Philosoph-Hadas et al., 1991; Bartoli et al., 1996). Hydrophilic and lipophilic antioxidants accumulate to detoxify ROS within different compartments of the plant cell (Baker and Orlandi, 1995; Hodges et al., 1996, 1997a, b; Wang et al., 2003), and the relation between leaf oxidative and antioxidative potential has been implicated in the dynamics of senescence (Kunert and Ederer, 1985) (Chapter 2). Together with the first changes in ROS, physiological events like the decrease in photosynthesis (Chapter 3) and chlorophyll degradation (all chapters) are activated (Munné-Bosch and Alegre, 2004; Yoshida, 2003; Noodén et al., 1997). Other processes, like lipid degradation, loss of antioxidants (Chapter 2) and loss of cellular integrity (Chapter 4 and 5) happen as a certain threshold is reached by these regulators.

In this work, we present evidence for the senescence related processes in the dark stored fresh-cut tissue, and how high sugar content, antioxidant potential and the oxidative potential of this tissue at harvest affect the dynamics of the senescence related processes.

4.3.1 Dark starvation

The presence of senescence processes in the dark stored tissue was confirmed by a rapid depletion of chlorophyll (Chapter 4 and 5), antioxidants, i.e. polyphenolic compounds (Chapter 2), carotenoids (Chapter 4 and 5) and AsA (Chapter 2, 4 and 5), and an increase in leakiness of membranes (Chapter 4 and 5). The absence of photosynthetic fixation of carbon resulted in utilization of secondary respiratory substrates, e.g. lipids, proteins and organic acids, as an energy source (Kays and Paull, 2004; Buchanan-Wollaston et al., 2005). The degradation of lipids, which are the main constituents of membranes, may lead to membrane disintegration, and therefore an increase in their leakiness (Thompson et al., 1998). The rapid decrease of AsA may have also contributed to the loss of membrane stability, as AsA is an antioxidant involved in the prevention of ROS-associated membrane lipid peroxidation (Borraccino et al., 1994; Hodges and Forney, 2000, Hodges et al., 2001; Jimenez et al., 1998). Since the cell membrane disruption has been reported to correlate with chlorophyll loss (Zhuang et al., 1994, 1997) and chlorophyll and proteins are not a fundamentally different measure (Peterson et al., 1973; Telek and Graham, 1983; Gan 2007), we speculate that in parallel to chlorophyll, other proteins were also degraded and used as an alternative energy source.

The importance for storability of the fresh produce of a high level of sugars as an energy source has been already suggested by several authors e.g. Lipton (1987), Klieber et al. (2002) and Clarkson et al. (2005). The accumulation of sugars in light, due to photosynthetic activity (light above the light compensation points (LCP)) and/or gluconeogenesis (light below and above LCP), extended the storability of the tissue. The positive effect of sugar was due to their role in i) increasing respiratory activity, ii) preserving of AsA, iii) acting as ROS scavengers (Bolouri-Moghaddam et al., 2010), iv) increasing membrane stability (Crowe et al., 1984; Koster and Carl, 1988) and, v) acting as an osmolyticum increasing cell turgescence. The increased respiratory activity may protect tissue from a depletion of ATP, which facilitates maintenance processes, and prevents tissue to enter the cell death mode. The presence of AsA may have reduced or delayed senescence processes, such as lipid peroxidation, protein breakdown, and as a consequence disintegration of membranes (as mentioned above), through the inhibition of H_2O_2 accumulation (Borraccino et al., 1994; Hodges and Forney, 2000, Hodges et al., 2001; Jimenez et al., 1998).

A general correlation between sugar content and the shelf-life of the fresh-cut lettuce was not established in this study (Chapter 3). For instance, the levels of sugars were about two times higher in GB than in RB, while the shelf-life of both genotypes was similar. Carbohydrate levels in the older GB were similar to those in the younger RB, whereas the shelf-life of the older GB was about 12 days shorter than that of the younger RB. However, the decrease in the levels of sugars with age correlated with the maturation dependent decrease in the shelf-life in each individual genotype. It appears that the level of the sugar in the tissue required for a good quality retention may be species- and variety-dependent, and may be linked to the reserves of other respiratory substrates, e.g. lipids, proteins and organic acids in the tissue. Therefore, species/ variety-dependent energy reserves at harvest, and their relation to the quality retention of fresh-cut produce should be further investigated. Perhaps, if systematically researched, the level of peculiar sugar, protein or lipid molecule could be used as a marker for predicting the storability of the fresh-cut tissue.

4.3.2 Oxidative and antioxidative potential

Most recent studies on antioxidants in fresh-cut leafy vegetables focuses on enhancing their levels for the benefits of human health (DellaPenna, 1999). In addition to the effects on human health, antioxidants might affect the storability of fresh-cut leafy vegetables that contain them, since antioxidant defence system has been suggested to play an important role in determining the onset and rate of senescence (Philosoph-Hadas et al., 1994; Meir et al., 1995; Hodges & Forney, 2003). Carotenoids and AsA are well known for scavenging of ROS (Cazzonelli et al., 2011), whereas polyphenolic compounds have been suggested to act as a backup to the primary AsA-dependent detoxification system (Sakihama, 2002), and they act as antioxidants themselves (Agati et al., 2009, Neill et al., 2002).

A relation between the antioxidant status and the shelf-life of the fresh-cut lettuce was not established in this study (Chapter 2 and section 4.1.1). The difference in post-harvest behaviour of wounded tissue appeared to be a function of the present pool of oxidative stress (as discussed in section 4.1.1). In detached and wounded tissue remobilisation of metabolic components is not possible, a hyper accumulation of toxic molecules, such as free radicals may therefore occur (Wagstaff et al., 2007). Given that the

levels of antioxidants decrease during storage in darkness, we suggest that in wounded dark stored tissue the ratio between ROS and antioxidants becomes unfavourable, the antioxidant system becomes overwhelmed and the tissue suffers degradation. In addition, the higher was the stress imposed on the tissue before harvest (high 70% blue light or 75% reduced light treatments) the quicker tissue deterioration will happen. We highly recommend further research on the qualitative and quantitative impact of oxidative status on tissue storability. This research should be done in relation to effects of the plant age, and growth conditions, as well as in association with breeding programs focused on stress resistant varieties.

In summary, a photosynthetic tissue is characterized by its oxidative status, which is a balance between oxidants and antioxidants present in the tissue. The oxidative status determines the potential of the tissue to withstand processing, and depends on the physiological age, growth conditions, and stress imposed during storage prior to processing. If the oxidative status becomes unfavourable for the tissue, senescence related processes are induced in the tissue. Under conditions that prevent a breakdown of energy reserves or lead to recycling of breakdown products back to sugars or antioxidants (as discussed above), i.e. storage in light, the tissue storability is extended (Figure 1).

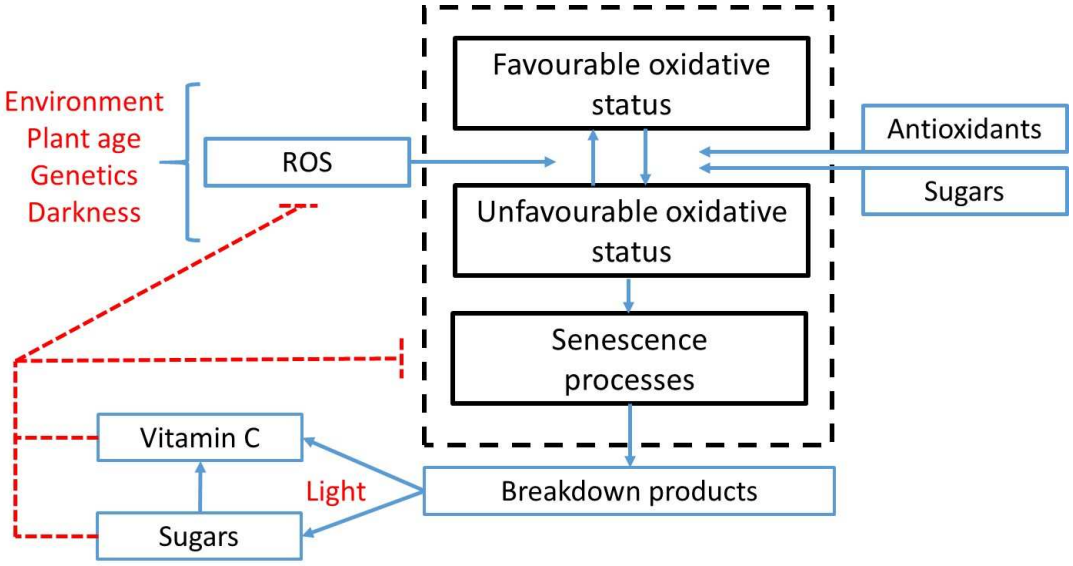

Fig. 1. The main factors and mechanisms determining the storability of the fresh-cut tissue. The arrows indicate an influence of one factor on another factor, and broken lines indicate inhibition of one factor on another.

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SUMMARY

Fresh-cut products are those fruit or vegetables that have been trimmed, peeled and/or cut into 100% usable product that is bagged or pre-packed to offer consumers high nutrition, convenience, and value while still maintaining its freshness. The quality of fresh-cut vegetables is offset by a rapid deterioration, due to processing operations, and the shelf-life of these products is seriously reduced in the market place. Hence, developing new, effective, non-invasive and non-chemical techniques for improving and maintaining quality are the timely questions of the industry. The subjects covered in this thesis focus on pre- and post-harvest methods with potential to improve the quality of fresh-cut butterhead lettuce (*Lactuca sativa* L.). The model species used for the experiment, *Lactuca sativa* L., is a commercially important leafy vegetable available worldwide over the whole year. Besides, lettuce also contains appreciable amounts of nutritionally important compounds (Caldwell, 2003; Cao et al., 1996; Chu et al., 2002; Vinson et al., 1998), and appears to exert a diversity of beneficial health effects (Caldwell, 2003; Llorach et al., 2004; Nicolle et al., 2004 a, b).

Chapter 2 describes a correlation between the level of major antioxidants in butterhead lettuce leaves at harvest and the storability of the fresh-cut tissue prepared from these leaves. We chose two related genotypes, i.e. green (GB, cv. Troubadour) and red butterhead (RB, cv. Teodore) differing in antioxidant concentration, and we exposed them to both quantitatively and qualitatively different light regimes during growth to introduce further variation in antioxidant content. Forty days after transplanting, lettuce heads were harvested, and leaves were processed. The fresh-cuts were monitored for changes in overall visual quality, pigmentation, and levels of main non-enzymatic antioxidants, i.e. carotenoids, ascorbic acid, polyphenolic compounds, as well as oxygen radical absorbance capacity (ORAC). The levels of main non-enzymatic antioxidants were higher in RB than GB. Compared to high light, reduced light resulted in lower levels of main non-enzymatic antioxidants in both genotypes, whereas use of LED lighting with high percentage blue light increased these levels. The evaluation of postharvest quality during storage revealed no consistent relation with the initial amount of antioxidants in the tissue. Samples with an ORAC value of about 3000 μmol TE 100g FW^{-1} displayed a shelf-life in the range of 6 to 20 days. Red tissue, high in antioxidants showed a similar shelf-life as green tissues with much lower antioxidants. The use of blue light increased amounts of antioxidants in green tissue, but severely compromised the shelf-life. We hypothesize that, instead of the level of antioxidants, the initial oxidative status and the response to wounding are more important determinants for fresh-cut storability.

Chapter 3 shows the importance of physiological maturity at harvest on storability of the fresh-cut tissue. We used two related genotypes, i.e. green (GB, cv. Troubadour) and red butterhead lettuce (RB, cv. Teodore) differing in their antioxidant levels. To investigate possible differences in shelf-life between fresh-cuts prepared from plants harvested at different ages, a number of physiological and nutritional parameters was determined at harvest. For both butterhead lettuce genotypes, the shelf-life of fresh-cuts prepared from younger plants was significantly longer than that of the fresh-cut prepared from the more mature plants. However, no simple one to one relationship emerged between any of the measured nutritional parameters, their change during maturation and the eventual shelf-life of the fresh-cut produce. The RB contained about two times more chlorophyll, carotenoids, and polyphenolic antioxidants than the GB, whereas their shelf-life was about similar. The content of chlorophyll, carotenoids, phenolic compounds, as well as total antioxidant capacity was not affected by maturity of the plants for either genotype. The content of ascorbic acid (AsA) decreased with maturation in GB; it was not influenced by maturity in RB. The net photosynthesis rate and carbohydrate reserves of RB were about half of that in GB. The net photosynthesis rate was not influenced by maturity in GB, whereas it decreased with maturation in RB. A decrease in sucrose and starch and, therefore, the total content of carbohydrates with aging was observed in both genotypes. This effect was more pronounced in RB than in GB. Although carbohydrate levels declined with age in each genotype, there was no apparent relationship between the absolute levels of the total carbohydrates and the shelf-life. Carbohydrate levels were about two times higher in GB than in RB, but the shelf-life of both genotypes was similar. Moreover, carbohydrate levels were similar for the older GB and the younger RB, whereas the shelf-life of the older GB was about 12 days shorter than that of the younger RB.

Chapter 4 focuses on the effects of storage conditions, i.e. darkness vs. light on the storability of fresh-cut butterhead lettuce. Storage of fresh-cuts in darkness caused a rapid depletion of chlorophyll and carotenoids, a depletion of ascorbic acid (AsA), and an increase in electrolyte leakage. Continuous illumination of fresh-cuts at intensities below (8 μmol m^{-2} s^{-1}) and above (30 μmol m^{-2} s^{-1}) the light compensation point (LCP) effectively increased the shelf-life of this product, and its visual and nutritional quality. Under light, AsA levels were preserved, carbohydrate accumulation occurred and the increase in electrolyte leakage observed in the dark was delayed. Chlorophyll and carotenoids breakdown, however, was not prevented by the light. The similar chlorophyll and carotenoids breakdown in all treatments, suggests that also lipids and proteins were broken down to a similar extent in the dark and in the light. The accumulation of carbohydrates, even under light levels below the LCP when no net photosynthesis was observed, indicates non-photosynthetic sugar production. It is hypothesized that, under low light levels, the break down products of non-carbohydrate substrates (proteins, lipids) may be recycled into sugars (gluconeogenesis). The increased sugar levels might evoke an increase in respiratory activity, and consequently improve ATP levels, which might facilitate maintenance processes, and delay cell death in wounded tissue. In addition, the soluble sugars may act as ROS scavengers and may preserve membrane properties. The maintenance of AsA in light may be due to either a coupling between photosynthetic electron transport and AsA biosynthesis or increased AsA production may be due to the increased sugar levels. The high AsA levels may benefit tissue in two ways, i.e. by reducing the browning of cut edges and by preventing ROS-associated membrane lipid

peroxidation. Together the above described mechanisms delay the senescence processes, and therefore quality deterioration of fresh-cut photosynthetic tissue.

Chapter 5 describes the effects of different storage duration and different lighting conditions during storage of whole butterhead lettuce heads prior to processing on the quality of both the intact heads and the fresh-cut produce made from these heads. Four related butterhead lettuce genotypes were used in this investigation. Depending on the experiment we evaluated the changes in overall visual quality, the level of energy reserves in the tissue, and some selected senescence markers, i.e. chlorophyll content and electrolyte leakage. The intact heads still had a good appearance even after 17 days of storage. However, a decline in the soluble sugars, a decrease in chlorophyll, and an increase in electrolyte leakage was observed. These changes in physiological parameters of the raw product had a major impact on the storability of the fresh-cuts made from these heads. In general, the longer the time between harvest and processing, the shorter was the shelf-life of fresh-cuts. The storage prior to processing of intact heads of green butterhead in light resulted in a slightly better quality retention of fresh-cuts compared to fresh-cuts prepared from heads stored in darkness. The lack of a visible effect of light on quality retention of red butterhead lettuce might be explained by a lack of visible symptoms of edge browning in this genotype, being a significant contributor to OVQ scores. Besides, the lesser light perception of this anthocyanin rich tissue may have an effect. Storage of heads prior to processing with roots attached did not affect the quality of fresh-cuts irrespective the presence of light during storage.

In **chapter 6** the observed effects of selected pre- and post-harvest factors affecting the storability of fresh-cut lettuce are discussed in a broader physiological perspective, and their practical implication as well as recommendations for future research are given. The chapter is concluded with a model depicting the main factors and mechanisms determining the quality deterioration of the wounded tissue.

SAMENVATTING

Voorbewerkte verse producten zoals gesneden sla, andijvie en worteltjes worden verpakt en kant-en-klaar aangeboden om te voldoen aan de behoefte bij consumenten aan een vers, gezond en gemakkelijk klaar te maken product. Het bewerkingsproces leidt tot veel verwonding en daarom wordt de levensduur door verwerking doorgaans sterk verkort. Er is daarom behoefte aan meer kennis over deze verwonding-geïnduceerde verouderingsprocessen en aan nieuwe methoden om de houdbaarheid van deze producten te verlengen. Binnen het kader van mijn onderzoek, beschreven in dit proefschrift, is gekeken naar zowel teelt factoren als naoogst factoren die een effect hebben op de houdbaarheid van gesneden botersla (Lactuca sativa L.). Sla is een belangrijk tuinbouwproduct en met name gesneden sla is een sterk groeisegment. Sla bevat relatief hoge gehalten aan diverse voedingstoffen en heeft een gunstig effect op de gezondheid (Caldwell, 2003; Cao et al., 1996; Chu et al., 2002; Vinson et al., 1998; Llorach et al., 2004; Nicolle et al., 2004 a, b).

In **Hoofdstuk 2** wordt de correlatie tussen de gehalten aan antioxidanten en de houdbaarheid van het gesneden product beschreven. Er is gewerkt met een groene en een rode botersla. De twee rassen zijn onder verschillende intensiteit en kwaliteit licht geteeld om verschillen in antioxidanten te bewerkstelligen. De kwaliteit van het gesneden product is bestudeerd; gekeken is naar de algemene visuele kwaliteit. Er is gekeken naar het verband tussen houdbaarheid van het gesneden product en de gehalten aan niet-enzymatische antioxidanten zoals carotenen, vitamine C, (poly)fenolen en de ORAC waarde (een maat voor totale antioxidant capaciteit). De antioxidanten niveaus waren hoger in de rode botersla dan in de groene botersla, onder lage lichtintensiteit bevatten beide rassen minder antioxidanten dan onder hoge lichtintensiteit. Onder LED belichting met hoog percentage blauw bevatten beide rassen een extra hoog gehalte antioxidanten. Er bleek geen consistente relatie tussen het antioxidant gehalte en de houdbaarheid van het gesneden product. Gesneden product van rode botersla met een veel hoger antioxidant gehalte dan groene botersla had een vergelijkbare houdbaarheid; ook binnen één ras was er geen langere houdbaarheid als het blad meer antioxidanten bevatte. Het gebruik van LED licht met veel blauw zorgde vooral in de groene botersla voor een hoog gehalte aan antioxidanten, maar de houdbaarheid van het gesneden product was korter in vergelijking met sla geteeld onder TL licht. We vermoeden dat niet het gehalte aan antioxidanten bepalend is voor de houdbaarheid maar dat het belangrijker is om de algehele oxidatieve status van het product te bepalen. Mogelijk ontstaat er ondanks de hoge initiële antioxidant waarde toch een ongunstige oxidatieve status als gevolg van verwonding.

In **Hoofdstuk 3** wordt het belang van de fysiologische leeftijd behandeld. Er werd weer met een groene en een rode botersla gewerkt. De planten werden op verschillende tijdstippen geoogst zodat gesneden product gemaakt kon worden van wat jongere en wat oudere kroppen. Doorgaans zat hier slechts 1 of 2 weken leeftijdsverschil tussen. De houdbaarheid van het gesneden product gemaakt van jongere planten van beide rassen was altijd aanmerkelijk langer dan wanneer oudere planten werden gebruikt. Er is uitgezocht waardoor dit verschil veroorzaakt wordt. Er is o.a. gekeken naar de initiële gehalten aan chlorofyl, carotenen, (poly)fenolen, vitamine C, suiker en zetmeel. Ook is gekeken naar de ademhaling en fotosynthese activiteit. Met toenemende ouderdom werd een daling van suiker en zetmeelgehalte in beide rassen gevonden en dit correleerde binnen het ras goed met de kortere houdbaarheid. Echter, het oudere blad van de groene botersla (korte houdbaarheid) bevatte nog steeds net zoveel suiker als het jongere blad van de rode botersla (lange houdbaarheid), maar er was een groot verschil in houdbaarheid van het gesneden product. Naast koolhydraat reserves zijn er dus nog andere factoren bepalend voor het verschil in houdbaarheid van gesneden product gemaakt van jonger en ouder blad. De andere gemeten inhoudstoffen vertoonden geen consistente relatie met de houdbaarheid.

In **Hoofdstuk 4** wordt het effect van licht tijdens de opslag van het gesneden product beschreven. Als het gesneden product wordt opgeslagen in het donker nemen de chlorofyl-, caroteen-, vitamine C- en suikergehalten snel af. De ionenlekkage (een maat voor verwelking) neemt toe. Als we het gesneden product opslaan bij een lage lichtintensiteit, ver onder het licht compensatiepunt, is de houdbaarheid belangrijk verlengd, soms wel verdubbeld. Dit gold vooral voor groene botersla, de effecten waren minder sterk in rode botersla. Onder deze condities wordt de afname van vitamine C geremd en wordt er een aanzienlijke hoeveelheid suiker extra geproduceerd. Licht heeft geen invloed op de afbraak van chlorofyl en caroteen., hetgeen impliceert dat er waarschijnlijk ook aanzienlijke afbraak van eiwit en lipiden plaatsvindt. De geproduceerde suiker en het hogere gehalte Vitamine C zijn waarschijnlijk verantwoordelijk voor de langere houdbaarheid en de verminderde bruinverkleuring bij bewaring onder licht. Fotosynthese metingen hebben aangetoond dat de extra suikers niet via fotosynthese gevormd worden; ook is er geen zetmeel voorraad aanwezig waaruit de suikers gevormd kunnen worden. Blijkbaar leidt de lichtbehandeling via een nog onbekend mechanisme tot productie van suikers uit alternatief substraat zoals eiwit of lipiden (neoglucogenesis).

In **Hoofdstuk 5** wordt het effect van bewaring van de kroppen voor bewerking tot gesneden product besproken. Indien de kroppen vóór bewerking enige tijd opgeslagen worden is de houdbaarheid van het gesneden product aanzienlijk verkort. De intacte kroppen worden echter niet zichtbaar door de bewaring beïnvloed. Er is nagegaan of de verminderde houdbaarheid van gesneden product van vooraf bewaarde kroppen samenhangt met energie reserves in het blad en of belichting van de kroppen tijdens bewaring een positief effect hierop heeft. Er is met 4 verschillende botersla rassen gewerkt in dit onderzoek. Tijdens opslag van de kroppen blijken suiker- en chlorofylgehalten af te nemen; de ionenlekkage (maat voor verwelking) neemt toe. Dit is echter met het blote oog niet te zien; na 2 weken bewaring van de kroppen zien zij er nog vers uit. Het afgenomen gehalte aan suikers na bewaring van de kroppen is waarschijnlijk de belangrijkste reden dat de kwaliteit van het gesneden product minder is. Het gebruik van lage intensiteit licht tijdens bewaring van de kroppen had een klein positief effect op de houdbaarheid van de

gesneden groene botersla. In de rode botersla was geen effect van licht waarneembaar, mogelijk doordat de licht perceptie door de grote hoeveelheid gekleurde anthocyanen wordt belemmerd.

In **Hoofdstuk 6** worden de effecten van teeltomstandigheden en naoogstcondities op de kwaliteit van gesneden product in een breder fysiologisch kader bediscussieerd. Het belang van de resultaten voor de praktijk en suggesties voor verder onderzoek worden besproken. Het hoofdstuk wordt afgesloten met een model dat de interactie aangeeft tussen de belangrijkste invloed factoren en het fysiologische mechanisme betrokken bij de kwaliteit van het gesneden product.

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Curriculum Vitae

Izabela Magdalena Witkowska was born on 5th January 1979 in Warsaw, Poland. In 1997 she started her study at Warsaw Agricultural University. She followed MSc-program Environmental Protection, and specialized in Environment, Food and Health at the Interdisciplinary Department of Environmental Protection. During the course of the study she was awarded a scholarship because of her performance in the course exams, and encouraged by the Professor of her Department to finish her Master degree at Wageningen University. Therefore, in 2002 she started the MSc-program Organic Agriculture, and specialized in Farm and the Rural Environment. Her MSc-thesis, entitled "Effect of season on fatty acid composition of herbage species in relation to fatty acid patterns of milk fat", was conducted at the Crop and Weed Ecology Group at WUR. This thesis work was supported by a grant from the Grassland Science Foundation. After graduating, she started her job as a scientific researcher at Grassland Science Foundation to continue her work on fatty acid in forages. From January 2009 until September 2012 she was employed as PhD student at the Horticultural Supply Chains Group at WUR, and conducted research on factors affecting quality of fresh-cut lettuce. The results of this work are presented in this thesis.

List of Publications

Papers published in refereed journals

Witkowska I.M., Wever C., Elgersma A. (2009). Effects of post-harvest treatments on concentration and profile of fatty acids in fresh perennial ryegrass (*Lolium perenne* L.). Animal Feed Science and Technology. 149: 60-69.

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Papers to be published in refereed journals

Witkowska I.M., Woltering, E.J. Pre-processing storage of intact heads limits the shelf-life of fresh-cut *Lactuca sativa* L., submited to ...

Witkowska I.M., Woltering, E.J. Low light levels during storage of fresh-cut *Lactuca sativa* L. prolong the shelf-life., submited to ....

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PE&RC PhD Training Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (5.6 ECTS)
- Factors responsible for the quality deterioration fresh-cut produce (2009)

Writing of project proposal (2.8 ECTS)
- Quality fresh-cut leafy vegetables: genetic and environmental factors affecting quality in fresh-cut lettuce

Post-graduate courses (3.2 ECTS)
- Advanced statistics; PE&RC (2006)
- Measuring techniques; ascorbic acid in leaf lamina; Leeds University, UK (2009)
- Photosynthesis laboratory: photosynthesis measurements; Tokyo University, Japan (2009)

Deficiency, refresh, brush-up courses (2.8 ECTS)
- Basic statistics; PE&RC
- Product quality and post-harvest physiology; only lectures; WUR

Competence strengthening / skills courses (3.7 ECTS)
- PhD Scientific writing; WGS (2006)
- PhD Competence assessment; WGS (2008)
- Supervising and teaching thesis students; DO (2009)
- NWO Talent class; NWO (2010)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.9 ECTS)
- Postharvest unlimited; Berlin, Germany (2008)
- ALW Meeting experimental plant sciences; Lunteren (2008)
- PE&RC Days (2008-2010)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)
- Excursion Rijk Zwaan; Fijnaart (2008)
- Excursion Fresh-Tomato (2008)
- Frontier Literature in Plant Physiology (2007-2011)

International symposia, workshops and conferences (5 ECTS)
- 6th International Postharvest Symposium; Antalya, Turkey (2009)
- 6th International Symposium on Light in Horticulture; Tsukuba, Japan (2009)

Lecturing / supervision of practical's/ tutorials; 25 days (3 ECTS)
- Organic agriculture (2009)
- Hortonomy (2009)
- Postharvest physiology (2010)
- Concepts in Environmental Plant Physiology (2010)

Supervision of 3 MSc students; 22 days
- Factors influencing quality attributes of butterhead lettuce genotypes
- Effects of light storage on postharvest quality of lettuce fresh produce
- The effect of pre-cut storage of intact lettuce heads under dark and light conditions on quality of fresh cut butterhead lettuce

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