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Review

# Browning disorders in pear fruit

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**Abstract**

Disorders occurring during long-term storage of pears can cause economic loss, especially when disordered fruit cannot be distinguished externally from sound fruit. A typical category of disorders in pear fruit is related to internal browning of the flesh and the presence of cavities. In this review, information which appeared in the literature in the last decade has been integrated into a generic model for the development of storage-related browning disorders in pear. In this model it is assumed that browning disorders are caused by an imbalance between oxidative and reductive processes due to metabolic gas gradients inside the fruit, leading to an accumulation of reactive oxygen species. The latter may induce loss of membrane integrity which becomes macroscopically visible through the enzymatic oxidation of phenolic compounds to brown coloured polymers. The development of disorders during postharvest ripening and storage of fruit also depends on a range of preharvest factors such as climate conditions and crop load. Methods to evaluate the incidence of browning disorders nondestructively have been reviewed.


*Keywords:* Pear; Browning; Core breakdown; Controlled atmosphere; Physiological disorder; Respiration; Gas exchange; Antioxidant system; Oxidative stress; Oxygen; Carbon dioxide

## Contents



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## 1. Introduction

In controlled atmosphere storage, the metabolic activity of pear fruit is decreased by controlling the $O_2$ and $CO_2$ partial pressures, usually in combination with a reduction of the temperature. The optimal storage conditions are always a compromise: e.g., temperature must be decreased to minimise metabolic activity while avoiding chilling or freezing damage, $O_2$ partial pressure must be decreased so as to minimise aerobic respiration yet avoiding fermentation, and an elevated $CO_2$ partial pressure helps maintain colour but may induce storage disorders. External factors can interrupt, restrict or accelerate normal metabolic processes and potentially cause physiological storage disorders. Besides postharvest environmental factors, adverse preharvest conditions during growth are important (Kays, 1991). Browning is an important disorder of pear fruit which can lead to considerable economic losses as the symptoms are internal and cannot be observed visually without cutting the fruit in half.

Many publications exist on pear browning disorders during storage, each one with its own approach and focus. In this article, we will first review the different symptoms that have been described in the literature, their terminology as well as methods to nondestructively measure their incidence. Next, the pre- and postharvest factors and possible ways to avoid the disorder will be discussed. Finally, the physiological and biochemical background will be reviewed and a general hypothesis for the development of browning disorders during storage of pears will be presented.

## 2. Symptoms and definitions

Browning disorders in pears can be present in different forms such as radial browning (Fig. 1A), asymmetrical browning (Fig. 1B), brown and/or dry spots (Fig. 1C), cavities (Fig. 1D), brown core, etc. Sound spots are often found in the extension of the five carpels in the brown zone. Cavities can be manifest in different ways: small spots in a star pattern in between the five carpels (Fig. 1C) or randomly localised dried lesions or randomly localised cavities, usually of a larger size (Fig. 1D). Roelofs and de Jager (1997) and Lammertyn et al. (2000) suggested that cavities arise from the brown tissue because of the time course of internal browning and the appearance of cavities. This was confirmed by magnetic resonance images of pears stored in browning-inducing conditions (Lammertyn et al., 2003b). The authors found that browning patterns in pear did not evolve or grow spatially over time, but became more severe during storage. They also showed that cavities eventually developed from brown tissue.

Recent research has focused on the cultivar 'Conference' (*Pyrus communis* L. cv. Conference) because of its

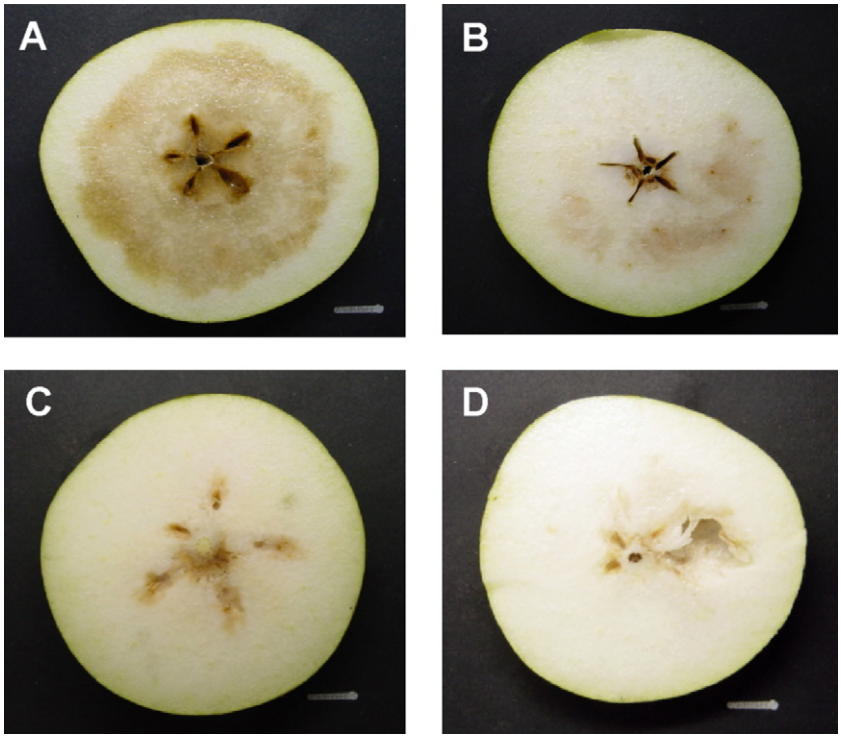


Fig. 1. Browning disorders in 'Conference' pears after 4 months in browning-inducing storage conditions (no cooling period, 1% $O_2$, 10% $CO_2$, −1 °C). The symptoms can be divided in four categories, which might not necessarily have the same origin. (A) Radial browning; (B) asymmetrical browning; (C) brown and dry spots in between the extension of the five carpels; (D) random cavities (bar = 1 cm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Table 1
Browning disorders in pears: names, symptoms and synonyms

| Name | Symptoms | Synonyms | Reference |
|---|---|---|---|
| Core breakdown | Brown, soft breakdown of the core and surrounding tissues | Senescent disorder | Blanpied (1975) |
| $CO_2$-injury | Core and flesh browning | Brown heart, brown core, pithy brown | Kader (1989) |
| Core breakdown | Brown, watery collapsed cortex tissue | – | Wang and Wang (1989) |
| Core breakdown | Browning/softening in and around the core | $CO_2$-injury, internal breakdown, brown core | Kadam et al. (1995) |
| Brown core | Tissue browning | – | Veltman et al. (2000) |
| Brown heart | Internal breakdown and/or cavities; cavities possible without browning | $CO_2$-injury | Giraud et al. (2001) |
| Core breakdown | Senescence disorder in over-mature/-stored pears; increased risk in large and late harvested fruit | – | Giraud et al. (2001) |
| Brown heart | – | Brown core, internal breakdown | Pinto et al. (2001) |
| Brown heart | Brown (upon 1 cm below peel) and dry tissue; no softening, sometimes cavities | – | Zerbini et al. (2002a,b) |
| Core breakdown | Browning around the flesh, especially around the core region, eventually with cavities because of dehydration | Brown core, internal breakdown | Verlinden et al. (2002) |
| Brown heart | – | Core browning, flesh browning, cavities | Saquet et al. (2003) |
| Core breakdown | Brown discoloration of the inner core tissue and development of cavities | – | Franck et al. (2003b), Lammertyn et al. (2003a,b) |
| Core browning | Flesh breakdown upon 1 cm from the peel | – | Larrigaudière et al. (2004) |
| Brown heart | Lesions which dry out | – | Larrigaudière et al. (2004) |

susceptibility to internal browning disorders, often accompanied by dried lesions or large cavities (Larrigaudière et al., 1998, 2001a; Veltman et al., 1999, 2000; Zerbini et al., 2002a; Lammertyn et al., 2003a). Browning disorders are also observed in other pear cultivars such as 'Williams Bon Chrétien' (Bain, 1961) and 'Bartlett' (Blanpied, 1975; Sugar and Powers, 1994). Externally, the damaged pears look perfectly normal, and therefore, unexpected economic losses may occur.

Table 1 illustrates the lack of standardisation in nomenclature of browning disorders of pears. The symptoms can be divided into three groups: (i) flesh browning, (ii) cavities and (iii) browning and cavities. Giraud et al. (2001) made a distinction between $CO_2$-injury ('brown heart') and a senescence-related injury ('core breakdown'). Larrigaudière et al. (2004) found different metabolic behavior between two browning disorders, 'core browning' and 'brown heart', and concluded that 'core browning' was mainly due to senescence and that storage and high $CO_2$ conditions only accelerated the symptom expression. Hence, the description of 'core browning' of Larrigaudière et al. (2004) corresponds well with the one of 'core breakdown' of Giraud et al. (2001) (both classify the disorders as 'senescence-related').

To conclude, the classification of the observed symptoms remains very subjective. We will, therefore, not make a distinction in the nomenclature in this review and address the disorder with the general term 'browning disorder', although there are indications that different mechanisms might be involved.

## 3. Nondestructive measurement of browning disorders

Browning disorders can cause economic losses due to the fact that damaged fruit cannot be distinguished externally from sound fruit. So far no nondestructive techniques are commercially available to measure browning disorders. Three techniques which have been used successfully on a laboratory scale are discussed here. A reliable and affordable nondestructive testing method for sorting and removing fruit with browning disorders from consignments for sale would be readily accepted by large co-operatives and commercial packing houses.

Research into near infra-red reflectance (NIR) spectroscopy for detecting internal browning was first reported in 1965 (Francis et al., 1965), but this technology has been revisited only in the last few years (Upchurch et al., 1997; Clark et al., 2003). Only detection of browning in apples has been addressed in the literature so far. McGlone et al. (2005) reported the use of near infra-red reflectance spectroscopy for brownheart measurements in 'Braeburn' apples at realistic grading speeds. Because of the limited penetration depth of NIR radiation in fruit tissue, transmission measurements are necessary. The advantage of this technology is that it is cheap and could possibly be combined with existing grading lines. Its accuracy at high grading speeds and robustness with respect to fruit variability remains to be shown, as well as its applicability to pears.

Time-resolved reflectance spectroscopy (TRS) is another nondestructive technique which has been used to measure

browning disorder in pears (Zerbini et al., 2002b). The novelty with TRS is the use of a pulsed laser source (in contrast with other spectroscopic techniques which are based on continuous waves), and the detection of the temporal distribution of re-emitted photons. The advantage of TRS is the fact that the absorption coefficient, as well as the transport scattering coefficient, can be measured simultaneously, while other continuous wave techniques are intrinsically dependent on the coupled effect of both of them. The TRS measurements carry more substantial information about the tissue since absorption is determined by pigments (chlorophyll, anthocyanins) or key constituents (water, sugars), while scattering is more related to the cellular structure and, hence, the presence of brown disorders. The equipment is relatively complex, and so far no on-line implementations have been attempted.

X-ray computer tomography imaging (Lammertyn et al., 2003a) and magnetic resonance imaging (MRI) (Wang and Wang, 1989; Lammertyn et al., 2003a) have been successfully used to detect brown heart in 'Conference' pears. The X-ray image is based on differences in mass density and absorption of the material and indicates water loss due to membrane damage. MRI, on the other hand, employs static fields and radio frequencies in order to obtain images of proton mobility (of the water fraction) in biological systems. Lammertyn et al. (2003a) concluded that MRI was the most appropriate technique to study the development of core breakdown disorder during postharvest storage since its sensitivity is higher compared with X-ray CT, especially in the case of incipient brown discoloration. They also discovered through nondestructive magnetic resonance images that incipient flesh browning was already present after two months of storage under browning-inducing conditions (no cooling period, 10% $CO_2$, 1% $O_2$, −1 °C) and that the brown zone did not grow spatially during storage but only the intensity of brown discoloration increased (Lammertyn et al., 2003b). The disadvantages of MRI and X-ray CT are the high capital cost of the equipment and the low speed of measurement. Also, in the case of X-ray CT, special safety measures are required because of the ionising radiation.

## 4. Preharvest factors

There are few postharvest disorders of fruit which are completely independent of preharvest factors (Ferguson et al., 1999). The incidence of disorders induced specifically by storage conditions such as low temperature or high $CO_2$ partial pressure will be modified by preharvest environmental conditions and orchard practice.

Preharvest factors can be divided into seasonal characteristics (temperature during growth, rainfall), orchard characteristics (including tree and soil characteristics, application of agro-chemicals, irrigation and geographical position) and the position of the fruit in the tree. Although several preharvest factors which affect the development browning disorders in apple fruit have been reported in the literature (references in Lau (1998), Elgar et al. (1999), Ferguson et al. (1999) and Streif and Saquet (2003)), there is little scientific literature about the effect of preharvest factors of pears. Seasonal characteristics are certainly important: 'Conference' pears grown in warm growing areas are less susceptible to browning disorders than pears grown in the cold areas (Magness et al., 1929; Hansen and Mellenthin, 1962; Zerbini et al., 2002a). Season to season variability is also considerable (Roelofs and de Jager, 1997; Verlinden et al., 2002). The application of boron has been shown to reduce browning incidence in 'Conference' pears in some cases (Xuan et al., 2001). Heavy cropping on the tree reduced browning incidence in 'Bartlett' fruit (Blanpied, 1975). 'Passe Crassane' fruit from less productive trees (Zerbini et al., 1977) and 'Conference' fruit from the top of the tree (Roelofs and de Jager, 1997; Franck et al., 2003b) have also been shown to be more susceptible to browning.

The combination of specific preharvest factors results in a particular set of intrinsic pear attributes at harvest which determine whether a pear is susceptible to disorders or not. As a consequence, evaluating the effect of single factors individually is insufficient, and future experiments should be carefully designed and statististically analysed to address not only direct effects but also interactions. The main pear attributes which are affected by preharvest factors and known to affect browning susceptibility are the fruit size, vitamin C and phenolics contents, and gas transport properties (Lentheric et al., 1999; Lammertyn et al., 2000; Hamauzu and Hanakawa, 2003).

A test for $CO_2$-related browning susceptibility of 'Fuji' apple involves a short-term storage of fruit under 20% $CO_2$ for three days (Volz et al., 1998). The assessment of flesh browning after this experiment is a good prediction of browning susceptibility and can be used to optimise storage of fruit from different orchards and harvest dates by sorting them according to their risk for CA-induced browning disorders. As far as the authors are aware of, no such test has been developed yet for pears.

## 5. Postharvest factors

Postharvest factors can be optimised in such a way that even when certain preharvest factors are suboptimal, browning incidence can be prevented to a large degree. Postharvest factors that influence the development of browning disorders are the picking date, the duration of the cooling period, the $CO_2$ and $O_2$ partial pressure, the storage temperature and storage duration (Blanpied, 1975; Lammertyn et al., 2000). Optimal storage conditions for several pear cultivars susceptible to browning disorders have been summarised by Richardson and Kupferman (1997) and Schenk (2004). The picking date has a large effect on postharvest quality of pear fruit (Hartman, 1925; Harley, 1929; Lammertyn et al., 2000; Giraud et al., 2001). In general, late-harvested fruit are far more susceptible to browning disorders. It is highly recom-

mended to start cooling immediately after harvest to reduce the respiration activity as soon as possible but to wait at least three weeks before applying the CA gas conditions (delayed CA, DCA) (Roelofs and de Jager, 1997; Verlinden et al., 2002). This procedure decreases core breakdown incidence efficiently, even for late-picked fruit. Also, the $O_2$ partial pressure during CA storage should not be too low (Lammertyn et al., 2000; Verlinden et al., 2002).

In general, large, more mature fruit, stored at lower $O_2$ and higher $CO_2$ partial pressures, at a higher temperature and for longer times are more susceptible to core breakdown (Hansen and Mellenthin, 1962; Lammertyn et al., 2000). However, the probability of core breakdown depends on several variables in a more complicated way than assumed before (Verlinden et al., 2002): DCA interacts with storage time meaning that eventually the beneficial effect of DCA will disappear when pears are stored too long. Another interaction term involves DCA and $O_2$ partial pressure suggesting that DCA works better when pears are subsequently stored at higher $O_2$ partial pressure or that the beneficial effect of DCA is decreased when the fruit is stored at lower $O_2$ partial pressures. Postharvest application of calcium chloride was found to be beneficial in reducing browning in 'Paternakh' Asian pears (Mahajan and Dhatt, 2004).

Lammertyn et al. (2000) and Verlinden et al. (2002) studied the development of browning disorders as a 'black box' concept: they found statistical indications that several factors influence the browning disorder incidence without knowing how these factors affect the fruit metabolism. In a following section, the possible physiological background of these factors in relation to storage related browning disorders will be discussed.

## 6. Physiological background

### 6.1. Gas exchange

From the statistical analysis of a large dataset on brown disorders in 'Conference' pears, Lammertyn et al. (2000) found that together with maturity and size, $O_2$ and $CO_2$ were the most important factors. This indicates that gas exchange plays a major role in the development of this disorder.

Gas exchange has a fundamental role in storage under controlled atmosphere and is determined by both the respiratory activity and the transport of gases from the storage atmosphere into the fruit. The rate of gas movement depends on the properties of the gas molecule, the concentration gradient and the physical properties of the intervening barriers (Burg and Burg, 1965). Burton (1982) determined four steps in gas exchange between the environment and a plant cell: (1) transport in the gas phase through the outer integument or skin, (2) transport in the gas phase through the intercellular system, (3) exchanges of gases between the intercellular atmosphere and the cellular solution and (4) transport in solution in the cell to or from the centers of consumption or production, respectively. In order to study gas transport in pear tissue, different systems to measure gas transport properties of skin and cortex tissue were developed (Cameron and Yang, 1982; Banks, 1985; Lammertyn et al., 2001a; Schotsmans et al., 2003; Ho et al., 2006a). As expected, the diffusivity of $O_2$ and $CO_2$ in the skin seem to be small compared with that of the cortex tissue (Lammertyn et al., 2001a; Schotsmans et al., 2003; Ho et al., 2006b). Along the equatorial radial direction of the pear, the $O_2$ and $CO_2$ diffusivity of the cortex tissue was almost constant. However, the axial $O_2$ and $CO_2$ diffusivity of the core tissue was much higher than that of the cortex tissue. The $O_2$ diffusivity was not influenced by temperature while temperature had a statistically significant effect on $CO_2$ diffusivity, although small compared with its biological variability (Ho et al., 2006b). The diffusion of $O_2$ was considerably smaller than that of $CO_2$. Picking date had no effect on the gas diffusivities. Diffusivities in brown tissue of disordered pears were smaller than diffusivities in sound tissue irrespective of whether the sound tissue came from a healthy or a disordered pear (Ho et al., 2006b). This latter observation can be explained by the fact that intracellular spaces are more filled with moisture in the case of brown tissue due to loss of cellular integrity. As such, this may aggravate the disorder by further restricting gas transport.

Lammertyn et al. (2003c,d) constructed and partially validated a respiration–diffusion model to predict the local $O_2$ and $CO_2$ concentrations in pear fruit. Respiration and fermentation kinetics accounting for $CO_2$ inhibition effects were incorporated, and the model took into account the full 3D shape of the pear. The model predicted considerable gas gradients which were also found in their validation experiments. Some typical simulations are shown in Fig. 2. As expected, the gas contours are concentric with the perimeter of the fruit but also with that of a typical brown area. This indicates that (limited) diffusion plays a major role in the development of browning disorders. The local $O_2$ and $CO_2$ partial pressure may be much lower and higher, respectively, than that of the storage atmosphere and cause drastic changes in the metabolism of the fruit which eventually may lead to browning disorders.

### 6.2. Browning is a consequence of membrane damage

The occurrence of browning is due to the enzymatic oxidation of phenolic compounds by polyphenoloxidase (PPO) to *o*-quinones, which are very reactive and form brown coloured polymers (Mathew and Parpia, 1971; Mayer, 1987). The initial reaction, catalysed by PPO, uses $O_2$ as co-substrate. The important factors involved in enzymatic browning are (i) the phenolics concentration, (ii) the PPO activity and (iii) other factors such as L-ascorbic acid (L-AA) (L-AA is able to convert *o*-quinones back to diphenols) and peroxidases (which react also with phenolics using $H_2O_2$ as co-substrate) (Amiot et al., 1992; Nicolas et al., 1994).

Hamauzu and Hanakawa (2003) found that 'Bartlett' and 'Conference' pears had less phenolic compounds and a lower

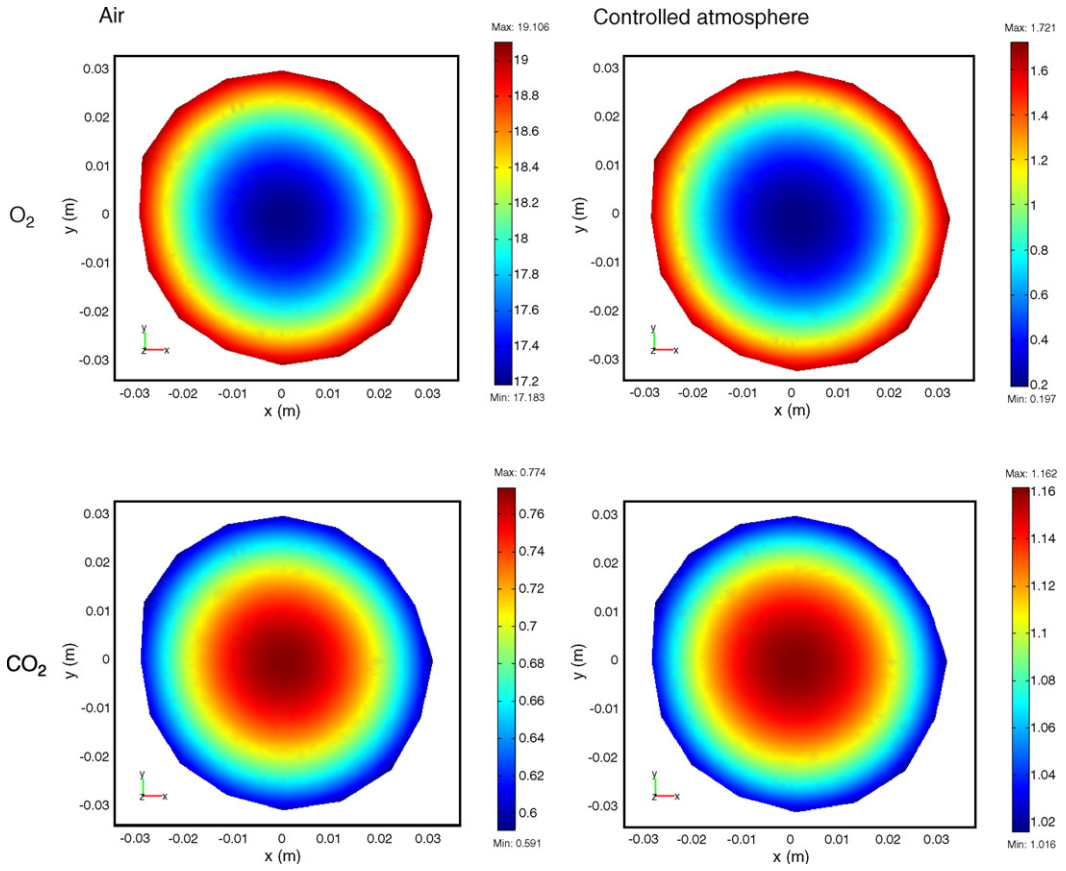


Fig. 2. Typical $O_2$ and $CO_2$ concentration profiles in a pear stored under air (left) and CA (right) conditions according to the model of Lammertyn et al. (2003c) (unpublished data).

degree of polymerisation of procyanidin, and were less susceptible to browning than 'General Leclerc', 'Beurre Hardy' and 'Josephine de Malines' pears. They concluded that the highly polymerised procyanidin plays an important role in tissue browning. Another important compound is chlorogenic acid, the main substrate of pear PPO (Gauillard and Forget, 1997). In a comparative orchard study, we found that the concentration of chlorogenic acid in pears from the most sensitive orchard was two-fold higher than the chlorogenic acid concentration in pears from an orchard yielding less susceptible fruit (Franck, 2004).

PPO activity was found not to be a limiting factor in the enzymatic browning (Amiot et al., 1992; Larrigaudière et al., 1998). Since PPO and its substrate are located in different cell compartments (cytoplasm/plastids and vacuole, respectively) (Nicolas et al., 1994; Dixon and Paiva, 1995), enzymatic browning is a direct consequence of membrane disintegration. Therefore, the causes of browning must be sought in processes which affect the membrane integrity.

### *6.3. An imbalance between oxidative and reductive processes may cause membrane damage*

Membrane disruption occurs when degradation (catabolic) processes exceed the maintenance (anabolic) processes. Maintenance is defined as the process in which damaged cellular components are removed and renewed. This involves biosynthetic reactions, which require energy. For example, for potato cells, Rawyler et al. (1999) calculated a threshold ATP production rate of 10 $\mu mol\ g^{-1}\ FW\ h^{-1}$ to preserve membrane integrity. Saquet et al. (2000) suggested a probable relationship between energy level expressed by ATP concentrations, ATP:ADP ratios and pyridine nucleotides and the development of browning disorders in 'Conference' pears and 'Jonagold' apples.

The current research into the origin of storage related browning disorders can be divided according to the focus on catabolic or anabolic processes, respectively. The latter focuses on insufficient respiration, hence, insufficient energy for maintenance (anabolic), while the former research path focuses on the prevention of damage (catabolic) by studying the antioxidant system. However, both hypotheses are complementary: what matters is the balance between the production of harmful reactive oxygen species (ROS), the efficiency of the antioxidant system and the available energy for maintenance, or, in other words, the balance between oxidative and reductive processes.

In normal plant cell metabolism, there is a certain production of ROS by the respiratory pathway through electron "leaks", by chloroplasts and auto-oxidative reactions (Gille

and Sigler, 1995; Foyer and Noctor, 2003). The first reaction product is usually the superoxide radical ($O_2^{\bullet -}$) which may be further reduced to hydrogen peroxide ($H_2O_2$) and reactive hydroxyl anion ($OH^{\bullet}$). While the reactivity of the former in aqueous solutions is rather limited, the hydroxyl anion is extremely reactive and may cause lipid peroxidation, DNA damage, protein oxidation and, finally, cell death (Gille and Sigler, 1995).

In optimal conditions, the produced ROS are efficiently removed by the antioxidant system. However, in stress conditions, ROS production increases (Möller, 2001). The cells will experience a more profound level of oxidative stress, which is more dramatic in the case of a deficient antioxidative system. When this oxidative state is too intense and/or lasts too long, abnormalities in cellular metabolism occur, resulting in, for example, loss of membrane integrity, and consequently, browning reactions.

Many papers have been published about the role of the antioxidant system, and in particular, L-AA, in the development of browning disorders (Lentheric et al., 1999; Veltman et al., 1999, 2000; Larrigaudière et al., 2001a; Zerbini et al., 2002a; Franck et al., 2003b). The general hypothesis is that L-AA protects against browning and that browning does not occur unless the L-AA concentration falls below a certain threshold value. L-AA functions as an antioxidant on its own or together with antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR). The combined action of these enzymes guarantees the neutralization of reactive oxygen species by converting them towards $H_2O$ (Davey et al., 2000). Phenolic substrates seem to play an ambiguous role with respect to browning disorders: they protect the fruit by scavenging ROS but the corresponding brown coloured oxidation products are the actual cause of the browning symptoms. Note that externally applied anti-oxidants such as DPA have been shown to prevent the development of browning in several apple cultivars (Meheriuk et al., 1984; Burmeister and Roughan, 1997; Colgan et al., 1999; Argenta et al., 2002a).

### 6.4. The influence of internal gas partial pressures on fruit metabolism

The internal gas partial pressures, which depend on the externally applied gas partial pressures and gas transport properties of the fruit, influence both the respiration rate, and, hence, the energy levels (Saquet et al., 2003), as well as the L-AA concentration and the antioxidant system (Agar et al., 1997; Larrigaudière et al., 2001b; Veltman et al., 1999; Franck et al., 2003a). Moreover, the L-AA metabolism is indirectly linked with respiration processes since NADPH is needed for regeneration of dehydroascorbic acid (the oxidised and non-active form of L-AA) which shows the difficulty of uncoupling different metabolic processes.

Storage of pears under (too) low $O_2$ conditions may induce metabolic adaptations to survive (induction of fermentation) or avoid anoxia (i.e., $O_2$ and ATP consuming pathways are retarded). Under low $O_2$ conditions fruit switch from a respiratory to fermentation metabolism. The latter pathway yields only 2 moles of ATP per mole glucose compared with 36 moles through respiration, and is, hence, far less efficient. Accumulation of typical fermentation end products such as acetaldehyde, ethanol and ethyl acetate was observed in 'Bartlett' pears kept at 0.25% $O_2$, 20% $O_2$ + 80% $CO_2$ and 0.25% $O_2$ + 80% $CO_2$ (Ke et al., 1994), but not at 1% or 0.5% $O_2$ in combination with 20% $CO_2$ (Ke et al., 1990). However, accumulation of fermentation metabolites such as ethanol and acetaldehyde was shown not to be the direct cause of cortex browning in apple (Fernandez-Trujillo et al., 2001; Argenta et al., 2002a).

Kader (1989) found that the $O_2$ concentration at which aerobic respiration of 'Bartlett' pears shifted to fermentation varied between 0.3% and 1.7% at temperatures between 0 °C and 25 °C. Lammertyn et al. (2003d) found that the value of the Michaelis–Menten constant $K_m$ for $O_2$ consumption of 'Conference' pears was 6.2 kPa. At such $O_2$ partial pressures cytochrome *c* oxidase, which is believed to be the rate determining enzyme in the respiratory pathways, is saturated (Cameron et al., 1995). This can be explained by diffusion limitations; a high diffusion resistance causes the internal $O_2$ partial pressure to be much lower than the external one. As a consequence, the $K_m$ value observed for $O_2$ consumption of intact fruit may be much larger than that of the cellular $O_2$ consumption. This was confirmed by Lammertyn et al. (2001b) who used modified Michaelis–Menten kinetics to describe the effect of the $O_2$ and the $CO_2$ concentration and temperature on the $O_2$ uptake rate of cell suspensions and intact fruit of 'Conference' pears. They found that the $K_m$ for intact pears was significantly larger than the one for protoplasts in suspension, which was in turn larger than the Michaelis–Menten constant obtained in mitochondrial respiration measurements described in the literature. Considerable gradients of $O_2$ and the $CO_2$ partial pressure in the intercellular space of 'Conference' pear tissue have been predicted and indirectly measured (Lammertyn et al., 2003d). However, the real partial pressure of $O_2$ and $CO_2$ in the cells may be considerably smaller and larger, respectively, than that of the surrounding intercellular space, particularly when the intercellular space is relatively small such as in 'Conference' pear and not every individual cell is surrounded by pores. At some positions the local $O_2$ partial pressure may then drop well below the $K_m$ of cytochrome oxidase and fermentation may occur. The consequence of this is that fermentation may be a very local phenomenon which occurs even at moderate storage $O_2$ partial pressures depending on the size and permeability of the fruit tissue. Note that Chervin et al. (1999) found typical fermentation metabolites such as ethanol and acetaldehyde in 'Packham's Triumph' pears when stored under atmospheric $O_2$ partial pressure. Further validation of this hypothesis, however, is required. Multiscale

models are currently developed to study gas transport at the microscopic level (Nicolaï et al., 2007) and might be useful for this purpose. It is interesting to note that some authors have assumed that plants possess an oxygen sensing system which causes plant cells to adapt their metabolism to decreasing $O_2$ even long before critical values are reached (Geigenberger, 2003).

The influence of an elevated $CO_2$ partial pressure is more complex: as an end product of the respiration, it is evident that $CO_2$ reduces respiration rates in apples and pears (Peppelenbos and van 't Leven, 1996; Hertog et al., 1998; Kerbel et al., 1998). Elevated $CO_2$ partial pressures reduces glycolysis (Kerbel et al., 1998) and Kreb's cycle (Shipway and Bramlage, 1973), induces fermentation (Ke et al., 1994) and reduces L-AA concentrations (Agar et al., 1997; Veltman et al., 1999; Larrigaudière et al., 2001b; Pinto et al., 2001; Franck et al., 2003a).

A further effect of gas gradients inside pear fruit became apparent when comparing L-AA maps of pears after three months storage under browning-inducing conditions with the maps of pears at harvest time (Franck et al., 2003b). The authors observed that mainly the central part of the fruit lost L-AA. This might be due to the fact that, because of respiratory activity and diffusion limitations, the internal $O_2$ can reach very low partial pressures, while $CO_2$ accumulates, especially at high temperatures. Under browning-inducing conditions (no cooling period, 1% $O_2$, 10% $CO_2$), $O_2$ drops to anoxic partial pressures in the center while the border is under hypoxic conditions (Lammertyn et al., 2003c). From the combination of the simulated gas profiles and L-AA maps, it appeared that there was a gas effect on the L-AA metabolism; contours of equal $O_2$ or $CO_2$ partial pressure and L-AA concentration were all concentric with the fruit perimeter. During long-term storage, it was found that enhanced partial pressures were much more disadvantageous for L-AA retention than low $O_2$ partial pressures (Franck et al., 2003a). However, it is not known how $CO_2$ interacts with the L-AA metabolism.

Internal gas partial pressures are strongly influenced by postharvest handling. The period just after harvest is crucial and determines many biochemical reactions. It has been found that fruit which were subjected to a cooling period before CA storage (delayed CA, DCA) contained higher ATP levels, presumably due to the fact that these fruit have higher respiration rates, resulting in a higher energy status (Saquet et al., 2003). Biochemical reactions, in turn, are influenced by the partial pressure of $O_2$ and $CO_2$. The effect of DCA was investigated by Lammertyn et al. (2003d) using a respiration–diffusion model. The authors showed that without DCA, the internal $O_2$ partial pressure drops very quickly to very low values due to the combination of the high temperature at the start of the cooling period with low storage atmosphere $O_2$ partial pressure. With DCA the storage gas conditions were only established after cooling, or when the respiration was sufficiently retarded to avoid extreme internal gas conditions.

### 6.5. Biochemistry of brown tissue

Larrigaudière et al. (2004) recently characterised and differentiated two disorders, core breakdown (CB) and brown heart (BH), based on the activity of enzymes involved in fermentative and oxidative metabolism. Their results suggest that CB and BH involve different metabolic pathways. In CB-damaged fruit, ethanol accumulates inducing the characteristic cell collapse observed in this fruit, whereas in BH-damaged fruit, oxidative processes were considered as the most important causes. The authors suggest that CB is mainly due to senescence (also pears stored in air may develop CB after long term storage) and that storage at high $CO_2$ partial pressures only accelerated symptom expression. This link with senescence was less clear for BH. A possible explanation could be that during senescence the cytoplasm of dying cells may fill up the intercellular space in such a way that gas transport is limited. Ho et al. (2006b) found indeed that the diffusivities of $O_2$ and $CO_2$ in brown tissue of disordered pears was smaller than that in sound tissue. This would mean that, while the initial events behind CB and BH might be very different, limited gas diffusion and, consequently, an imbalance between oxidative and reductive processes might be the actual cause of browning in both cases. This hypothesis is supported by recent research of Argenta et al. (2002b) who found that 'Fuji' apples that had smaller intracellular air spaces suffered from watercore in the tissue. Further, the more severe the watercore, the higher the levels of fermentative metabolites such as acetaldehyde and ethanol and the more severe the browning symptoms.

In order to gain new insights into the origin of browning disorders, the research must be diversified: instead of focusing on ethylene *or* respiration *or* particular target metabolites such as L-AA, a more global biochemical profiling approach is required, preferably by combining information about both enzyme and metabolic studies. Metabolic profiling studies using unbiased and simultaneous analytical techniques, such as GC–MS, to measure metabolites can be useful to obtain a global view on biochemical changes in disordered fruit. Franck (2004) evaluated the composition of polar metabolites in sound and brown tissue of pears stored during 3 months under browning inducing conditions (1% $O_2$, 10% $CO_2$, $-1\,^{\circ}C$). A dramatic increase in GABA concentration and a loss of malic acid was observed in brown tissue compared with the surrounding sound tissue. In plants, GABA is considered to be (1) a regulator of cytosolic pH, (2) a reserve of C and/or N and (3) a signaling molecule in case of exposure to biotic stress (Kinnersley and Turano, 2000), and its biosynthesis is linked with the Kreb's cycle (Fig. 3). GABA:pyruvic acid transaminase is known to be inhibited in anaerobic conditions (Streeter and Thompson, 1972). It has been shown that anoxia and other stress conditions lead to cytosolic increases in $Ca^{2+}$ which, on turn, stimulate glutamic acid decarboxylase activity (Ferreira de Sousa and Sodek, 2002). It is reasonable to expect that GABA may therefore accumulate in anaerobic conditions. The measured GABA accumulation in brown tis-

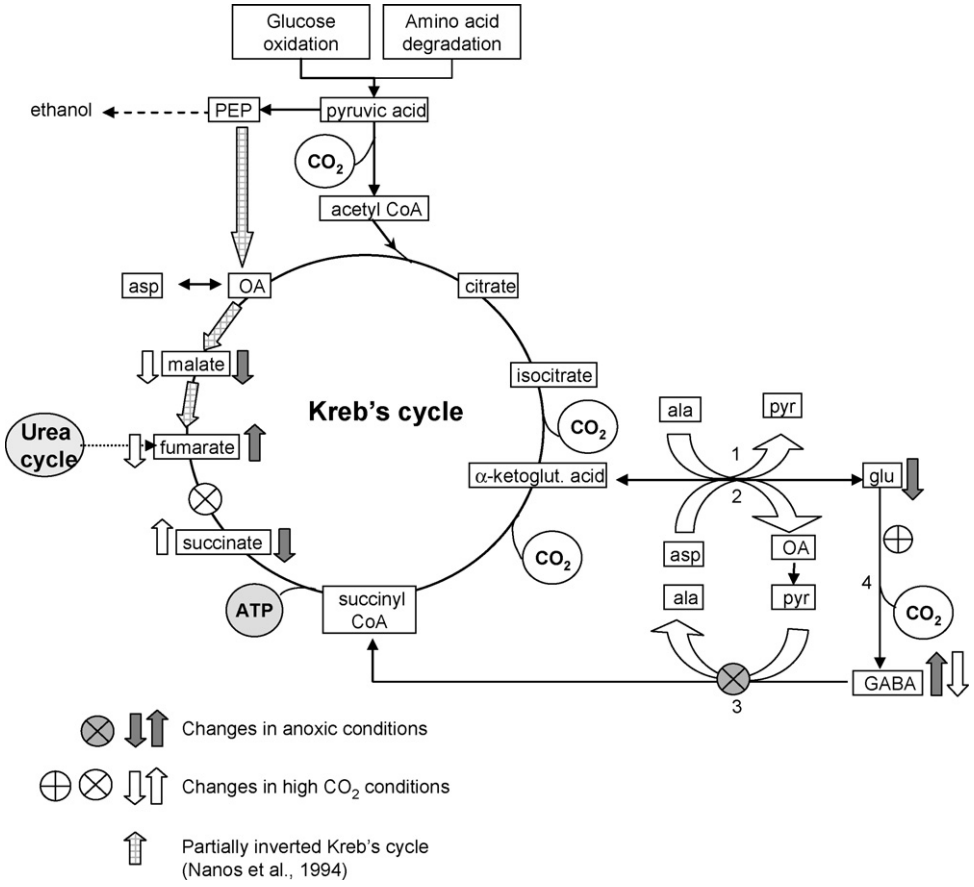


Fig. 3. Changes in Kreb's cycle in anaerobic conditions (full block arrows) and under high $CO_2$ (open arrows). ⊗ indicates inhibition of enzyme activity, ⊕ indicates stimulation of enzyme activity. Enzymes 1, 2 and 3 are transaminases, enzyme 4 is glutamic acid decarboxylase.

sue, therefore, gives biochemical evidence for the presence of anoxic zones in pears.

Apart from the increase in GABA, the increased fumaric acid concentration was another characteristic feature of brown tissue (Franck, 2004). It is known that high $CO_2$ conditions result in a depletion of malic acid and an accumulation of succinic acid as a consequence of an increased malic oxidation and an inhibition of succinic acid dehydrogenase, respectively (Shipway and Bramlage, 1973; Ke et al., 1993). It is likely that these metabolic changes in the Kreb's cycle also occur in browning-inducing conditions, however, they cannot explain the increase in fumaric acid concentration. Possible explanations for the rise in fumaric acid concentration are the following: (i) fumaric acid is a by-product of the urea cycle, which serves to eliminate excess nitrogen (indication of protein breakdown) and is present in certain amino acid degradation pathways; (ii) in anoxic fruit, a partial reversal of the Kreb's cycle has been reported (references in Nanos et al. (1994)) and exposure of suspension-cultured pear fruit cells to hypoxia resulted in an increased PEP carboxykinase activity (Nanos et al., 1994). High $CO_2$ partial pressures during hypoxia might facilitate this reaction. However, these hypotheses remain highly speculative and more research is required.

The biochemical facts described above are the result of analyses on brown tissue samples, which give indications of cumulative metabolic activities. It is clear that the Kreb's cycle is disturbed and that degradation processes are going on. The measured metabolic profile is the result of all these degradation processes. In order to obtain more information about direct causal relationships between metabolite concentrations and enzyme activities on one hand, and browning development on the other, future research should focus on a time series of samples taken in the center of fruit from harvest on, in order to monitor the biochemical changes *preceding the onset of browning*. However, the difficulty of these experiments is the fact that biochemical analyses are unavoidably destructive and, hence, it can never stated with full certainty whether a particular sample is taken from a pear which would develop browning disorders. Even when fruit are stored under severe browning-inducing conditions, the browning percentage is very variable from year to year (e.g., in 2003, we observed 77% of disordered pears, while in 2004 only 43%; in both seasons, the same browning-conditions were applied and the pears came from the same orchard, unpublished results).

Another possible research route is metabolic flux analysis on cell suspensions which allows characterisation of the activity of different metabolic pathways in a quantitative way

(Stephanopoulos et al., 1998). This is often accomplished using $^{13}C$ labeling experiments in which the isotopic enrichment of labeled metabolites is measured using NMR or MS. In theory this would allows elucidation of which anabolic or catabolic pathways are downregulated in for example anaerobic conditions. This technique has not been used yet in postharvest physiology as far as we are aware.

### 6.6. A model for browning

Based on the above evidence and hypotheses, a model for storage related browning disorders in pear can now be constructed. The main factor which initiates the chain of events which eventually result in browning symptoms is the storage atmosphere composition. While in normal air storage the respiratory activity and the diffusion resistance of pear tissue may cause gas partial pressure gradients in the fruit, a too low $O_2$ partial pressure in combination with a too high $CO_2$ partial pressure in the storage atmosphere may lead to local anoxic conditions in the center of the pear. The latter cause oxidative stress and changes in the normal cellular metabolism from the respiratory to the energetically far less efficient fermentation pathways so that insufficient energy becomes available for normal maintenance processes and repair of membrane damage by ROS in particular. The latter are a by-product of respiration and are in normal conditions efficiently removed by the cellular antioxidant system based on the L-AA—glutathione cycle which, however, may be impaired in oxidative stress conditions. When membrane damage occurs, the normal cellular compartmentalisation is lost and phenolic substrates may be enzymatically oxidised to *o*-quinones and, eventually, brown coloured polymers which are responsible for the actual browning symptoms. The cytoplasm of the leaky cells first fills up the intercellular space, thereby reducing its diffusitivity for metabolic gasses and indirectly causing more extreme local gas conditions. Eventually the moisture diffuses towards the boundary of the fruit where it is lost to the environment, and cavities remain.

Preharvest factors affect fruit attributes such as porosity or cell density, which are directly related to gas diffusion characteristics (Schotsmans et al., 2004). Fruit with a small internal air space are likely to be more susceptible to browning, in particular when in combination with a high respiratory activity. Fruit size is of particular importance, as the total diffusive resistance is proportional to the diffusion path length and, hence, the diameter of the fruit (Lammertyn et al., 2000). Fruit weight is mainly established during the temperature-responsive cell division growth phase (Stanley et al., 2000). The potential maximum fruit size is determined by the total fruit cell number, which is produced during a temperature-responsive cell division growth phase. Given no limitations in water and carbohydrate supply (which is determined by crop load and weather conditions), the cells would expand to their optimum size to provide the maximum fruit weight achievable for that total cell number (Stanley et al., 2000). Late picking in general gives larger fruit and this may additionally increase browning susceptibility.

Increasing maturity at harvest is negatively correlated with L-AA concentrations and activity of antioxidant enzymes in pear (Lentheric et al., 1999). In 'Conference' pears the L-AA and glutathione concentrations and SOD and CAT activity significantly decreased with increasing maturity, while APX and POD activity increased. These results provide evidence that a later harvest is accompanied by a decline in non-enzyme and enzyme antioxidative systems resulting in accumulation of cytotoxic superoxide anions and $H_2O_2$. Further, exposure to sunlight increases the L-AA content of fruit (Davey et al., 2000). This might explain why 'Conference' pears grown in the Mediterranean area are less susceptible to browning disorders than pears grown in the northwest of Europe (Zerbini et al., 2002a), and 'Bartlett' pears from cool growing districts in the USA are more susceptible than from warm districts (Magness et al., 1929). Other preharvest factors such as the application of boron have a positive effect on the L-AA content of fruit, probably by helping to maintain membrane integrity and thereby decreasing the need for protection by anti-oxidants (Xuan et al., 2001).

## 7. Conclusions

Browning disorders are a common storage disorder in several pear fruit cultivars, particularly in 'Conference' pears. It is generally assumed that flesh browning and the presence of cavities are one and the same disorder. However, some authors prefer to distinguish between them, and biochemical data which gives evidence for a metabolic different origin between flesh browning and cavities have been published recently. Due to the lack of clarity in the diversity of names and definitions, we prefer to use the most general term, namely 'browning disorder'.

Browning disorders are a typical postharvest problem, induced by adverse storage conditions. However, the development of disorders during postharvest ripening and storage of fruit depends also on a range of preharvest factors. These factors explain the large variability in susceptibility between different orchards, regions and seasons. These differences are reflected in the antioxidant system and the overall metabolic activity and we believe that browning disorders are caused by an imbalance between oxidative and reductive processes due to metabolic gas gradients inside the fruit. This may lead to an accumulation of reactive oxygen species which, in turn, may induce loss of membrane integrity which becomes macroscopically visible through the enzymatic oxidation of phenolic compounds to brown coloured polymers. Future research should focus on the following topics:

- Affordable nondestructive techniques for measuring brown disorders.
- A multivariate statistical approach towards analysing the relationship between browning disorders and pre- and

postharvest factors. There are clearly interactions between the different factors which may not be revealed by a univariate approach.
- Improved gas transport models which address multiple scales down to the subcellular scale.
- Integration of biochemical data from enzyme (proteomics) and metabolite (metabolomics) oriented research into quantitative models.
- Development of models to describe generation of ROS, the antioxidant system and the browning process, and coupling with the multiscale gas transport models.

Ultimately this should lead to a better understanding of the phenomenon and means to control its incidence.

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