The use of gas exchange characteristics to optimize CA storage and MA packaging of fruits and vegetables

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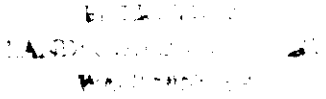

Cover: Part of the equipment for the measurement of gas exchange and diffusion resistance.

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Stellingen

1. Het gasuitwisselingsgedrag van groente en fruit is goed te simuleren met een beschrijving op enzymniveau[1,2].
   Dit proefschrift.
   [1] Chevillotte, 1973, J. Theor. Biol., 39: 277-295.
   [2] Cameron et al., 1995, HortSci., 30: 25-34.

2. Aerobie en anaerobie zijn onbruikbare begrippen als oxidatieve en fermentatieve processen gelijktijdig voorkomen.
   Dit proefschrift.

3. De toename van de snelheid van fermentatieve processen bij lage zuurstofconcentraties kan zowel worden beschreven met behulp van een afname van de zuurstofconcentratie als met behulp van een afname van de ATP produktie.
   Dit proefschrift.

4. Het Anaerobic Compensation Point[1] kan niet worden gebruikt om de optimale zuurstofconcentraties voor de bewaring van appels te voorspellen.
   Dit proefschrift.
   [1] Boersig et al., 1989, J. Am. Soc. Hort. Sci., 113: 869-873.

5. Het idee dat fermentatie pas begint beneden een bepaalde zuurstofconcentratie[1,2], is het gevolg van de gebruikte technieken om fermentatieve metabolieten te meten.
   Dit proefschrift.
   [1] Kader, 1989, Acta Hort., 258: 161-167.
   [2] Knee, 1991, in: Plant life under oxygen deprivation, M.B. Jackson, D.D. Davies and H. Lambers (eds.): 229-243.

6. Grote lijnen alleen bestaan voor een onderzoeker niet. Creatieve gedachten komen juist voort uit het combineren van details, vaak uit verschillende randgebieden[1].
   [1] R. Plasterk, Volkskrant 4-9-1993, p. 15 wetenschap.

7. Het knotten van wilgen heeft meer met bonsai dan met natuur te maken.

8. Het plaatsen van kunstvoorwerpen in woonwijken om de leefkwaliteit te verhogen is omstreden. Om een verdere achteruitgang in de waardering van openbare kunst te voorkomen, moet voor plaatsing de bewoners niet alleen inspraak worden gegeven over het type kunstobject, maar ook over de wenselijkheid ervan.

9. Als de uitvoering van een reorganisatie afhankelijk wordt van het succes van de voorgaande reorganisatie, zal dit verschijnsel veel aan aantrekkingskracht verliezen.

10. Door het grote aantal doe-het-zelf zaken in Nederland ontstaat de indruk dat er maar weinig goede huizen zijn.

11. Omdat het veroorzaken van slachtoffers door middel van een voertuig altijd veel milder wordt bestraft dan wanneer dit gebeurt door middel van een wapen, wordt asociaal gedrag met voertuigen aangemoedigd.

12. Door de toename van intelligente literatuurzoeksystemen wordt het tijdschrift waarin gepubliceerd wordt steeds onbelangrijker.

13. Door de toename van de hoeveelheid t.v. uitzendingen met voetbal en zogenaamde reality-t.v. wordt de kans groter dat ook een combinatie van beide zal ontstaan, wat de t.v. kijker zal verlossen van voetbalcommentatoren.

Stellingen behorende bij het proefschrift getiteld "The use of gas exchange characteristics to optimize CA storage and MA packaging of fruits and vegetables", door Herman W. Peppelenbos.

Wageningen, 22 November 1996

Aan Jolijn, Gideon en Lucas
Aan mijn ouders

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1

Introduction.
The influence of low oxygen and high carbon dioxide concentrations on stored fruits and vegetables

Fresh fruits and vegetables are important elements in the human diet. These harvested plant products are a main source for carbohydrates and proteins, and contain essential biomolecules such as vitamins. The appreciation, however, of fruits and vegetables is not only based on these functional elements, but also on features like firmness, colour, taste, aroma and appearance. Immediately after harvest changes in plant products occur which can be attributed to maturation, ripening and senescence (Watada et al., 1984; Kader et al., 1989). Although a physiological distinction between ripening and senescence has never been finally drawn, ripening hastens the onset of senescence and the probability of cell injury and cell death (Brady, 1987). From a consumers point of view postharvest changes often negatively affect the mentioned quality attributes. The rate at which fruits and vegetables lose quality is influenced by environmental conditions, such as temperature, humidity and gas composition. Studies on these factors have lead to ways that prolong the storage period and shelf-life of numerous fruits and vegetables.

Early research

Storage techniques based on altered gas conditions have a long history. Ancient chinese writings report the transport of fruits in sealed clay pots with fresh leaves and grass added. This generated a low oxygen and high carbon dioxide atmosphere which retarded the ripening of the fruit (Floros, 1990; Jameson, 1995). During the time of the Roman Empire modified gas atmospheres were created by sealing underground pits

filled with grain, thereby protecting it from insects and rodents (Kays, 1991). In the beginning of the nineteenth century Berard demonstrated that fruit placed in closed containers did not ripen (Berard, 1819). Extensive research on the use of altered gas conditions started early this century, with the work of Kidd and West (1923), Thomas (1925) and Blackman (1928). Kidd and West's discovery of the climacteric and Blackman's studies of respiration in apples established the basis of modern postharvest physiology (Laties, 1995). Commercial storage under altered gas conditions started in England in 1929, when apples were stored in 10% CO_2 and ambient O_2 (Kays, 1991).

CA storage and MA packaging

Reduced O_2 concentrations and increased CO_2 concentrations also proved to be beneficial for other products than apples. Nowadays low O_2 and high CO_2 concentrations are involved in several techniques. The most commonly used for fruits and vegetables are Controlled Atmosphere storage (CA) and Modified Atmosphere packaging (MA). 'Controlled' refers to a (large) storage room with monitoring and active adjustment of the gas composition. 'Modified', however, refers to a difference in gas composition as compared with ambient air, without any active control of the gas composition. The gas composition within the package is the result of a balance between metabolic rates of the packed product and diffusion characteristics of the package. At the moment 60 to 70% of the Dutch apple harvest and 40 to 50% of the pear harvest is stored in CA facilities. MA packages are increasingly used in supermarkets for products such as broccoli, corn, and minimally processed endive, spinach, vegetable mixes and salads.

Regarding the suitability for CA-storage and MA-packaging large differences between harvested plant products are found. There are products, like apples, where storage under low O_2 conditions can increase the storage period by months. Also products are known, like carrots (Weichmann, 1977), that do not respond positively to low O_2 and high CO_2 concentrations. In general altered gas conditions are regarded as positive only within a certain range of concentrations, the so called 'optimum concentrations'. Much research has been directed toward the determination of optimum concentrations (Kader, 1986), and at the moment for many products these concentrations are known (Stoll, 1975; Isenberg, 1979; Smock, 1979; Kader, 1993; Meheriuk, 1993; Saltveit, 1993). At the moment CA and MA are used for products such as apples, pears,

bananas, kiwi, strawberries, currants, cabbage and broccoli (Kader et al., 1989).

Disorders

When plant products are stored at very low O_2 or very high CO_2 concentrations, increased fermentation rates are found together with disorders like necrotic and discoloured tissues (Fig. 1), and off odours and off taste (Kader et al., 1989). Disorders in apples related to gas conditions are generally referred to as 'brown heart', in the case of CO_2 injury, and 'low oxygen breakdown' in the case of O_2 injury (Smock, 1977). In postharvest research the general idea is that a direct relation exists between fermentation and the occurrence of disorders. Thomas (1925) mentioned that as a result of acetaldehyde production the surfaces of apples become brown. Kader (1986) stated that the decarboxylation of pyruvate to form acetaldehyde, CO_2 and ultimately ethanol, results in the development of off-flavours and tissue breakdown. O_2 concentrations are considered to be optimal when respiration rates are reduced without the development of fermentation (Kader, 1989; Banks et al., 1993). Nevertheless the assumption that ethanol is toxic under certain conditions is still not generally accepted (Pfister-Sieber and Brändle, 1994). For carrot cells Perata and Alpi (1991) found that

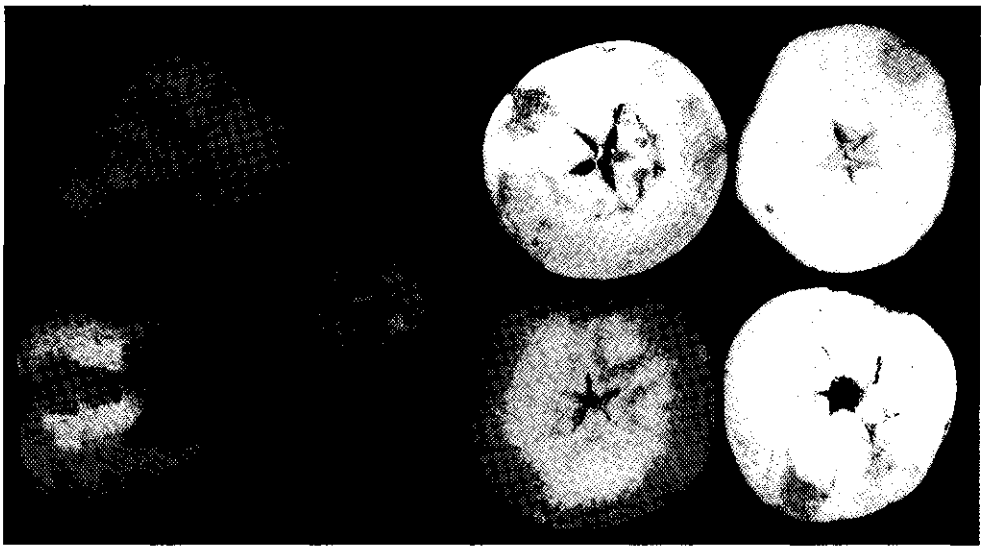

Figure 1. *Disorders found in Boskoop apples stored at 0.5% O_2 for 6 months (with kind permission of Dr. S.P. Schouten, ATO-DLO).*

observed toxic effects of ethanol cannot be ascribed to ethanol per se but to acetaldehyde. Unclear, however, is whether acetaldehyde can normally accumulate in high enough quantities to cause damage.

From the beginning research on CA storage was focused on avoiding fermentation. Throughout the years several concepts were used to describe fermentative metabolism in fruits and vegetables. The first concept was the Extinction Point (EP), defined as the highest O_2 concentration with no fermentative metabolites found (Blackman, 1928). Very soon, however, ethanol was also found at normal O_2 concentrations (Fidler, 1933), suggesting fermentative processes to occur in situations with sufficient oxygen. This was recognized by Boersig et al. (1988), who proposed the Anaerobic Compensation Point (ACP), defined as the O_2 concentration at which CO_2 production is minimal. The ACP can be explained as the O_2 concentration where an increase in fermentative CO_2 production compensates for the decrease in oxidative CO_2 production. This implies fermentation to occur at higher O_2 concentrations than the ACP. No explanation is known for fermentation being active at high O_2 concentrations, and fermentative metabolites found at these conditions are commonly referred to as 'background concentrations' (Leshuk and Saltveit, 1991).

Optimal gas concentrations

Traditionally, lists of recommended storage conditions have been evolved by national research organizations by extensive laboratory research (Jameson, 1995). The common experimental procedure is to store products under a range of O_2 and CO_2 concentrations, and to monitor quality changes. The lower O_2 limit for stored fruits is accomplished empirically by lowering the storage O_2 concentration until intolerable damage occurred. Each commodity and new cultivar required a large investment in time, equipment and materials (Wollin et al., 1985; Gran and Beaudry, 1993). By repeating the trials year after year, it is possible to sense the importance of climatic variation on product behaviour (Jameson, 1995).

Some caution is needed with the application of optimal concentrations. For the various apple cultivars, for instance, the advised optima differ per country (Meheriuk, 1993). Growing conditions like climate and orchard factors influence crop growth and contribute to these differences (Bramlage et al., 1980; Chen et al., 1986; Luton and Holland, 1986). Although this is probably also the case for plant products other than apples, this is never specified.

Another important aspect of optimal values for temperature, O_2 and CO_2 concentrations is that they are often established separately, although interactions between temperature, O_2 and CO_2 concentrations (and probably also humidity) are known. Optimal O_2 concentrations are found to shift to a higher value when CO_2 concentrations are increased (Thomas, 1925; Lougheed, 1987; Kader et al., 1989; Beaudry, 1993), when products are more mature (Thomas and Fidler, 1933; Lidster et al., 1985; Boersig et al., 1988; Kader, 1989), at a higher temperature (Thomas, 1925; Saltveit and Ballinger, 1983; Ke et al., 1991, Beaudry et al., 1992), or when products sensitive for chilling injury are stored at low temperatures (Kader, 1986; Lougheed, 1987). For apples it is found that the CO_2 limit at low O_2 concentrations decreases when the temperature is decreased (Kidd and West, 1923). Also the effect of the relative humidity is often an interacting factor as a cause for, or in the symptom expression of, a disorder. Relative humidity, however, is often not mentioned and frequently not (accurately) measured (Lougheed, 1987).

The conclusion is that it is very hard to advise an absolute value for the optimum O_2 and CO_2 concentrations or the O_2 and CO_2 limits for a product without knowledge of other factors (Peppelenbos, 1995). In fact, despite a long history of CA and MA research, the knowledge of the actual physiology behind the CA and MA effects remains sketchy and empirically based (Banks, 1989). The processes and mechanisms behind the influence of O_2 and CO_2 and the reasons for the differences between products are still quite uncertain. For a better understanding it is necessary to focus on the biophysical and biochemical processes actually occurring within the plant tissues exposed to extreme gas conditions. The main processes involved in gas exchange processes are respiration, fermentation and diffusion.

Low oxygen and respiration

After harvest fruits and vegetables stay metabolically active, which is expressed in high respiration rates. Respiration is a central process in living cells that mediates the release of energy and the formation of carbon skeletons necessary for maintenance and synthetic reactions after harvest (Kays, 1991). Respiration provides the energy and metabolites for maturation and ripening. A reduction of respiration results in a lower energy supply and a reduced rate of changes within the product (Kader et al., 1989). Conditions around the product that reduce respiration, like low temperature and low O_2 concentrations, should be created.

In general the reduction of the respiration rate is regarded the most important affected process by altered gas conditions (Banks et al., 1993; Lee et al., 1995), although it is not the only beneficial effect of CA and MA (Kader, 1986). Laties (1995) for example, criticizes postharvest studies on ripening focussing on respiration without regard to the role of ethylene. Nevertheless he also mentions fruits not exhibiting an ethylene-induced climacteric on ripening, and ethylene induced respiratory responses without concomitant ripening. Thus, the course and nature of respiration in ripening fruit remains of paramount interest (Laties, 1995). A good quantification of the effect of reduced O_2 on respiration rates is also essential for MA, as this effect generates the equilibrium concentrations inside MA packages. Therefore for a good application of CA and MA it is important to have good insight in the respiratory characteristics as they are affected by O_2 and CO_2 concentrations.

Respiration pathways

Respiration can be divided into three closely related pathways; glycolysis, TCA cycle and oxidative phosphorylation. The term glycolysis, meaning lysis of sugar, was introduced in 1909 to describe the breakdown of sugar to ethanol. Nowadays glycolysis is described as the group of reactions that converts glucose (or fructose) to pyruvate and in which 2 ATP and 2 NADH molecules per glucose are produced (Salisbury and Ross, 1985). The breakdown of pyruvate takes place in a cycle of reactions called the Krebs cycle (or TCA cycle). The Krebs cycle accomplishes removal of some of the electrons from organic acid intermediates, and transfer of these electrons to NAD^+ or FAD. Per molecule of pyruvate 4 NADH and 1 $FADH_2$ is generated. Also one molecule of ATP is formed during the conversion of succinyl CoA to succinic acid (Salisbury and Ross, 1985). The final pathway involved in respiration is the oxidative phosphorylation. In this pathway the NADH and $FADH_2$ produced in glycolysis and Krebs cycle are oxidized to produce ATP. This oxidation step involves O_2 uptake and H_2O production.

Fermentation

Glycolysis can function well without O_2, only the further oxidation of pyruvate and NADH requires this gas. Lowering O_2 concentrations around plant tissues inevitably leads to a decrease in oxidative phosphorylation. The NADH produced in glycolysis cannot be oxidized to NAD^+. Thus when O_2 is limiting, NADH and pyruvate are accumulating (Salisbury and Ross, 1985). Fermentation enables the production of

NAD^+ from NADH, which ensures the production of (glycolytic) ATP, and an increased rate of glycolysis (Stryer, 1981). Under very low O_2 conditions the glycolytic pathway replaces the Krebs cycle as the main source of the energy needed by the plant tissues (Kader, 1986). The main products of fermentation in plant tissues are ethanol, lactate and alanine, all derived from pyruvate, the end-product of glycolysis. Ethanol is the major product in higher plant tissues (Ricard et al., 1994). Especially under prolonged anoxia ethanol is the most abundant fermentation product (Pfister-Sieber and Brändle, 1994; Ricard et al., 1994).

Gas exchange rates

Because respiration has a central position in the overall metabolism of a plant (part), its measurement is often used as a general measure of metabolic rate (Kays, 1991; Lee et al., 1995). Specific metabolic changes, however, may occur without measurable changes in net respiration (Kays, 1991). Measurements on gas exchange were already conducted by Kidd (1917) on pea and mustard seeds. In 1928 Blackman first measured the CO_2 production of apple fruits. Thornton (1933) was the first who measured O_2 consumption of a range of harvested plant products other than apples. Platenius (1943) combined the measurement of both O_2 consumption and CO_2 production, and was able to derive respiration quotients (RQ). After the second world war gas exchange rates are extensively measured, under various O_2, CO_2, temperature and humidity conditions. To estimate the increase of fermentation rates at low O_2 conditions, the measurement of only O_2 consumption or carbon dioxide production is not adequate. The measurement of both O_2 consumption and CO_2 production under various combinations of fixed O_2 and CO_2 concentrations, however, is rarely done. Thusfar only data are known on apples (Fidler and North, 1967), tomato (Yang and Chinnan, 1988; Chinnan and Pendalwar, 1990) and mushrooms (Peppelenbos et al., 1993). In addition also data are known measured with a clever technique using MA packages to derive various gas conditions: tomato (Cameron, 1989) and blueberry (Beaudry et al., 1992; Beaudry, 1993).

Diffusion

Some experimental data on gas exchange rates cannot be understood if diffusion is not taken into account (Chevillotte, 1973). Normally respiration rates of products are compared with O_2 and CO_2 concentrations from the atmosphere around the product.

However, it is known that concentrations inside a product differ from the concentrations outside. There are diffusion barriers between the external atmosphere and the actual place where respiration takes place, the mitochondria. This leads to a different response of intact fruits to O_2 concentrations compared to cell cultures (Boersig et al., 1988). Although the terminal oxidase of respiration is saturated at O_2 concentrations well below 5%, apple respiration is still increasing at higher O_2 concentrations. This behaviour is often explained by the combination of respiratory responses with diffusion limitations within fruits (Burton, 1974; Cameron et al., 1995).

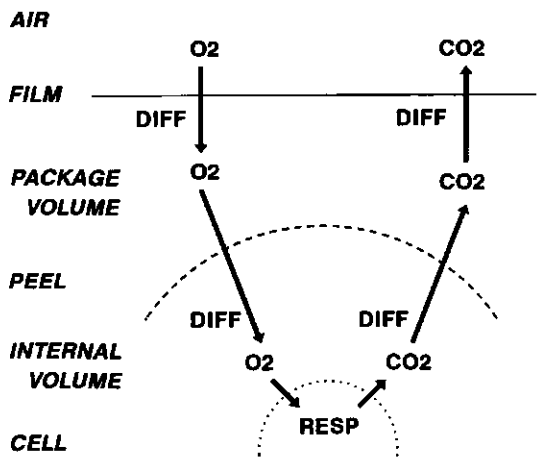

Figure 2. *Main gas exchange processes inside a MA package. O2 = Oxygen, CO2 = Carbon dioxide, DIF = diffusion, RESP = respiration (adapted from Peppelenbos, 1995).*

Diffusion of the metabolic gasses O_2 and CO_2, between the external and internal atmosphere of both package and product, is often considered to follow Ficks first law (Burg and Burg, 1965; Cameron and Yang, 1982; Emond et al., 1989). This is correct if the skin is the major barrier for diffusion (Knee, 1991a,b), and if the rate of CO_2 production is uniform throughout the tissue (Solomos, 1987). From research by Rajapakse et al. (1990) it is known that for some products diffusivity in flesh tissues must be taken into consideration. In apples, however, the percentage of the total O_2 gradient between the external atmosphere and the internal core cavity caused by flesh was found to be very low. A difference in the resistance to gas diffusion is one of the

reasons for differences between products in their response to altered gas conditions. Often the diffusion resistance between the intercellular space volume and the atmosphere around the product is assumed to be constant, although Rodriguez et al. (1989) found a relationship between ripeness of fruits and the resistance to gas diffusion. Also internal gas volumes and internal concentrations in a product are not homogeneous (Solomos, 1987; Weichmann and Brückner, 1989; Rajapakse et al., 1990). These findings stress that for a correct estimation of the relationship between O_2 and CO_2 concentrations and respiration rates, internal gas concentrations should be known. This information can be used to improve knowledge on changes in optimum storage conditions during storage of products. When the considerations on gas exchange, fermentation and diffusion are combined, a product like an apple seems to resemble a MA-package itself (Dadzie et al., 1993; Peppelenbos, 1995): optimum O_2 and CO_2 concentrations seem to depend most on the combination of the respiration rate, the diffusion resistance and the internal volume of the product (Fig. 2).

Models

Both respiration and fermentation result in mass fluxes of O_2 and CO_2. To relate those fluxes to gas concentrations, several equations were developed (Hayakawa et al., 1975; Yang and Chinnan, 1988; Mannapperuma et al., 1989). These empirical descriptions of O_2 uptake can cause serious prediction errors, especially at low O_2 concentrations (Cameron et al., 1995). At the moment the most widely used and appreciated equation to describe respiration of a whole fruit is based on a mathematical description of the kinetics at enzyme level. This Michaelis-Menten type of kinetics have been used by several authors (Chevillotte, 1973, Banks et al., 1989, Lee et al., 1991, Andrich et al., 1991):

$$V_{O2} = \frac{Vm_{O2} * O_2}{Km_{O2} + O_2} \qquad (1)$$

where V_{O2} is the O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Vm_{O2} is the maximum O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), O_2 is the O_2 concentration (%) and Km_{O2} is the Michaelis constant for O_2 consumption (% O_2). In equation 1 it is assumed that the whole respiratory chain can be described by one enzyme mediated reaction, with the substrate glucose considered as non-limiting, and the substrate O_2 as limiting. The total reaction is considered to be in a pseudo steady-state. Steady-state refers to the

assumption that the enzyme-substrate complex reaches a constant value soon after the start of the reaction (Chang, 1981). 'Pseudo' is added by Rubinow and Segel (1991), because they expect the enzyme-substrate complex to be changing slowly in time. The Km_{O2} in the equation refers to the O_2 concentration where the reaction rate (O_2 uptake rate) is half the maximum rate. Vm_{O2} is the maximum reaction rate, in case O_2 is also non limiting. Because not only low O_2 concentrations reduce respiration rates, but also high CO_2 concentrations (Fig. 3), equation 1 was modified to describe this effect.

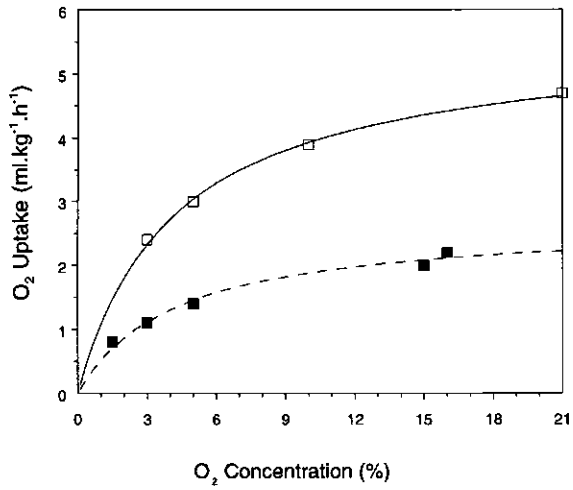

Figure 3. *Gas exchange data of Fidler and North (1967), combined with equation 2, with Vm_{O2} = 6.0, Km_{O2} = 4.2 and Km_{CO2} = 4.0 (□ = data at 0% CO_2, ■ = data at 5% CO_2, ——— = model at 0% CO_2, – – – = model at 5% CO_2).*

Although it was unclear which type of inhibition was caused by high CO_2, the non-competitive type was used by Lee et al. (1991):

$$V_{O2} = \frac{Vm_{O2} * O_2}{(Km_{O2} + O_2) * (1 + \frac{CO_2}{Km_{CO2}})} \qquad (2)$$

where Km_{CO2} is the Michaelis constant for CO_2 inhibition of O_2 consumption (% O_2). The limitation of the models thusfar is that they can be used only for the calculation of

oxidative processes: respiration. If a package or the transport and storage conditions are not optimal and fermentation takes place, even during a small period, the calculated concentrations will differ from the actual concentrations. For the enzyme kinetics model specific parameters must be known for every product; Vm_{O2}, Km_{O2} and Km_{CO2}. Accurate measurements of O_2 consumption and CO_2 production under various O_2 and CO_2 concentrations are needed to calculate these values. Thus far only few data are published that meet this need.

For the MA technique the most suitable package can be calculated by combining product data (optimum levels of O_2 and CO_2) and package data (diffusion characteristics) with descriptions of the underlying physical and physiological processes (respiration, fermentation and diffusion). Simulations of changing O_2 and CO_2 concentrations in a MA-package are shown in Fig. 4.

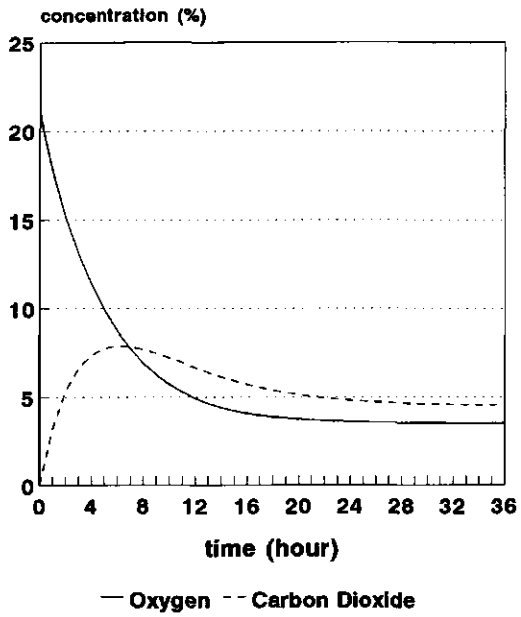

Figure 4. Simulation of the change in O_2 and CO_2 concentrations in a MA-package (adapted from Peppelenbos, 1995).

Outline of the thesis

Respiration is the key process behind the positive effects of CA and MA technology, while fermentation is thought to be closely related to disorders. Optimum gas concentrations for storage are often a compromise between decreased respiration rates and increased fermentation rates. The exact reason for a specific optimum concentration is unclear. It is expected, however, that a good quantification of metabolic products, including fermentative metabolites and energy carriers, will clarify an important part of the tolerance of harvested plant products to low O_2 concentrations. Models are essential to simplify all the processes involved, and to generate predictions on metabolic rates and needs.

The thesis is focussed on the analysis of the quantitative meaning of respiration and fermentation for the decrease in quality of stored produce. To integrate basic physiological knowledge models are developed that quantify the influence of the metabolic gasses O_2 and CO_2 on gas exchange rates. First respiration is described, with a special focus on the influence of high CO_2 concentrations, and by which type of inhibition this influence can be described best (chapter 2). In the next chapter (3) two models are derived, as the O_2 uptake model based on a description of enzyme kinetics, enabling the description of CO_2 production. The models make a distinction between CO_2 produced by oxidative or fermentative processes. The functioning of the two models is then compared with two previous described models. Because analysis of the CO_2 production models revealed that CO_2 not only influences respiration (chapter 2), but also fermentation, this influence is further analyzed in chapter 4. To include this aspect of gas exchange one of the CO_2 production models was modified. Together chapters 2, 3 and 4 address the influence of various O_2 and CO_2 concentrations on O_2 uptake and CO_2 production of a range of harvested plant products.

In the mentioned chapters gas exchange rates were related to gas conditions outside a product, although the metabolic processes themselves take place in cell organelles experiencing different gas conditions. Therefore chapter 6 is addressing the role of resistances to gas diffusion in the functioning of the derived gas exchange models. First (chapter 5) a newly developed method is described to measure both gas exchange rates and diffusion resistance simultaneously and on the same object. The data obtained by this method are used to calculate internal O_2 concentrations. The functioning of gas exchange models is analyzed using both the external as well as the calculated internal O_2 concentrations (chapter 6). Chapter 7 is focussed on the use of

gas exchange models for the prediction of the tolerance to low O_2 concentrations. This was done by comparing changes in specific gas exchange characteristics, like the Anaerobic Compensation Point and the Respiration Quotient to changes in optimal O_2 concentrations during storage as observed in literature. Based on the models also energy (ATP) production was calculated and compared to estimated maintenance energy requirements for the prediction of optimal O_2 concentrations for the storage of apples. Although the models described are based on enzyme kinetics, they are merely used as mathematical tools only. To have some idea of their physiological relevance, predicted fermentation rates under various O_2 concentrations are compared with actual measurements on fermentative metabolites (chapter 8). The last chapter (9), the general discussion, is summarizing and integrating the results of the other chapters.

2

Evaluation of four types of inhibition for modelling the influence of carbon dioxide on oxygen consumption of fruits and vegetables

H.W. Peppelenbos, J. van 't Leven.
1996, Postharvest Biol. Techn., 7:27-40.

Abstract

High carbon dioxide (CO_2) concentrations can reduce the oxygen (O_2) consumption rate of a number of fruits and vegetables. This reduction can be modelled by four types of inhibition in an enzyme kinetics model: 1. the competitive type, 2. the uncompetitive type, 3. a combination of both previous types and 4. the non-competitive type. These different types of inhibition were tested for describing the CO_2 influence on O_2 consumption using experimental data supplemented with data from literature. The gas exchange rates of apples (cv Golden Delicious and Elstar), asparagus, broccoli, mungbean sprouts and cut chicory were measured under a wide range of O_2 and CO_2 concentrations. With the range of CO_2 concentrations used, no influence was found on gas exchange rates of apples. There was a clear influence of high CO_2 on the gas exchange rates of the other produce. A good estimation of O_2 consumption could be obtained with the inhibition models. This supports the use of Michaelis-Menten kinetics for modelling O_2 consumption. Depending on the product the statistical analysis gave good results for the competitive and the uncompetitive type of inhibition. Based on gas exchange data only, no distinction between the competitive and uncompetitive type of inhibition could be made. It suggests the simultaneous existence of both types of inhibition. Therefore the combined inhibition equation seems most closely related to what is actually occurring in plant tissues. However, for reasons of simplicity the non-competitive type of inhibition is preferred, showing similar results to the combined

inhibition, and giving good results for all the products tested.

Introduction

Low O_2 concentrations are commonly used for the extension of the storage period and shelf life of a number of fruits and vegetables. The decrease in the gas exchange rates under these conditions is considered to be the most important, although not the only factor for the retarded deterioration of the products (Kader et al., 1989). For an effective application of gas conditions in systems like Controlled Atmosphere (CA) storage or Modified Atmosphere (MA) packaging, the relationship between gas concentrations and gas exchange rates must be known. The relationship between O_2 concentrations and O_2 consumption rates can be described using Michaelis-Menten kinetics (Chevillotte, 1973; Banks et al., 1989). Although this description is a simplification, based on one (limiting) enzymatic reaction instead of all the enzymes involved, the relation fits well with experimental data (Banks et al., 1989; Andrich et al., 1991; Lee et al., 1991). The relation between O_2 concentration and O_2 consumption is described as:

$$V_{O2} = \frac{Vm_{O2} * O_2}{Km_{O2} + O_2} \quad (1)$$

where V_{O2} is the O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Vm_{O2} is the maximum O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), O_2 is the O_2 concentration (%) and Km_{O2} is the Michaelis constant for O_2 consumption (% O_2). For some products, both O_2 and CO_2 concentrations have an influence on quality and shelf life. At high CO_2 concentrations, quality loss could be reduced for products such as broccoli (Lebermann et al., 1968), pear (Ke et al., 1990), figs (Colelli et al., 1991) and mushrooms (Peppelenbos et al., 1993). An influence on the gas exchange rate is found for CO_2 as well (Kidd, 1917; Thomas, 1925; Thornton, 1933; Fidler and North, 1967; Shipway and Bramlage, 1973; Kerbel et al., 1990). This is often explained as an influence of CO_2 on the activities of enzymes in the glycolysis (Shipway and Bramlage, 1973, Kerbel et al., 1990; Hess et al., 1993) and the Krebs cycle (Bendall et al., 1960; Frenkel and Patterson, 1973; Shipway and Bramlage, 1973). Bendall et al. (1960) suggested a direct competitive effect of CO_2 on succinic dehydrogenase. Shipway and Bramlage (1973), however, state that the effect of CO_2 is not due to an effect on a single enzyme. They suggest

that the effect of CO_2 on many enzymes can probably be attributed to pH changes. This is confirmed by Hess et al. (1993), who found an indirect effect of CO_2 on ATP- and PPi phosphofructokinase due to changes in pH.

The question arises whether the overall influence of CO_2 can be modelled using the kinetics of a single enzyme, as is done with O_2 influence on O_2 consumption. In general three types of inhibition on the reaction rate of an enzyme are distinguished: competitive, non-competitive and uncompetitive (Chang, 1981). The competitive type of inhibition occurs where both inhibitor (CO_2) and substrate (O_2) compete for the same active site of the enzyme. An increase of O_2 at high CO_2 concentrations would then strongly influence the O_2 consumption rate. The model with competitive inhibition can be described as:

$$V_{O2} = \frac{Vm_{O2} * O_2}{O_2 + Km_{O2} * \left(1 + \frac{CO_2}{Kmc_{CO2}}\right)} \qquad (2)$$

where CO_2 is the CO_2 concentration (%) and Kmc_{CO2} is the Michaelis constant for the competitive CO_2 inhibition of O_2 consumption (% CO_2). The uncompetitive type of inhibition occurs where the inhibitor (CO_2) does not react with the enzyme, but with the enzyme-substrate complex. In this case the increase of O_2 at high CO_2 concentrations has almost no influence on the O_2 consumption rate (when O_2 concentrations are not very low). The model with uncompetitive inhibition can be described as:

$$V_{O2} = \frac{Vm_{O2} * O_2}{Km_{O2} + O_2 * \left(1 + \frac{CO_2}{Kmu_{CO2}}\right)} \qquad (3)$$

where Kmu_{CO2} is the Michaelis constant for the uncompetitive CO_2 inhibition of O_2 consumption (% CO_2). The non-competitive type of inhibition occurs where the inhibitor reacts both with the enzyme and with the enzyme-substrate complex. This leads to O_2 consumption rates at high CO_2 concentrations which lie in between those obtained by the previously described inhibition models. The model with non-competitive inhibition can be described as:

$$V_{O2} = \frac{Vm_{O2} * O_2}{(Km_{O2} + O_2) * \left(1 + \frac{CO_2}{Kmn_{CO2}}\right)} \quad (4)$$

where Kmn_{CO2} is the Michaelis constant for the non-competitive CO_2 inhibition of O_2 consumption (% CO_2). When enzyme reactions are described, only one enzyme is involved. The complete respiratory pathway, however, involves many enzyme reactions. This means that the 'overall' type of inhibition describing gas exchange can be a combination of both competitive and uncompetitive types. The non-competitive type of inhibition describes such a combination, but in such a way that assumes both types to be equally active. Therefore an equation is given with a combination of the competitive and uncompetitive type, where each type differs in its relative activity:

$$V_{O2} = \frac{Vm_{O2} * O_2}{Km_{O2} * \left(1 + \frac{CO_2}{Kmc_{CO2}}\right) + O_2 * \left(1 + \frac{CO_2}{Kmu_{CO2}}\right)} \quad (5)$$

Although Bendall et al. (1960) suggested a competitive type of inhibition by CO_2 in *Ricinus* mitochondria, to our knowledge only the other types of inhibition have been actually used for gas exchange models of whole products, i.e. the uncompetitive (Lee et al., 1991) and the non-competitive type of inhibition (Peppelenbos et al., 1993). To make a discrimination between these types of inhibition based on gas exchange data, datasets are needed on O_2 consumption measured at least at two different CO_2 concentrations and at least three O_2 concentrations. We found six datasets that meet this requirement, published by Fidler and North (1967), Yang and Chinnan (1988), Lee et al. (1991), Talasila et al. (1992), Beaudry (1993) and Peppelenbos et al. (1993). The data of Talasila et al. (1992), Beaudry (1993) and Peppelenbos et al. (1993), however, show no clear influence of CO_2 on O_2 consumption.

The four described inhibition models were tested with gas exchange data from experiments and literature. The objective of this was to obtain a generic model describing both the influence of O_2 and of CO_2 on O_2 consumption. The model should be able to describe gas exchange for different types of products.

Material and methods

Model testing

To test the reliability of the different types of CO_2 inhibition, data on O_2 consumption measured at different O_2 concentrations (including very low O_2 concentrations) and CO_2 concentrations are necessary. These data sets were obtained from literature and from gas exchange measurements. The data were compared with the models using the facilities for non-linear regression in the statistical package Genstat (release 5). The O_2 consumption was fitted using equations 1 to 5. In all cases, the non-linear equations were fitted directly without any transformation, using an iterative method to maximize the likelihood, rather than first linearizing the equations as is often done. Linearizing the equations is equivalent to changing the weight given to the data in the estimation procedure. The two methods will only tend to coincide when the residuals are small (Ross, 1990). Normally, when fitting linear models, an *F*-test is used for comparing them when one model is a subset of the other. This cannot be applied for non-linear models (Draper and Smith, 1981), which is the case here. Still, the variance ratio for comparing two models when one has extra parameters, gives a guideline as to whether a model gives any real improvement.

Literature data

From the literature, three datasets were obtained with an influence of CO_2 on O_2 consumption: data on apple (Fidler and North, 1967; Table 2), tomato (Yang and Chinnan, 1988; Fig. 1, day 9) and broccoli (Lee et al., 1991; Table 3).

Product information

The products selected for the gas exchange measurements were apples (*Malus domestica* Borkh., cv. Golden Delicious and Elstar), broccoli (*Brassica oleracea* L., *var. Italica*), asparagus (*Asparagus officinalis* L., *var. Altilis*), mungbean sprouts (*Phaseolus aureus, syn. mungo*) and, representing a processed vegetable for MA packaging, cut chicory (*Cichorium intybus* L., *var. Foliosum*).

The experiment with apples was carried out twice, once with Golden Delicious and once with Elstar. Golden Delicious was harvested on 27 September 1993 and stored in air for 4 weeks at 1 °C. Elstar was harvested on 31 August 1993 and stored in air for 9 weeks at 1 °C. Also the experiments with broccoli and asparagus were carried

out twice. Broccoli was harvested on 16 November 1992 and on 30 November 1992. It was stored in air for one day at 2 °C. The asparagus was harvested on 14 May 1994 and on 28 May 1994. It was stored in air for two days at 1 °C. The mungbean sprouts were harvested on 31 January 1994 and stored in air for two hours at 18 °C. The chicory was harvested on 3 January 1994 and stored in air for two hours at 18 °C. The chicory leaves were cut into pieces of about 4 cm^2 one hour before the experiment.

Gas exchange measurements

The fresh weight of the products was measured. Then the products were placed in 1.5 l flasks (20 l desiccators for broccoli). The flasks were stored in a temperature controlled room and connected to a flow through system. A temperature and a range of gas conditions were selected (Table 1), simulating storage temperatures for MA packages of the tested products. The temperature was recorded automatically every 15 minutes with a Vaisala temperature probe (HMP 31 UT). The gas coming into a flask was humidified by leading the gas through a 500 ml water flask. The relative humidity in all experiments was close to saturation (97-99%). In the flow-through system, pure N_2, O_2 and CO_2 were mixed using mass flow controllers (Brooks, 5850 TR series). The flow rate used in the experiments was 400 $ml.min^{-1}$. For all experiments a complete factorial design was used with all the combinations of gas concentrations as listed in Table 1.

Table 1. ***Experimentory setup of the gas exchange measurements.***
Temp = average temperature (°C, ± 0.4 °C), O_2 = O_2 concentrations (%), CO_2 = CO_2 concentrations (%), nr = number of products per flask/desiccator, rep = number of replications, weight = average weight of measured products and standard deviation (g), day = days of storage when respiration rates were measured (these measurements were averaged).

Product	Temp	O_2	CO_2	nr	rep	weight	day
Apple (G. Delicious)	19.0	0, 0.5, 1.5, 2.5, 8, 21	0.5, 5	1	2	168.4 ± 13.1	2, 3, 4
Apple (Elstar)	19.6	0, 0.5, 1.5, 2.5, 8, 21	0.5, 5	1	2	147.1 ± 10.1	2, 3, 4
Asparagus	18.6	0, 3, 10, 20	0, 10, 20	3	2	153.2 ± 7.2	2, 3, 4
Broccoli	18.7	1, 3, 5, 21	0, 2, 10	3	2	593 ± 101	2, 3, 4
Mungbean sprouts	17.9	0, 0.5, 2, 6, 21	0, 5		2	30.6 ± 0.5	2, 6, 7
Cut chicory	8.1	0, 0.5, 1.5, 5, 20	0, 5, 20		1	76.2 ± 2.1	2, 3, 6

The gas concentrations selected in the experiments were based on previous experiments (not shown) or literature data. For measuring gas exchange, the air

stream through the flasks was stopped. O_2, CO_2 and N_2 concentrations were measured twice with a Chrompack CP 2001 gas-chromatograph (GC). Gas was led directly from the flasks to the GC. The exact time of the measurement was logged. For every measurement two samples were taken, and only the second sample was used. The time period between first and second measurement was 2 hours at 8 °C and 1 hour at 18 °C. The difference in concentration between the two measurements never exceeded 0.5% O_2 or CO_2 at high O_2 and 0.3% at low O_2 concentrations. The total amount of air taken from the flasks was 7.2 ml, which resulted in a pressure drop of 6 mbar in the flasks (measured with a Druck PDCR 930). Afterwards a correction was made for pressure loss. After the two measurements the flasks (desiccators) were again connected to the flow through system. The O_2 consumption rates, measured on different days, were averaged (Table 1). Data were only used from days showing almost no difference in gas exchange (O_2 consumption and CO_2 production, not shown).

Gas exchange rates were calculated by expressing the concentration differences between two measurements as volume difference (ml) per unit time (hour) and per unit weight (kg fresh weight at the start of the experiment). The free volume of the desiccators and the flasks was calculated by subtracting the estimated product volume from the measured desiccator or flask volume. The product volume was estimated by multiplying the fresh weight with the average density, which was 1.16 $ml.g^{-1}$ for broccoli, 1.05 $ml.g^{-1}$ for asparagus, 1.18 $ml.g^{-1}$ for cut chicory (Verbeek, 1988) and 1.05 $ml.g^{-1}$ for mungbean spruots. For apples the density was calculated for each individual apple using the method of Baumann and Henze (1983).

Results

The models with the different types of inhibition were tested using both literature data (Table 2) and experimental data (Table 3). Models 1 (no inhibition) and 5 (combined inhibition) were compared with the other models using an *F*-test.

A clear effect of CO_2 on O_2 consumption is found for literature data on apples (Fidler and North, 1967) and tomato (Yang and Chinnan, 1988). For both products model 1 showed poorer results then the inhibition models (Table 2). The non-competitive and uncompetitive models gave slightly better (not significant) results then the competitive model (Table 2). For the broccoli data (Lee et al., 1991) the CO_2 influence was very small and probably (considering the *F*-test) not significant.

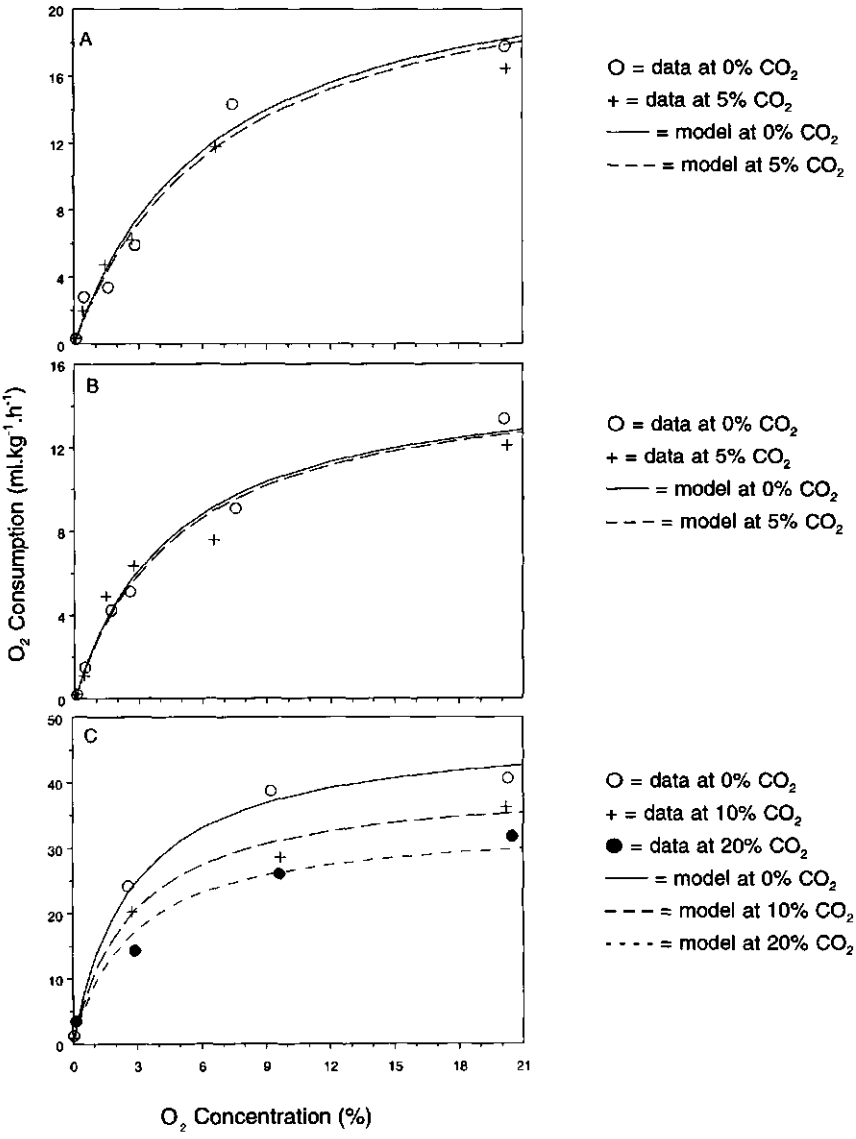

Figure 1. *Average values for O_2 consumption data ($ml.kg^{-1}.h^{-1}$) at several O_2 and CO_2 concentrations, fitted with the non-competitive type of inhibition. A: Apple cv. Golden Delicious, B: Apple cv. Elstar, C: Asparagus.*

The CO_2 concentrations used showed no influence on O_2 consumption rates for the experimental data on apples. None of the four types of inhibition, compared to the model without inhibition, increased the percentage of explained variance (R^2, Table 3). Possibly the CO_2 concentrations used were too low for apples or, alternatively, the period of measurement was too short to show any effect. The gas exchange data of asparagus, broccoli, mungbean sprouts and cut chicory showed a clear influence of CO_2 on O_2 consumption rates. The inhibition models gave better results then the model without inhibition (Table 3). The competitive and the combined type of inhibition showed the best results for broccoli and asparagus. For chicory, however, the competitive type showed the poorest results. For mungbean sprouts, all inhibition types gave equally good results, with high values for R^2. In conclusion, none of the inhibition models used showed the best results for all products measured.

Table 2. ***Regression analysis of data on O_2 consumption obtained from literature.***
*Model 1 = No influence of CO_2, Model 2 = Competitive inhibition, Model 3 = Uncompetitive inhibition, Model 4 = Non-competitive inhibition, Model 5 = Combined inhibition, R^2 = Percentage variance accounted for (indication for the goodness of fit) and $_{adj}$ = adjusted for the number of parameters. v.r. = variance ratio, with 1 = comparison with model 1 and 5 is comparison with model 5 (+ is significantly different, with P = 0.05), est = estimated values, se = standard error, * = could not be estimated, Lee = Estimated values of Lee et al. (1991) with uncompetitive inhibition.*

Product	Model	R^2_{adj}	v.r.		Vm_{O2}		Km_{O2}		Kmc_{CO2}		Kmu_{CO2}		Kmn_{CO2}	
			1	5	est	se	est	se	est	se	est	se	est	se
Apple (Cox's)	1	37.2		+	4.18	1.02	4.24	3.09	-		-		-	
Fidler, North	2	89.6	+	-	4.84	0.45	2.30	0.96	0.82	0.39	-		-	
1967	3	91.2	+	-	5.59	0.55	4.16	1.17	-		3.05	0.81	-	
	4	93.3	+	-	5.32	0.40	3.45	0.81	-		-		4.49	0.96
	5	92.4	+		5.25	0.50	3.28	1.07	3.45	3.28	5.03	2.58	-	
Tomato	1	52.3		-	16.0	8.2	17.4	16.1	-		-		-	
Yang, Chinnan	2	80.7	+	-	18.2	6.5	17.1	11.1	23.0	12.3	-		-	
1988	3	84.2	+	-	22.4	8.9	24.1	15.1	-		15.5	8.7	-	
	4	82.9	+	-	19.1	6.1	18.4	10.3	-		-		41.4	16.6
	5	80.2	-		23.0	12.3	25.1	21.4	*	*	14.0	17.3	-	
Broccoli	1	59.7		-	229	16	1.92	0.63	-		-		-	
Lee et al.	2	61.8	-	-	230	16	1.63	0.75	29.5	50.4	-		-	
1991	3	64.6	-	-	245	23	2.06	0.66	-		87.0	86.0	-	
	4	64.2	-	-	242	20	1.93	0.60	-		-		112	114
	5	60.2	-		245	24	2.06	0.70	*	*	86.8	91.0	-	
	Lee	-			219		1.4		-		115		-	

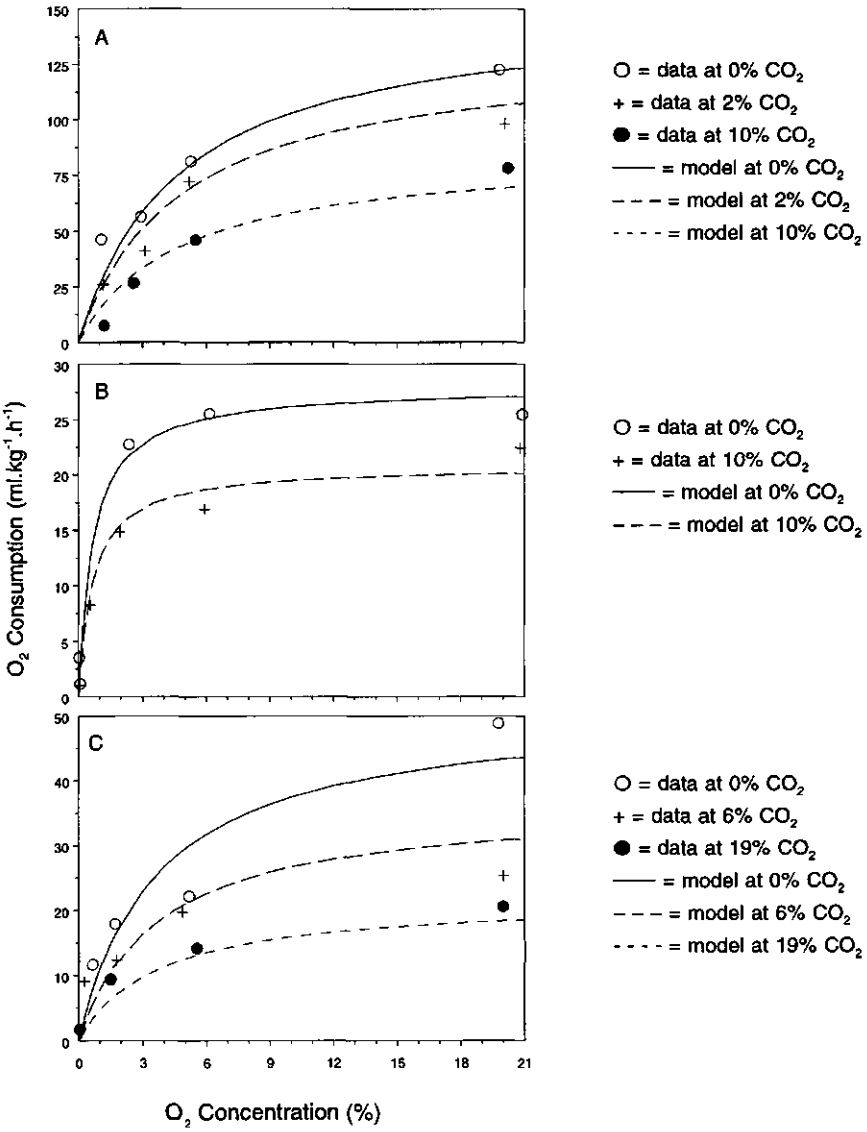

Figure 2. *Average values for O_2 consumption data ($ml.kg^{-1}.h^{-1}$) at several O_2 and CO_2 concentrations, fitted with the non-competitive type of inhibition. A: Broccoli, B: Mungbean sprouts, C: Cut chicory.*

Table 3. ***Regression analysis of data on O_2 consumption from gas exchange measurements.***
*Model 1 = No inhibition incorporated in the model, Model 2 = Competitive inhibition, Model 3 = Uncompetitive inhibition, Model 4 = Non-competitive inhibition, Model 5 = Combined inhibition, R^2 = Percentage variance accounted for and $_{adj}$ = adjusted for the number of parameters. est = estimated values, v.r. = variance ratio, with 1 = comparison with model 1, 5 = comparison with model 5 (+ is significantly different, P = 0.05), se = standard error, * = could not be estimated*

Product	Model	R^2_{adj}	v.r. 1	v.r. 5	Vm_{O2} est	Vm_{O2} se	Km_{O2} est	Km_{O2} se	Kmc_{CO2} est	Kmc_{CO2} se	Kmu_{CO2} est	Kmu_{CO2} se	Kmn_{CO2} est	Kmn_{CO2} se
Apple G.Del.	1	96.0		-	23.0	1.5	6.40	0.98	-		-		-	
	2	96.1	-	-	23.1	1.5	6.17	1.03	47.8	70.0	-		-	
	3	96.4	-	-	24.9	2.0	6.96	1.08	-		33.8	22.7	-	
	4	96.3	-	-	24.1	1.7	6.50	0.96	-		-		64.1	49.8
	5	96.2	-		24.9	2.0	6.96	1.11	*	*	33.8	23.3		
Apple Elstar	1	93.1		-	15.2	1.1	4.57	0.86	-		-		-	
	2	93.1	-	-	15.2	1.1	4.55	0.96	429	*	-		-	
	3	93.4	-	-	16.1	1.4	4.88	0.94	-		42.7	43.0	-	
	4	93.3	-	-	15.7	1.2	4.61	0.85	-		-		91	126
	5	93.1	-		16.1	1.5	4.88	0.96	*	*	42.7	44.0	-	
Asparagus	1	88.1		+	41.2	2.2	2.95	0.60	-		-		-	
	2	96.3	+	-	43.0	1.3	1.22	0.27	5.0	1.3	-		-	
	3	94.8	+	+	50.4	2.3	3.24	0.46	-		37.2	6.1	-	
	4	95.7	+	+	49.3	1.8	2.76	0.34	-		-		45.1	6.1
	5	96.5	+		44.9	1.8	1.57	0.37	8.19	3.17	135	81	-	
Broccoli	1	70.9		+	124	14	4.94	1.47	-		-		-	
	2	94.2	+	-	132	7	2.51	0.47	2.37	0.59	-		-	
	3	87.7	+	+	169	18	6.05	1.28	-		8.03	2.37	-	
	4	92.0	+	+	159	11	4.72	0.72	-		-		11.5	2.3
	5	94.0	+		137	9	2.82	0.63	3.00	1.15	59.9	78.2	-	
Mungbean sprouts	1	85.3		+	25.0	1.7	0.86	0.30	-		-		-	
	2	95.2	+	+	24.9	0.8	0.19	0.08	0.71	0.33	-		-	
	3	93.9	+	+	28.4	1.5	0.81	0.21	-		13.1	3.3	-	
	4	95.0	+	+	28.1	1.2	0.67	0.16	-		-		14.2	3.1
	5	96.5	+		26.1	0.9	0.26	0.10	1.41	0.74	27.5	11.5	-	
Cut chicory	1	49.2		+	34.1	7.4	2.81	1.97	-		-		-	
	2	72.7	+	+	44.8	7.9	2.61	1.56	3.41	2.31	-		-	
	3	85.7	+	-	59.0	9.4	5.20	1.94	-		8.05	2.90	-	
	4	83.4	+	-	52.1	7.4	3.68	1.36	-		-		13.5	4.8
	5	83.9	+		59.0	9.9	5.20	2.05	*	*	8.04	3.07	-	

The maximum O_2 consumption rate (fitted as Vm_{O2}) was highest for broccoli, and lowest for Elstar apples. The Km_{O2} varied widely between the products, ranging from 0.20 (mungbean sprouts) to 6.96 (Golden Delicious). This difference in Km_{O2}, probably

related to the different resistance for diffusion of the products, is reflected in the differently shaped curves (shown in Fig. 1 and 2). Two different apple cultivars were measured and their respiration curves were compared (Fig. 1a and 1b). There seems to be only a minor difference in the maximum O_2 consumption rate (Vm_{O2}) and Km (Km_{O2}) between Golden Delicious and Elstar apples. The different types of inhibition are compared in Fig. 3, where the equations (models) 2, 3 and 4 fitted on broccoli data are shown. The Fig. shows the different response of the inhibition models to elevated CO_2 concentrations. As expected the competitive type shows the strongest inhibition of the three types at low O_2 concentrations, whereas it is the uncompetitive type which shows the strongest inhibition at high O_2 concentrations. The non-competitive type of inhibition shows a response which lies between that of the other two types of inhibition.

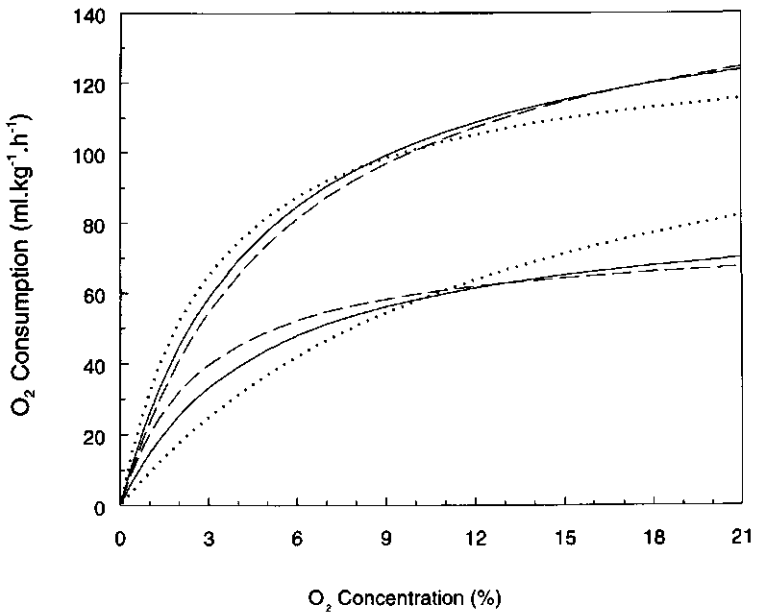

Figure 3. *Comparison of three inhibition models fitted on O_2 consumption of broccoli.: the competitive (.....), non-competitive (———) and uncompetitive (– – –) type of inhibition at 0% CO_2 (upper three lines) and 10% CO_2 (lower three lines).*

Discussion

Enzyme kinetics of the Michaelis-Menten type are employed to describe the influence of gas concentrations on gas exchange. In fact enzyme kinetics describe processes at the level of a cell organelle. In an agricultural product a series of resistances exist to gas diffusion between a cell organelle and the atmosphere around the product. When enzyme kinetics are used for these products, as it is done here, diffusion resistances influence the model results in two ways. Firstly they influences substrate (O_2) availability, thereby affecting the Vm_{O2}, Km_{O2} and Km_{CO2}. Secondly, concentration gradients along the tissue of the products can occur and it can be assumed that this will result in different respiration rates within a product. For the model it is important to establish whether the overall reaction is still of the Michaelis-Menten type. According to Chevillotte (1973) this is not the case, but he states that the single enzyme representation can still be satisfactory. This is confirmed by the percentage of explained variance (R^2 in Table 2) found for all models. The models used give a good description for the O_2 consumption of all the products measured. This supports the use of Michaelis-Menten kinetics for modelling O_2 consumption at product level.

When the CO_2 influence on O_2 consumption is investigated more closely, a difference between literature data and the present measurements becomes clear. Fidler and North (1967) found an influence of CO_2 on O_2 uptake of apples, but the current data do not show this effect. On the other hand, the data of Lee et al. (1991) show no clear influence of CO_2 on broccoli, while the current measurements do show this.

Lee et al. (1991) selected the uncompetitive type of inhibition for modelling the influence of CO_2. The analysis of the current measurements on broccoli shows a higher percentage of explained variance for the competitive and the combined type of inhibition (Table 3). Moreover, the lower (more important) value for Kmc_{CO2} compared to Kmu_{CO2} in the combined inhibition model suggests a major relative importance for the competitive type of inhibition. The difference in the values for Vm_{O2}, Km_{O2} and Kmu_{CO2} given by Lee et al. (1991) and the values obtained from the current statistical analysis (Table 2) is probably caused by the different methods of model fitting used, as the former authors linearized data and models before fitting.

Based on gas exchange data only, no selection between the competitive and uncompetitive type of inhibition can be made (Table 3). It suggests the simultaneous existence of both types of inhibition, at least for broccoli, but probably also for asparagus and mungbean sprouts, where the combined inhibition equation gave the

best results (Table 3). The idea is that combined inhibition (equation 5) is most closely related to what is actually occurring in plant tissues. For reasons of simplicity the non-competitive type of inhibition is preferred, as it gives very similar results to the combined inhibition, and good results for all the products tested.

The duration of the gas exchange experiments on apples was quite short (4 days). This is in contrast to the experiments carried out by Fidler and North (1967) which lasted 50 to 200 days. This difference could be responsible for the fact that no effect of CO_2 on O_2 consumption of Golden Delicious and Elstar apples was found in the current measurements, in contrast to the results of Fidler and North (1967). On the other hand, a clear CO_2 influence was found for broccoli (average values of 4 consecutive days), where Lee et al. (1991) found only a minor influence (measured after 1 day). In addition to the question as to which type of CO_2 inhibition is involved, this raises the question as to when the inhibition begins to take effect. Being a weak acid, dissolved CO_2 influences the pH in the cytoplasm (Brown, 1985). An influence of CO_2 on glycolytic enzymes by changing the pH was found by Hess et al. (1993). During a longer storage period, the influence on pH could be larger. It also means that the influence of CO_2 on the metabolic rate could be of another type than the four previously described, which are all based on a direct influence on enzymatic intermediates.

Acknowledgements

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3

Modelling oxidative and fermentative carbon dioxide production of fruits and vegetables

H.W. Peppelenbos, L.M.M. Tijskens, J. van 't Leven, E.C. Wilkinson
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Abstract

Two models have been developed to describe carbon dioxide (CO_2) production of fruits and vegetables in response to oxygen (O_2) and CO_2 concentrations. The models describe a combination of oxidative and fermentative processes. In both cases the ATP production rate, representing ATP concentration, functions as an inhibitor of fermentative CO_2 production. The difference between the models is that ATP production is calculated by using oxidative processes only or by a combination of oxidative and fermentative processes. The models are compared with two published ones which use the O_2 concentration as an inhibitor of fermentative CO_2 production. The comparison is made using gas exchange data of apples, asparagus, broccoli, mungbean sprouts and cut chicory. All four models allow for increased CO_2 production at low O_2 concentrations. However, high percentages of accountable variance were found only for one published model and the new one which uses oxidative ATP. The performance of the other two models is considerably poorer. The results do not clarify whether increased fermentation rates can be attributed to decreased O_2 levels or energy fluxes. The approach used, however, enables the calculation of CO_2 production rates of different types of commodities stored under various gas conditions. This facilitates a better prediction of CO_2 conditions inside storage rooms and MA packages.

Introduction

In Controlled Atmosphere storage (CA) or Modified Atmosphere packaging (MA), fruits and vegetables are exposed to low O_2 and high CO_2 concentrations. These gas conditions reduce the respiration rate, which is often regarded as the main cause for a slower rate of maturation and change of quality. Several models have been developed to enable the prediction of gas concentrations in MA packages. The relation between O_2 and CO_2 concentrations and O_2 consumption rates can be described using Michaelis-Menten kinetics (Chevillotte, 1973; Peppelenbos and van 't Leven, 1996):

$$V_{O2} = \frac{Vm_{O2} * O_2}{(Km_{O2} + O_2) * (1 + CO_2/Kmn_{CO2})} \quad (1)$$

where O_2 is the oxygen and CO_2 the carbon dioxide concentration (%), V_{O2} the O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Vm_{O2} the maximum O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Km_{O2} the O_2 concentration where the O_2 consumption is 50% of the maximum rate (%), and Kmn_{CO2} the CO_2 concentration (%) where the O_2 consumption is inhibited for 50%. Equation 1 is also often applied to describe CO_2 production (Lee et al., 1991; Song et al., 1992). This approach assumes no CO_2 production at 0% O_2, which is correct if no fermentation takes place or if all of the fermentation which occurs leads to the formation of products like lactate or alanine. In most plant tissues, however, it is primarily ethanol which is formed in fermentation (Perata and Alpi, 1993; Ricard et al., 1994). This leads to additional CO_2 production at low O_2 concentrations. When O_2 concentrations are not too low, this extra CO_2 production can be accommodated for some products by adjusting Vm_{O2} and Km_{O2}. However, equation 1 becomes unsuitable for products with increasing CO_2 production at low O_2 concentrations, such as asparagus and carrots (Platenius, 1943), pear (Boersig et al., 1988), cherry (Cameron, 1989) and blueberry (Beaudry et al., 1992).

To overcome this problem, four models have been published (Banks et al., 1993; Beaudry et al., 1993; Peppelenbos et al., 1993; Andrich et al., 1994) that make distinctions between CO_2 produced by oxidative metabolism and by fermentative metabolism:

$$V_{CO2} = V_{CO2(ox)} + V_{CO2(f)} \quad (2)$$

where V_{CO2} is the total CO_2 production rate ($ml.kg^{-1}.h^{-1}$), $V_{CO2(ox)}$ the oxidative CO_2 production rate ($ml.kg^{-1}.h^{-1}$), and $V_{CO2(f)}$ the 'fermentative' CO_2 production rate ($ml.kg^{-1}$.

h^{-1}). Beaudry et al. (1993) and Andrich et al. (1994) use exponential functions to describe fermentative CO_2 production. Banks et al. (1993) and Peppelenbos et al. (1993), however, base their equations on an extension of equation 1, which is based on enzyme kinetics. These latter authors calculate oxidative CO_2 production by multiplying the O_2 consumption with a specific RQ value, RQ_{ox}, which is the ratio between oxidative CO_2 production and O_2 uptake:

$$V_{CO2(ox)} = RQ_{ox} * V_{O2} \quad (3)$$

RQ_{ox} is assumed to be independent of O_2 concentrations. Banks et al. (1993) as well as Peppelenbos et al. (1993) regard O_2 as an inhibitor of fermentative CO_2 production. The type of inhibition, however, is different. Banks et al. (1993), referred to as model 1, describe total CO_2 production as:

$$V_{CO2} = RQ_{ox} * V_{O2} + \frac{RQ_{ox} * Vm_{O2} * 10^{-10}}{(O_2 + a)^b} \quad (4)$$

where a and b are empirical constants. In a slightly modified version of the model of Peppelenbos et al. (1993), total CO_2 production is calculated as:

$$V_{CO2} = RQ_{ox} * V_{O2} + \frac{Vmf_{CO2}}{1 + O_2 / Kmf_{O2}} \quad (5)$$

where Vmf_{CO2} is the maximum fermentative CO_2 production rate ($ml.kg^{-1}.h^{-1}$) and Kmf_{O2} the Michaelis constant for the inhibition of fermentative CO_2 production by O_2.
Model 1 was not fitted to experimental data, whereas model 2 was fitted to gas exchange data for mushrooms only. Therefore the degree to which either model describes CO_2 production of different products is unknown. The objective of this work was the development of a generic model describing the production of CO_2 under various gas conditions for different types of products.

Material and methods

First, measurements on gas exchange for a range of products are described. Then the development of two new CO_2 production models is explained. The two new models and the two published models were tested using the gas exchange data.

Gas exchange measurements

Gas exchange rates were measured on a wide variety of agricultural products under a range of O_2 and CO_2 concentrations. The products selected for the gas exchange measurements were apples (*Malus domestica* Borkh., cv. 'Golden Delicious' and 'Elstar'), broccoli (*Brassica oleracea* L., *var. Italica*), asparagus (*Asparagus officinalis* L., *var. Altilis*), mungbean sprouts (*Phaseolus aureus, syn. Mungo*) and cut chicory (*Cichorium intybus* L., *var. Foliosum*), representing a processed vegetable for MA packaging. Measurements on O_2 consumption and CO_2 production were performed as described in Peppelenbos and van 't Leven (1996). The conditions for the measurements are shown in Table 1.

Table 1: The range of products and gas atmospheres used.
O_2 = O_2 concentrations (%), CO_2 = CO_2 concentrations (%), rep = number of replications, n = number of data values, weight = average weight of measured products in g. (and standard deviation), day = days of storage when respiration rates were measured (these measurements were averaged).

Product	O_2	CO_2	rep	n	weight	day
Apple (Golden Delicious)	0, 0.5, 1.5, 2.5, 8, 21	0.5, 5	2	24	168.4 ± 13.1	2,3,4
Apple (Elstar)	0, 0.5, 1.5, 2.5, 8, 21	0.5, 5	2	24	147.1 ± 10.1	2,3,4
Asparagus	0, 3, 10, 20	0, 10, 20	2	24	153.2 ± 7.2	2,3,4
Broccoli	1, 3, 5, 21	0, 2, 10	2	24	593 ± 101	1,2,3,4
Mungbean sprouts	0, 0.5, 2, 6, 21	0, 5	2	20	30.6 ± 0.5	3,7,8
Cut chicory	0, 0.5, 1.5, 5, 20	0, 5, 20	1	15	76.2 ± 2.1	2,3,6

Model development

An increase of CO_2 production at low O_2 concentrations can be attributed to increased rates of glycolysis and fermentation. Adenine nucleotides play a key role in the regulation of the metabolic rates. For example, a relation between increased CO_2 production and reduced ATP concentration was found in potato tubers (Amir et al., 1977). Although the ATP concentration has no direct influence on the activity of alcohol dehydrogenase (Davies et al., 1974; Ke et al., 1995), it can influence fermentation rate indirectly. Both phosphofructokinase and pyruvate kinase are inhibited by high levels of ATP (Stryer, 1981). At low ATP levels, these enzymes become more active, and the glycolytic rate is increased. This will result in increased ATP and NADH production. When the low ATP levels were caused by low O_2 concentrations (Tesnière et al., 1994), the NADH produced in glycolysis cannot be oxidized to NAD^+. Fermentation enables the production of NAD^+ from NADH, which ensures the production of

(glycolytic) ATP, and the increased rate of glycolysis (Stryer, 1981). In a model summarizing these processes in one overall reaction, ATP concentration can be considered as the main inhibitor of fermentative CO_2 production. The following equation, based on enzyme kinetics, can be derived if carbohydrates are considered to be non-limiting in the fermentation process:

$$V_{CO2(f)} = \frac{Vmf_{CO2}}{1 + \left(\frac{ATP}{Kmf_{ATP}}\right)} \quad (6)$$

where ATP is the ATP concentration ($mg.kg^{-1}$) and Kmf_{ATP} the Michaelis constant for the inhibition of fermentative CO_2 production by ATP. If only gas exchange data are used for testing the models, no information is available on ATP concentrations. As ATP consumption of plant cells is closely tied to ATP production (Pradet and Raymond, 1983), a direct proportionality between ATP production and ATP concentration was assumed. This allows the use of ATP production to represent ATP concentration, and ATP production can be estimated using gas exchange rates. The use of the ATP production rate in equation 5 was tested in two different models. One model was based on the main production route for ATP, oxidative phosphorylation, which can be directly derived from the O_2 consumption rate:

$$V_{ATP(ox)} = V_{O2} * 6 * 41.87 \quad (7)$$

where $V_{ATP(ox)}$ is the oxidative ATP production rate ($\mu mol.kg^{-1}.h^{-1}$), 6 is the assumed ATP/O_2 ratio, and 41.87 is a conversion factor ($\mu mol.ml^{-1}$) based on the ideal gas law (at 18°C and 101.3 kPa). Combining equation 3, 6 and 7 gives the following equation for total CO_2 production, referred to as model 3:

$$V_{CO2} = V_{O2} * RQ_{ox} + \frac{Vmf_{CO2}}{1 + V_{ATP(ox)} / Kmf_{ATP}} \quad (8)$$

Oxidative ATP production is only part of total ATP production, especially at low O_2 concentrations when fermentation is increased. Therefore both oxidative and fermentative ATP production were used to represent ATP concentration in a second model. It was assumed that the main fermentation route leads to the production of ethanol and CO_2 (Perata and Alpi, 1993; Ricard et al., 1994) allowing the calculation of fermentative ATP production from fermentative CO_2 production:

$$V_{ATP(total)} = V_{O2} * 6 * 41.87 + V_{CO2(f)} * 41.87 \quad (9)$$

where $V_{ATP(total)}$ is the total ATP production rate ($\mu mol.kg^{-1}.h^{-1}$). By combining equations 3, 6 and 9 the following equation (referred to as model 4) for total CO_2 production can be derived:

$$V_{CO2} = V_{O2} * RQ_{ox} + \frac{Vmf_{CO2}}{1 + V_{ATP(total)} / Kmf_{ATP}} \quad (10)$$

Model testing

The models were fitted directly to CO_2 production data using O_2 uptake (Peppelenbos and van 't Leven, 1996) by non-linear regression in the statistical package GENSTAT (Rothamstead, U.K.). The fitting of model 4 was complicated by the fact that fermentative ATP production is a function of fermentative CO_2 production (equation 9), while at the same time fermentative CO_2 production is a function of ATP (equation 6). The problem was solved by using initial values for fermentative CO_2 production, and then iteratively optimizing both calculations. The initial values were obtained by subtracting the product of the O_2 consumption values (Peppelenbos and van 't Leven, 1996) and an estimated RQ_{ox} from the measured (total) CO_2 production. RQ_{ox} was estimated by dividing the measured CO_2 production by the measured O_2 uptake in ambient air, where fermentation is expected to be very low. In all cases the non-linear equations were fitted directly without any transformation of the equations or the data, using an iterative method to maximize the likelihood. Models were compared using R^2 values for the percentage variance accounted for, and the standard errors found for the parameter estimations. As additional criteria on the applicability of the various models, RQ values were derived by combining fitted CO_2 production from each of the four mentioned models with the fitted O_2 consumption given by equation 1 for the dataset of apple cv. Golden Delicious (after Peppelenbos and van 't Leven, 1996). These four sets of estimated RQ's were compared with RQ values calculated directly from the measurements.

Results

CO_2 production data

The behaviour of the commodities that were investigated allow them to be divided into two groups. In one group consisting of the two apple cultivars and asparagus (Figs. 1a, 1b and 1c), the CO_2 production increased at low O_2 concentrations. In the other group, consisting of broccoli, mungbean sprouts and cut chicory (Figs. 2a, 2b and 2c), CO_2 production was lowest at the lowest O_2 concentration applied, but did not appear to cease at O_2 concentrations close to 0%. When the measurements on asparagus and cut chicory were examined more closely, a reduction of CO_2 production at O_2 concentrations close to 0% was found when CO_2 concentrations were higher (Figs. 1c and 2b). Because almost all of the CO_2 produced at such O_2 concentrations is the result of fermentation, this suggests an influence of CO_2 on fermentation rates.

CO_2 production models

Models 2 (Peppelenbos et al., 1993) and 3 (oxidative ATP) consistently accounted for more than 75% of the variance (R^2, Tables 2 and 3). Model 2 almost always resulted in the highest values for R^2, although the results of model 3 were only slightly lower (Tables 2 and 3). It is not possible to say whether model 2 gives a statistically significant better fit than model 3. The fitting of model 2 on broccoli data resulted in a high R^2, but also led to an unreliable value for the RQ_{ox} (0.68, Table 2) and a high standard error for Kmf_{O2}. For all products model 1 (Banks et al., 1993) resulted in low R^2 values, and for 'Elstar' apples it was inapplicable. Also the standard error of parameter b could not be established for any of the commodities because the estimates for the parameters a and b are highly correlated with each other (Table 2). When total ATP production (model 4) was used instead of oxidative ATP production (model 3) to represent ATP concentration, the results were considerably worse (Table 3). The results of the two models were only comparable for products which showed almost no increase in CO_2 production at low O_2 concentrations (e.g. broccoli, mungbean sprouts and cut chicory). An inhibition of oxidative CO_2 production by increased CO_2 concentrations was found for asparagus, broccoli, mungbean sprouts and cut chicory (Figs. 1c, 2a, 2b and 2c). This influence was especially clear at high O_2 concentrations, where fermentation was very low or negligible. In all the models this influence of CO_2 was incorporated in the term V_{O2} (eq. 1).

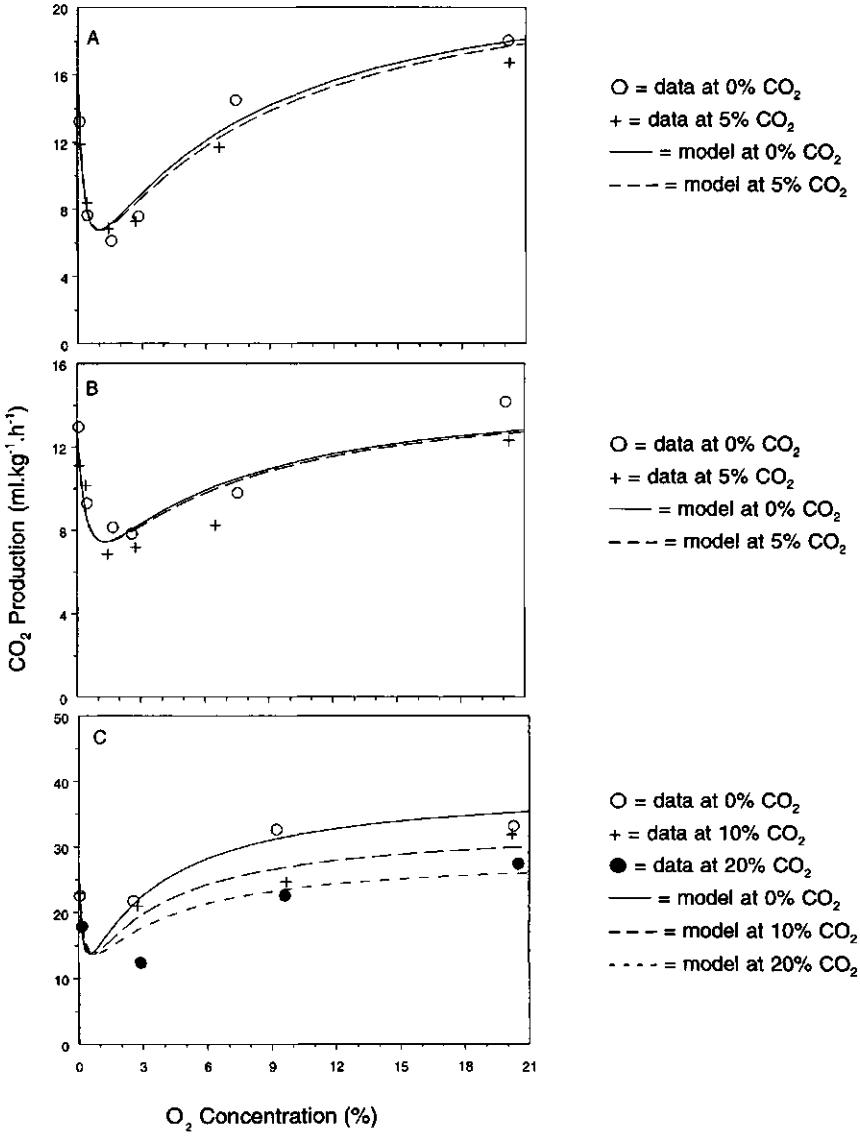

Figure 1. *Average values for CO_2 production data ($ml.kg^{-1}.h^{-1}$) combined with a model fit. The fit is a combination of model 3 and equation 1 using the same gas exchange data (after Peppelenbos and van 't Leven, 1996). A: Apple cv. Golden Delicious, B: Apple cv. Elstar, C: Asparagus.*

For two products, asparagus and mungbean sprouts, CO_2 production at an O_2 concentration near 0% was also reduced by higher CO_2 concentrations (Figs. 1c and 2b). An influence of CO_2 on fermentative CO_2 production was not incorporated in any of the models. Therefore fitting the models to the data of asparagus and mungbean sprouts resulted in erroneous results. For instance model 3 predicts a small increase of CO_2 production at low O_2 concentrations for mungbean sprouts, whereas the data do not show this (Fig. 2b).

Modelled fermentation

Using data for apples cv. Golden Delicious the predictions of the four models at low O_2 concentrations were more closely examined. When the calculated total CO_2 production was compared to the data (Fig. 3a), two of the models seemed to be slightly incorrect. Model 1 gave a very low CO_2 production rate at 0.2% O_2, where the measurements showed much higher rates. In contrast, the total CO_2 production predicted by model 4 never became as low as the measurements, and neither did it rise as the measured levels for fruit kept at

Table 2. Results of the regression analysis for CO_2 production.
*Model 1 = model Banks et al. (1993), a and b = empirical constants, Model 2 = model Peppelenbos et al. (1993), Vmf_{CO2} = the maximum fermentative CO_2 production rate ($ml.kg^{-1}.h^{-1}$), Kmf_{O2} = Michaelis constant (expressed in % O_2), $R^2_{adj} = R^2$: Percentage variance accounted for (indication of the goodness of fit), adj: adjusted for the number of parameters, est = estimated values, se = standard error, # = could not be established, * = standard error not available due to singularity.*

Product	Model	R^2_{adj}	RQ_{ox}		a		b	
			est	se	est	se	est	se
Apple (Golden Delicious)	1	20.6	1.07	0.02	0.283	0.003	19.8	*
Apple (Elstar)	1	#	1.16	0.01	0.270	0.003	19.6	*
Asparagus	1	34.2	0.87	0.02	0.324	0.002	19.8	*
Broccoli	1	71.2	0.93	0.04	0.007	*	5.0	*
Mungbean sprouts	1	68.5	0.98	0.04	0.334	0.002	19.8	*
Cut chicory	1	66.7	1.01	0.02	0.002	0.003	5.63	*
					Vmf_{CO2}		Kmf_{O2}	
Apple (Golden Delicious)	2	91.4	0.99	0.03	16.6	1.71	0.233	0.062
Apple (Elstar)	2	82.1	0.98	0.04	14.7	0.97	0.492	0.095
Asparagus	2	92.2	0.84	0.01	26.1	1.57	0.190	0.050
Broccoli	2	92.2	0.68	0.07	20.7	2.62	88	150
Mungbean sprouts	2	89.9	0.92	0.03	12.2	1.04	0.367	0.153
Cut chicory	2	98.9	0.99	0.02	9.82	1.44	0.310	0.134

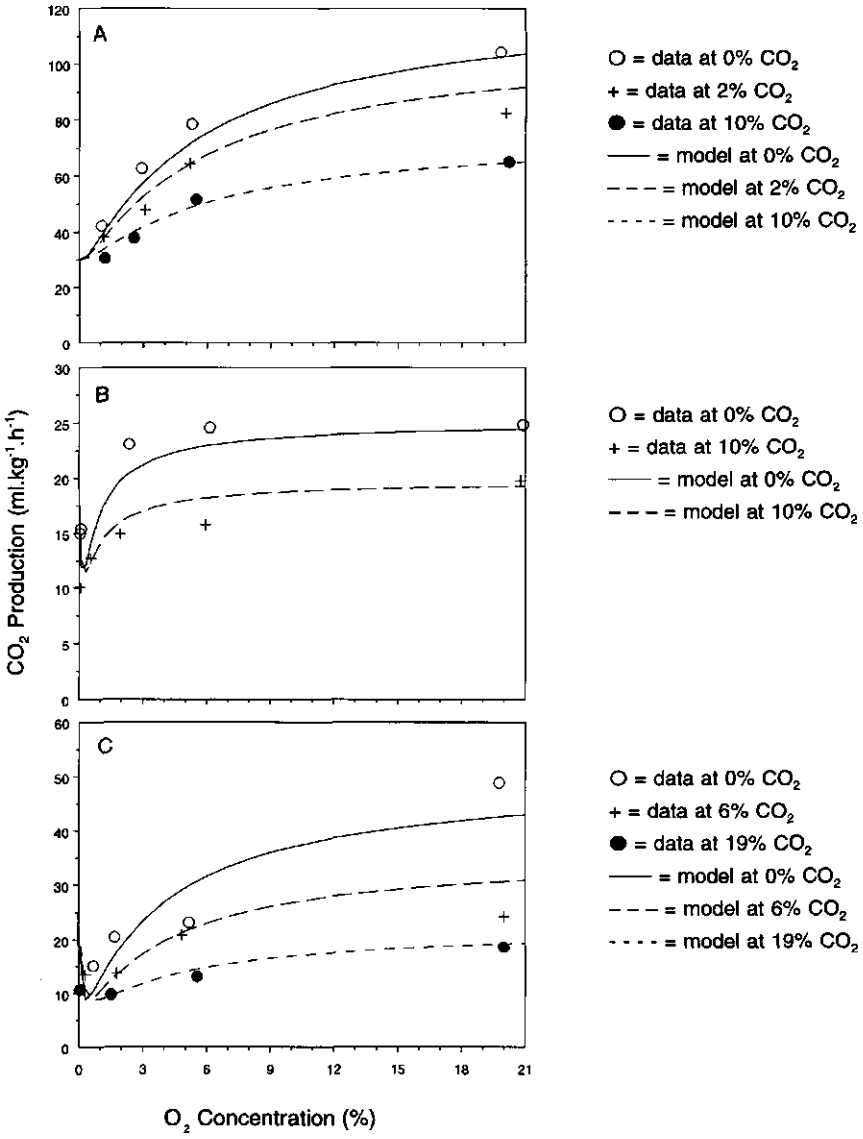

Figure 2. *Average values for CO_2 production data ($ml.kg^{-1}.h^{-1}$) combined with a model fit. The fit is a combination of model 3 and equation 1 using the same gas exchange data (after Peppelenbos and van 't Leven, 1996). A: Broccoli, B: Mungbean sprouts, C: Cut chicory.*

Table 3. *Results of the regression analysis for CO_2 production.*
Model 3 = inhibition of fermentative CO_2 production by oxidative ATP production, model 4 = model 3 + fermentative ATP production, Vmf_{CO2} = the maximum fermentative CO_2 production rate ($ml.kg^{-1}.h^{-1}$), Kmf_{ATP} = Michaelis constant (expressed in ATP production rate, $mmoles.kg^{-1}.h^{-1}$), $R^2_{adj} = R^2$ = Percentage variance accounted for (indication of the goodness of fit), $_{adj}$ = adjusted for the number of parameters, est = estimated values, se = standard error.

Product	Model	R^2_{adj}	RQ_{ox}		Vmf_{CO2}		Kmf_{ATP}	
			est	se	est	se	est	se
Apple (Golden Delicious)	3	90.2	0.95	0.03	17.0	1.8	0.22	0.06
Apple (Elstar)	3	78.3	0.88	0.05	13.5	0.8	0.42	0.09
Asparagus	3	87.2	0.79	0.02	28.4	2.1	0.70	0.19
Broccoli	3	93.1	0.78	0.04	30.3	7.3	8.90	6.40
Mungbean sprouts	3	83.4	0.83	0.05	18.9	5.5	0.96	0.63
Cut chicory	3	97.2	0.97	0.03	23.7	19.3	0.27	0.30
Apple (Golden Delicious)	4	56.0	0.82	0.07	15.3		0.66	0.14
Apple (Elstar)	4	29.1	0.65	0.09	14.1		1.03	0.20
Asparagus	4	68.0	0.68	0.03	27.3		2.10	0.30
Broccoli	4	92.9	0.76	0.04	26.8		14.8	6.31
Mungbean sprouts	4	77.3	0.73	0.07	13.0		3.62	1.48
Cut chicory	4	96.0	0.95	0.04	9.09		1.39	0.62

0% O_2. Fermentative CO_2 production at ambient air, as calculated by models 2, 3 and 4, was considerably higher than calculated by model 1 (Fig. 3b). Model 4 in particular gave a high estimated fermentation rate. When model 1 was applied, fermentative CO_2 production became negligible at an O_2 concentration greater than 0.2% (Fig. 3b). While models 2, 3 and 4 resulted in a maximum fermentative CO_2 production at 0% O_2, a maximum was never reached by model 1. When the RQ values calculated from the measurements were compared with the RQ's derived from the models, a good visual relation was found for models 2, 3 and 4 (Fig. 3c). The figure also shows that the models describe a gradual increase of the RQ when O_2 concentrations are decreased, suggesting a gradual increase of fermentation rates.

Discussion

The impossibility of using O_2 consumption models (such as equation 1) for calculating CO_2 production at low O_2 concentrations is underlined by the current results. For all the products considered in this article CO_2 production did not approach zero when O_2 concentrations were close to 0%. The four tested models overcome this problem, but to different degrees. The models can be compared on statistical grounds using R^2

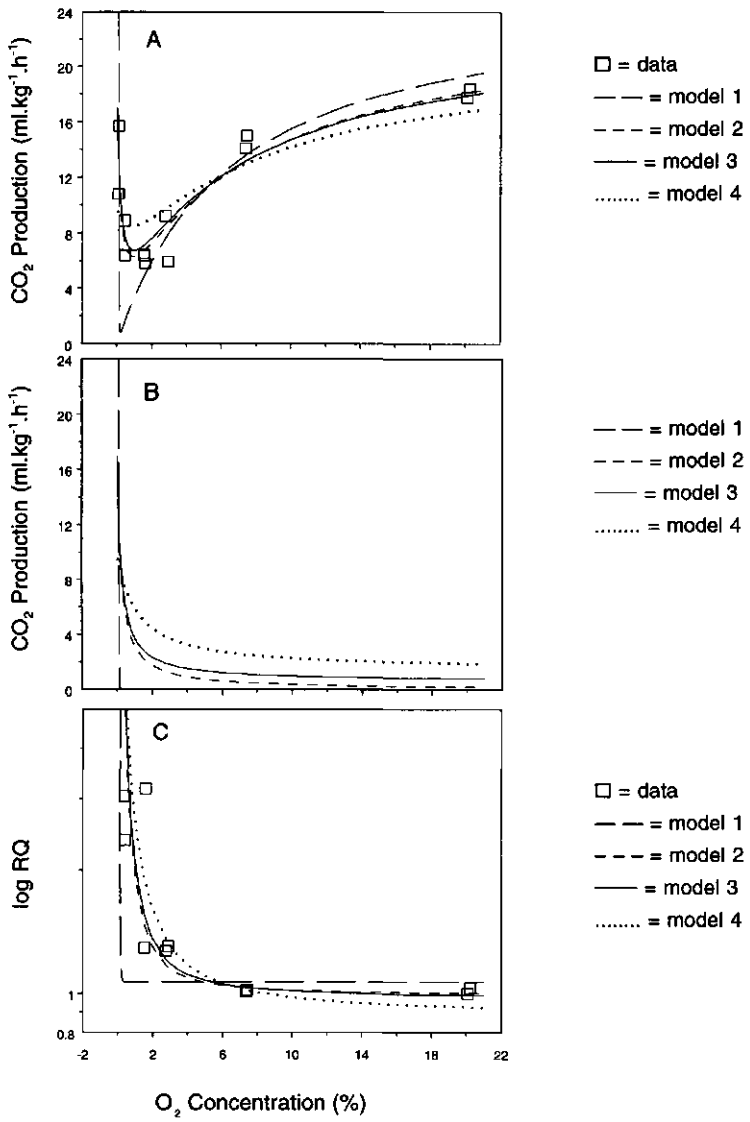

Figure 3. *Comparison of the four models tested on data of apple cv. Golden Delicious. A: Total CO_2 production data and models, B: Modelled fermentative CO_2 production, C: RQ values derived from data and gas exchange models.*

values and standard errors (of parameter estimates), but their physiological relevance also needs to be taken into account. When considering statistical aspects, models 2 and 3 result in higher correlations than models 1 and 4. The parameter standard errors for model 2 are on the whole lower than those of model 3, except for broccoli. This suggests a slight preference for model 2, when statistical aspects are considered. For a comparison of the physiological relevance of the models, the parameters used are more closely examined.

RQ_{ox} is used to describe the ratio between oxidative CO_2 production and O_2 consumption. An RQ based on actual measurements also includes fermentative CO_2 production. In case of fermentation being active at high O_2 concentrations, the RQ values derived from such measurements will tend to overestimate oxidative CO_2 production. On the other hand, the low RQ_{ox} values found for model 4 (Table 3) seem an underestimation of oxidative processes, and most likely result from the difficulty of simulating increased CO_2 production at low O_2 concentrations using this model. It was solved by the statistical package by estimating a high fermentative CO_2 production at 21% O_2 (Fig. 3b).

Model 1 results in unrealistically high values for the maximum fermentative CO_2 production rate, making it inappropriate for use at O_2 concentrations close to anoxia. The Vmf_{CO2} values of models 2, 3 and 4 (Tables 2 and 3) are close to the data found on CO_2 production at low O_2 concentrations (Figs. 1a to 2c). Therefore it seems that Vmf_{CO2} can be used as a measure for the maximum fermentation rate when mainly ethanol is formed, which is the case for most plant tissues (Perata and Alpi, 1993; Ricard et al., 1994). The influence of CO_2 on fermentative CO_2 production, found for mungbean sprouts and asparagus, was not incorporated in any of the models. Given that a similar influence was found by Patterson and Nichols (1988) on apples, the models should be extended.

Models 3 and 4 relate energy production to fermentation rates. The parameter used is Kmf_{ATP}, the ATP production rate which inhibits fermentative CO_2 production to 50% of its maximum value. Although there is an indirect relation between ATP levels and fermentation rates (see Material and methods), it is unclear whether ATP fluxes are a correct representation of ATP levels. In models 3 and 4 ATP production drops to 0 (model 3) or becomes very low (model 4). Normally in anaerobic situations, ATP concentrations only temporarily drop to values close to 0 (Raymond and Pradet, 1980; Trippi et al., 1989; Tesnière et al., 1994). Using this information, a strong increase of

fermentation (and fermentative CO_2 production) is not allowed for with the current models. It seems that the high fermentation rates at low O_2 predicted by model 3 and 4 cannot be explained merely by a shift from oxidative to fermentative pathways. An increase of the maximum fermentative rate would then be necessary. Indeed it is found that at low O_2 concentrations the synthesis of alcohol dehydrogenase can increase (Andrews et al., 1993; Sun Chen and Chase, 1993, Bucher et al., 1994), which implies that Vmf_{CO2} can also increase. Interestingly Bucher et al. (1994) suggest that ADH transcription is triggered directly through an oxygen-sensing system. This implies an influence of the O_2 concentration on Vmf_{CO2}, which is described in models 1 and 2. Parameter Kmf_{O2}, used in model 2, describes the O_2 concentration where fermentative CO_2 production is 50% of its maximum rate. It is unclear, however, whether enzyme kinetics are relevant in describing the oxygen-sensing system. The parameters used in model 1, a and b, are strictly empirical and do not directly describe an aspect of the fermentation process.

Models 2, 3 and 4 describe a gradual increase in fermentation rates when O_2 concentrations are lowered and even suggest a considerable fermentation rate in ambient air (Fig. 3b). Indeed, for several products (apple, pear, strawberries, fig, avocado), ethanol and lactic acid production has been found in ambient air (Fidler, 1933; Boersig et al., 1988; Li and Kader, 1989; Colelli et al., 1991; Nanos et al., 1992). It seems that fermentation is active under all O_2 concentrations, although ethanol is not necessarily to be found due to remetabolization (Bucher et al., 1994). Another explanation of fermentation at relatively high O_2 concentrations is that the models involved the gas concentrations outside the products, whereas the actual concentrations inside the product can be considerably lower. This can explain the poor performance of model 1, which was originally meant to incorporate internal O_2 concentrations. The value of 10^{-10}, used in model 1 (Banks et al., 1993), would probably increase when external O_2 concentrations were used.

In conclusion, statistical results indicate a much better performance of models 2 and 3. Considering the physiological relevance of Kmf_{O2} and Kmf_{ATP}, no preference between models 2 and 3 can be made. It seems that both decreased O_2 levels and energy fluxes attribute to increased fermentation rates. The approach used for both models enables the calculation of CO_2 production rates of different types of commodities stored under various gas conditions. This facilitates a better prediction of CO_2 conditions

inside storage rooms and MA packages.

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4

The influence of carbon dioxide on gas exchange rates of mungbean sprouts at aerobic and anaerobic conditions

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(Submitted)

Abstract

To predict gas conditions inside MA packages of fresh plant products, gas exchange models are often used. Current models describing CO_2 production of harvested plant products do not account for an inhibition of high CO_2 concentrations on fermentation rates, whereas such an inhibition on plant metabolism is well documented. One existing model, based on inhibition of the alcoholic fermentation rate by O_2, was therefore modified to include this inhibition by CO_2. Gas exchange data of mungbean sprouts were collected under various O_2 and CO_2 concentrations and used to validate the model. With the modification applied, CO_2 production rates were described better. Although CO_2 production at low O_2 concentrations was reduced by high CO_2 concentrations, no influence was found on ethanol and acetaldehyde levels. The data found indicate large differences between gas exchange rates of different batches of mungbean sprouts. It is suggested that microbial metabolism attributes substantially to total CO_2 production rates found, and might explain these differences.

Introduction

Highly perishable plant products, such as mungbean sprouts, must be kept under specific conditions to increase the time period in which the quality remains acceptable. The use of MA packaging offers the possibility of reducing the O_2 concentration and increasing the CO_2 concentration around the product. Altered gas conditions are found to increase the shelf-life of mungbean sprouts (Varoquaux et al., 1996), who found optimal conditions of about 5% for O_2 and 15% for CO_2. Increased concentrations of carbon dioxide can influence metabolic rates in harvested plant parts. In aerobic conditions high carbon dioxide concentrations are found to decrease respiration rates of apples, broccoli, asparagus and mungbean sprouts (Fidler and North, 1967; Lee et al., 1991; Peppelenbos and van 't Leven, 1996). Under these conditions, also an increased fermentation rate has sometimes been found, for products such as strawberry, pear, lettuce and blueberry (Li and Kader, 1989; Ke et al., 1990; Mateos et al., 1993; Beaudry, 1993). Under anoxic conditions, high CO_2 concentrations have been found to decrease fermentation rates of apples (Thomas, 1925; Patterson and Nichols, 1988) but increase fermentation rates of sweet potato roots and pears (Chang et al., 1983; Ke et al., 1993). No single explanation is known that covers all these findings, although the influence of CO_2 on pH may induce different enzyme responses dependent on the plant tissue affected (Shipway and Bramlage, 1973). Davies et al. (1974) found a shift from lactic acid production to ethanol production when pH was decreasing. This latter finding indicates that CO_2 may affect the fermentative route, but leaves unanswered whether it also inhibits the total fermentative rate as well.

Because the main fermentation route in higher plants is assumed to be leading to ethanol (Pfister-Sieber and Brändle, 1994; Ricard et al., 1994), the lower CO_2 production at anoxic conditions can be explained as an influence of CO_2 on fermentation rates. For an effective application of altered gas conditions in systems like Controlled Atmosphere (CA) storage or Modified Atmosphere (MA) packaging, the relationship between gas concentrations and gas exchange rates of the produce must be known. Mungbean sprouts are known to show a decrease in the CO_2 production in response to elevated CO_2 concentrations both in ambient air and under anoxic conditions (Peppelenbos and van 't Leven, 1996; Peppelenbos et al., 1996). At the moment it is not possible to calculate gas exchange rates of mungbean sprouts in MA packages (Varoquaux et al., 1996). The purpose of this work was to develop a gas exchange model which incorporates the influence of CO_2 on both oxidative as well as

fermentative pathways.

Material and methods

Gas exchange rates

Two batches of mungbean sprouts (*Vigna radiata* (L.) Wilczek) were obtained freshly harvested with a 12 days interval from a local grower and were subsequently stored in air for three hours at 2 °C before the experiments started. Storage during the experiments was at 8 °C. In addition all the possible combinations of 0, 0.5, 2, 6 and 21 % O_2 with 0 and 10% CO_2 were applied using a flow through system. Measurements on gas exchange were carried out on the first batch, while fermentative metabolites were measured in material from both batches. For gas exchange measurements about 100 g of mungbean sprouts were placed in 1.5 l flasks. Two flasks per gas condition were used. Gas exchange rates, as described by Peppelenbos and van 't Leven (1996), were measured after 2, 3, 6 and 9 days of storage.

Fermentative metabolites

Plastic trays with 50 g of mungbean sprouts each were placed inside 65 l metal containers. The containers were connected to a flow through system, using the same gas conditions as for the flasks with a humidified flow of 400 ml per hour. After 7 and 10 days of storage, two trays were used for the measurement of fermentative metabolites. To this end 15 g of mungbean was grinded (Bühler type HO4) in 10 ml of demiwater at 35000 rpm for 1 to 2 minutes, until the mixture was homogenous. The mixture was poured into a closed cuvette, and boiled for 6 minutes to inactivate enzyme activity. After boiling, the samples were placed on ice to cool down. Then they were centrifuged for 30 min at 20000 rpm at 4°C. The supernatant was filtered using Syrfil MF 0.22 µm filters. One ml of sample was mixed together with 1 ml a solution containing a known amount of phenoxyacetic acid (internal standard). Lactic acid, acetate, acetaldehyde and ethanol were measured by HPLC (Shodex Ionpack KC-811) as described by Gosselink *et al.* (1995). Lactic acid, acetaldehyde and acetate were assessed with UV and RI detectors. Ethanol was measured using a RI detector only. Recovery tests for the metabolites mentioned, carried out before actual measurements took place, showed a recovery above 98%.

Modelling gas exchange

The experimental data on gas exchange rates were compared with an O_2 consumption model and a CO_2 production model. For O_2 consumption, and the CO_2 influence on O_2 consumption, a model using a noncompetitive type of inhibition by CO_2 (Peppelenbos and van 't Leven, 1996) was used:

$$V_{O2} = \frac{Vm_{O2} * O_2}{(O_2 + Km_{O2}) * \left(1 + \frac{CO_2}{Kmn_{CO2}}\right)} \quad (1)$$

where V_{O2} is the actual O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Vm_{O2} is the maximum O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Km_{O2} is the Michaelis constant for the inhibition of O_2 consumption by O_2 (%O_2), and Kmn_{CO2} is the Michaelis constant for the noncompetitive inhibition of O_2 consumption by CO_2 (%CO_2). Peppelenbos et al. (1996) gave two models that adequately describe CO_2 production. The one using O_2 as an inhibitor of fermentative CO_2 production is used here. It includes CO_2 produced by oxidative and fermentative pathways:

$$V_{CO2} = V_{O2} * RQ_{ox} + \frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}}\right)} \quad (2)$$

where V_{CO2} is the CO_2 production rate ($ml.kg^{-1}.h^{-1}$), RQ_{ox} is the RQ of oxidative processes, Vmf_{CO2} is the maximum fermentative CO_2 production rate ($ml.kg^{-1}.h^{-1}$) and Kmf_{O2} is the Michaelis constant for the inhibition of fermentative CO_2 production by O_2. The influence of CO_2 on CO_2 production is only accounted for oxidative pathways by the incorporation of actual O_2 uptake in both models. An influence on fermentative pathways, however, was not accounted for, and is introduced in equation 2 to give:

$$V_{CO2} = V_{O2} * RQ_{ox} + \frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}}\right) + \left(\frac{CO_2}{Kmf_{CO2}}\right)} \quad (3)$$

where Kmf_{CO2} is the Michaelis constant for the inhibition of fermentative CO_2 production by CO_2.

Statistical analysis

The O_2 uptake and CO_2 production rates measured were compared with the models describing gas exchange (equations 1, 2 and 3), using the facilities for non-linear regression in the statistical package Genstat. The non-linear equations were fitted directly without any transformation, using an iterative method to maximize the likelihood. RQ_{ox}, the RQ of the oxidative pathways, was not estimated by the statistical package but fixed at 0.872, being the average RQ value found at O_2 concentrations exceeding 20% (where fermentation is thought to be negligible). The concentrations of fermentative metabolites were analyzed for significant differences by analysis of variance (ANOVA) with Genstat as well.

Results and discussion

O_2 and respiration

O_2 uptake rates of mungbean sprouts, assessed during a 9 days storage period at 8°C, were reduced only at very low O_2 concentrations, resulting in low Km_{O2} values, which were found to be ranging between 0.46 and 1.11 % of O_2 (Table 1). After prolonged storage (longer than 3 days) O_2 uptake showed a dramatic increase, especially at higher O_2 concentrations. Such an increase is comparable to results of Varoquaux et al. (1996) who, however, also found a decrease in O_2 uptake after 5 days of storage (Fig. 1). When also data used in Peppelenbos et al. (1996, not shown previously) are compared, it is obvious that the storage period after which a strong increase in O_2 uptake is found can vary widely. Although these results were found at 21% O_2, which is higher than used in MA-packages, this variability between batches needs to be taken into account for a good application of MA.

Table 1. ***Results of the regression analysis for O_2 consumption (equation 1).***
est = estimated value, se = standard error, R^2 = Percentage variance accounted for (indication for the goodness of fit) and $_{adj}$ = adjusted for the number of parameters.

	day 2		day 3		day 6		day 9	
	est	se	est	se	est	se	est	se
R^2_{adj}	76.2		86.9		92.3		97.6	
Vm_{O2}	15.9	1.5	18.1	1.2	59.9	4.2	159.8	8.6
Km_{O2}	0.77	0.30	0.46	0.13	1.11	0.25	0.96	0.12
Kmn_{CO2}	17.6	6.7	17.3	4.5	3.49	0.82	2.44	0.40

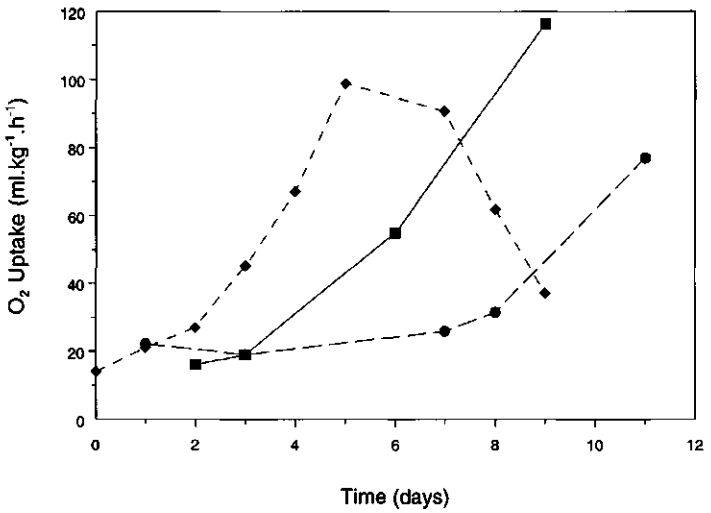

Figure 1. *Comparison of change in O_2 uptake of mungbean sprouts stored at 8°C and in ambient air. Data from Varoquaux et al., 1996 (♦), Peppelenbos et al., 1996 (●) and current measurements (■).*

Because tissue damage was found from day 6 onwards at 6 and 21 % O_2 (data not shown), oxidative reactions other than respiration likely contributed to the increased O_2 uptake rates. Mungbean sprouts are known for their high microbial counts at harvest, and therefore also microbial metabolism might have contributed to some extent to the increased gas exchange rates observed (after Varoquaux et al., 1996). A difference in initial microbial counts could than explain the difference between the batches as shown in Fig 1. This parameter, however, was not measured in the current experiment.

CO_2 and respiration

The highest CO_2 concentration used (10%) inhibited respiration at high O_2 concentrations (Fig. 2). When the O_2 uptake model (eq 1) was used to quantify the inhibition term Kmn_{CO2}, values between 2.4 and 17.6% CO_2 were found (Table 1), which is in the range of the average Kmn_{CO2} (14.2) as found by Peppelenbos and van 't Leven (1996). Interestingly the inhibition by CO_2 increased in importance after a

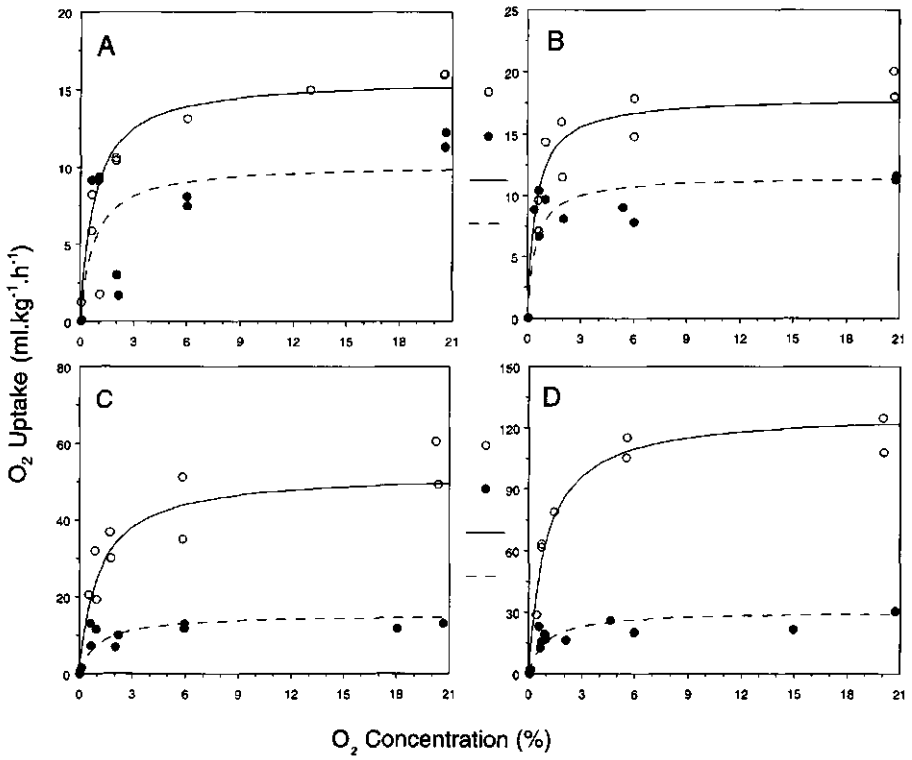

Figure 2. *Measured O_2 uptake (○ = at 0% CO_2, ● = at 10% CO_2) and modelled O_2 uptake (——— = at 0% CO_2, – – – = at 10% CO_2) of mungbean sprouts at various O_2. A: after 2 days of storage, B: after 3 days of storage, C: after 6 days of storage, D: after 9 days of storage.*

prolonged storage period (Table 1). This might be explained by an accumulating effect of high CO_2 concentrations, as this gas easily dissolves in plant tissues. On the other hand, it is also known that increased CO_2 concentrations can reduce microbial growth and metabolism (Dixon and Bell, 1989; Bennik et al., 1995), also on mungbean sprouts (Varoquaux et al., 1996). If microbial metabolism is consistently contributing to the total gas exchange rates found, and with microbial metabolism increasing strongly under low CO_2 but not under high CO_2, this could explain the decreased Kmn_{CO2} values.

CO_2 production models

At low O_2 concentrations CO_2 production was also decreased at the highest CO_2 concentration (Fig. 3), which confirms earlier data of Peppelenbos et al. (1996). Fermentative CO_2 production under 0% O_2 remained equal during storage (Fig. 3a to 3d). Because for most plant tissues ethanolic fermentation decreases during the first hours or days after transfer from air to anoxia (Ricard et al., 1994), it could mean that for mungbean sprouts this change already occurred before the first measurement (day 2). After two and three days of storage, the unmodified CO_2 production model (equation 2) accounts for low amounts of the found variation, with R^2 of 41.2 and 47.0 (Table 2). The modification of the model (equation 3) increased these numbers. Thus the modification improves the prediction of gas conditions inside packages when O_2 concentrations (temporarily) drop to very low values. Remarkebly the R^2 at day two and three, when metabolic rates were comparable to data of Peppelenbos et al. (1996, Fig. 1), was considerably lower than found by these latter authors when using the same unmodified model. Maybe the lower CO_2 concentrations used by Peppelenbos et al. (1996), 5% at maximum, contributed to this difference.

Table 2. ***Results of the regression analysis for CO_2 production.***
est = estimated value, se = standard error, R^2 = Percentage variance accounted for (indication for the goodness of fit) and $_{adj}$ = adjusted for the number of parameters.

	day 2		day 3		day 6		day 9	
	est	se	est	se	est	se	est	se
Without CO_2 inhibition (equation 2):								
R^2_{adj}	41.2		47.0		94.5		95.1	
Vmf_{CO2}	10.6	1.7	6.83	1.80	9.43	1.58	15.0	3.5
Kmf_{O2}	1.12	0.64	3.52	4.40	2.59	1.86	1.51	1.39
With CO_2 inhibition (equation 3):								
R^2_{adj}	61.8		73.4		96.1		95.8	
Vmf_{CO2}	12.8	1.6	11.0	1.8	12.5	1.8	19.1	3.7
Kmf_{O2}	1.98	1.02	3.98	2.70	3.45	2.13	2.50	1.87
Kmf_{CO2}	6.95	3.34	2.99	1.79	7.96	3.86	9.35	5.86

Change of model parameters in time

Gas exchange calculated with models based on enzyme kinetics suggest the description of actual physiological processes. The use of such models to describe the changes in gas exchange rates in time, however, can be questioned. Although the

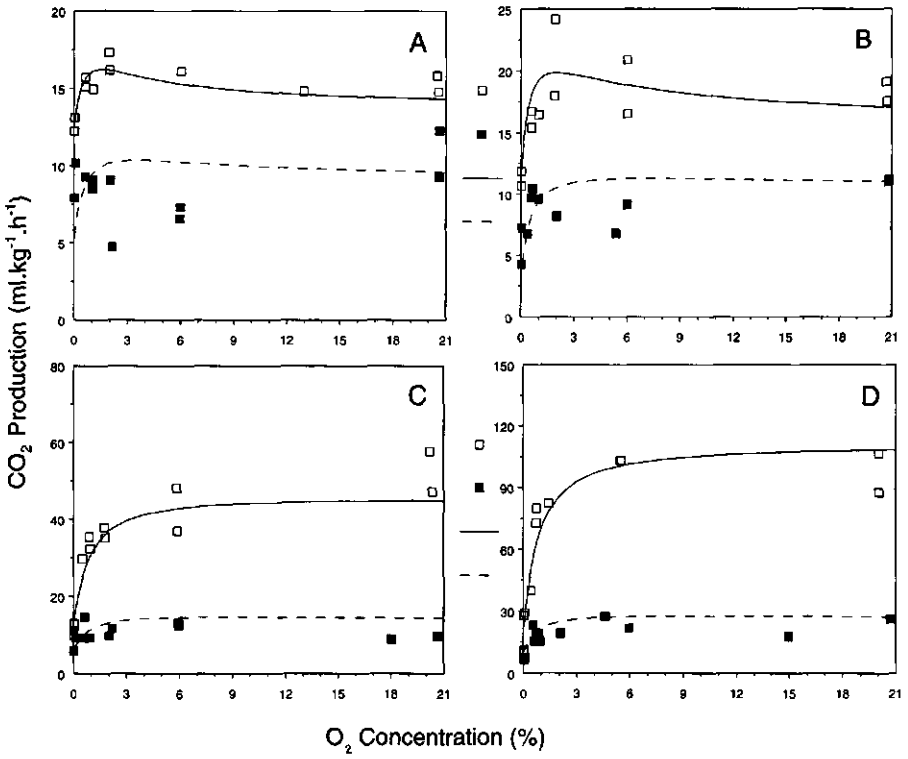

Figure 2. *Measured CO_2 production (○ = at 0% CO_2, ● = at 10% CO_2) and modelled CO_2 production (——— = at 0% CO_2, – – – = at 10% CO_2) of mungbean sprouts at various O_2. A: after 2 days of storage, B: after 3 days of storage, C: after 6 days of storage, D: after 9 days of storage.*

absolute age of mungbean sprouts stored at the various O_2 concentrations is equal, this is surely not the case with the physiological age; maturation and/or senescencewill be different. Nevertheless, the models better fit to data measured after a longer storage time (Tables 1 and 2). It seems that the present models can be used well to describe the 'overall' metabolic processes, especially after prolonged storage. To relate, however, predicted gas exchange rates to actual physiology (fermentation rates at tissue level, energy metabolism), more processes have to be taken into account.

Talasila et al. (1994) already suggested to describe Km_{O2} as $Km_{1/2}$, to express the fact that Km values found at plant product level (fruit, vegetable) are not the Km values at enzyme level due to diffusion processes. Varoquaux et al. (1996) suggested that it could also be important to take into account the contribution of microbial gas exchange rates. When only gas exchange rates are calculated or predicted, this is not essential considering the good fit of the modified model. In such a case it is more relevant to consider the changes in time of the model parameters. The parameters for O_2 uptake change substantially during storage (see earlier). The parameters for fermentative CO_2 production, however, hardly change (Table 2). Although there is some variation in Vmf_{CO2}, Kmf_{O2} and Kmf_{CO2}, the values stay within the same ranges and within the standard errors found. This means that once fermentative CO_2 production is known, it can also be estimated throughout the complete storage period of a MA package of mungbean sprouts.

Fermentative metabolites

In Table 3 the O_2 and CO_2 concentrations actually measured during the storage period are given. When the fermentative metabolites arising under 0% O_2 were examined, no significant difference between ethanol, acetaldehyde or acetate concentrations were found under low CO_2 or high CO_2 (Table 3). When ANOVA was repeated using CO_2 concentrations as the only variable, acetaldehyde and acetate concentrations appeared to be significantly lower at a high CO_2 concentration (Table 3). The influence on acetaldehyde is the only indication found of an influence of CO_2 on ethanolic fermentation of mungbean sprouts. The reduced acetate levels suggest a CO_2

Table 3. ***Concentrations of fermentative components at day 10.***
Concentrations ($mg.g^{-1}$) with different letters (a, b) are significantly different ($p<0.05$).

	Component	Lactic acid		Acetaldehyde		Ethanol		Acetate	
O_2 (%)	CO_2 (%)	0.26	9.89	0.26	9.89	0.26	9.89	0.26	9.89
0.05		0.40a	0.09b	0.43a	0.47a	1.34a	1.25a	0.35b	0.23b
0.54		0b	0b	0.53a	0.10a	0b	0b	0.27b	0.04b
0.94		0.14b	0b	0.63a	0.14a	0b	0b	0.38b	0.06b
1.93		0.10b	0b	0.53a	0a	0.05b	0b	0.77a	0.15b
5.81		0b	0b	0a	0.02a	0.01b	0b	0.13b	0.03b
20.4		0b	0b	0a	0a	0b	0b	0.03b	0.05b
average		0.11a	0.02b	0.35a	0.12b	0.23a	0.21a	0.32a	0.09b

influence on microbial metabolism, since acetate is not an abundant fermentative end product in higher plants (Ricard et al., 1994). At 0.05% O_2 concentrations of lactic acid were lower when CO_2 was increased (table 3). Because after prolonged storage under anoxia the main fermentative route in plant tissues should lead to ethanol (Pfister-Sieber and Brändle, 1994; Ricard et al., 1994), the high lactic production again suggests a high metabolic rate of microorganisms.

Estimation of microbial CO_2 production

Microbial metabolism is mentioned several times, although its contribution to the total CO_2 production is unknown. Using measurements at anoxia an estimation of microbial CO_2 production can be made. This was done by relating concentrations of ethanol and acetaldehyde (in $\mu mol.g^{-1}$) at day 10 to mungbean metabolism, and acetate to microbial metabolism. Using such a calculation, microbial metabolism covers 13.2% of the total CO_2 production at 0% CO_2, and 9.34% at 10% CO_2. Although these numbers do not cover the difference in CO_2 production due to high CO_2 as found at the lowest O_2 concentrations, they are surprisingly high. It underlines the need for a good quantification of microbial metabolism.

Conclusions

The modification of the CO_2 production model of Peppelenbos et al. (1996) results in an improved description of fermentative CO_2 production of mungbean sprouts. Although CO_2 production at low O_2 concentrations was reduced by high CO_2 concentrations, no influence was found on ethanol and acetaldehyde levels. The data found showed large differences between gas exchange rates of different batches of mungbean sprouts. It is suggested that microbial metabolism attributes substantially to total CO_2 production rates found, and might explain the differences between batches.

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5

A method for the simultaneous measurement of gas exchange and diffusion resistance under various gas conditions

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Abstract

A method was developed for measurements of metabolic gas exchange rates and gas diffusion resistance in apples simultaneously, under various gas conditions. For this purpose the trace gas neon was selected. In closed flasks containing apples in a specific gas condition, neon was added so as to bring its partial pressure up to 110 Pa. Changes in the oxygen and carbon dioxide concentrations were used to calculate gas exchange rates, and the decrease in neon concentration was used to calculate gas diffusion resistance. Resistance values have been compared with data from the literature, and estimations values of gas diffusion of O_2 and CO_2 were made.

Introduction

The use of Modified Atmosphere Packaging (MAP) and Controlled Atmosphere (CA) storage systems for fruits and vegetables is focused on so called optimum gas concentrations, i.e. gas conditions which positively affect quality and shelf-life. Fruits can be considered as MA packages themselves, with an internal atmosphere different in composition from the external atmosphere (Dadzie et al., 1993). Internal gas concentrations are determined by gas exchange rates and the resistance to gas diffusion. Although optimal gas concentrations are related to the external atmosphere, the actual metabolic processes occurring in plant tissues are more related to internal concentrations. Changes in gas exchange rates and/or the resistance to gas diffusion in stored products will influence the internal concentrations, also when external concentrations remain close to optimal values. For future applications in CA storage, like dynamic control systems where storage conditions react on product physiology, knowledge about these changes is needed to provide truly optimal external gas concentrations. This knowledge will also be useful in developing MA packages not only designed to provide optimal concentrations, but also to keep the gas concentrations within certain limits.

To determine the relationship between the time change of gas exchange rates and diffusion resistance, both should be measured on the same fruit or vegetable, because diffusion resistance shows large variations between individual fruits of the same species and cultivar (Banks, 1985; Rodriguez et al., 1989). A method is needed enabling this simultaneous measurement under various gas conditions without influencing the product. The use of non-influencing measurements enables repeating the measurements in time. Changes of both gas exchange and diffusion resistance, and their influence on optimal storage conditions, can then be monitored. A good method of determining diffusion resistance is to measure the efflux of a trace gas (ethane) from the fruit some period after the fruit was loaded with the gas (Cameron and Yang, 1982; Knee, 1991). This method, however, cannot be combined with gas exchange measurements under various gas conditions, because gas exchange measurements need a period of time to let the product adjust to the gas conditions. One option to avoid preloading is to start with products already adjusted to various gas conditions, and then add a trace gas in order to measure the flux of the gas into the product. This latter option is addressed in this study.

Material and methods

For the measurement of a gas flux into the product the trace gas neon was selected, as it was easy to detect on the gaschromatograph (GC) used. Also, neon does not interfere with ethylene peaks, as was the case with ethane. Neon is an inert gas, present in normal air in a concentration of 18.18 ppm (Greenwood and Earnshaw, 1984).

Produce information and storage conditions

Three cultivars of apples (*Malus domestica* Borkh.) were used: 'Golden Delicious', 'Elstar' and 'Cox's Orange Pippin'. 'Golden Delicious' was harvested on 27 September 1993 and stored in air for 4 weeks at 1 °C. 'Elstar' was harvested on 31 August 1993 and stored in air for 9 weeks at 1 °C. 'Cox's Orange Pippin' was harvested on 8 September 1993 and stored in air for 9 weeks at 1°C. Several gas conditions were applied to the apples. A complete factorial design was used with all the combinations of the following gas concentrations: 0, 0.5, 1, 2, 6 and 21 % O_2 and 0 and 5% CO_2 in two replicates, resulting in 24 apples per cultivar. After 4 and 5 days under the mentioned gas conditions gas exchange and neon afflux were measured.

Measurements of gas exchange and neon afflux

The method of Baumann and Henze (1983) was used to establish product weight, product volume and the internal gas volume (V_i) of each individual apple. A small amount of grease was put on the floral end of the apple to avoid a possible flow of water (during the underwater measurements) or air (during the measurements of diffusion resistance) into the central cavity. The apples were placed in 1.5 L flasks containing small ventilators in a piece of PVC pipe that supported the apple. The flasks were connected to a flow through system to achieve various gas conditions.
Temperature was controlled and recorded every 15 minutes (Vaisala HMP 31 UT). In all experiments the temperature was 18-19 °C (s.e. was 0.4 °C, see Table 1). The gas entering each flask was humidified, resulting in a relative humidity close to saturation.

After closing of the flasks, 1.5 ml of neon was added into each flask resulting in an initial partial pressure close to 110 Pa. The air in the flasks was mixed by the ventilators for 30 seconds. Then the ventilators were stopped (to prevent a local increase in temperature), and the first measurement on gas concentrations was carried

out. In total 5 measurements were made, and each measurement consisted out of three samples. The exact time of the measurements after closing of the flasks was 1, 19, 37, 127 and 289 minutes. After the fifth measurement the flasks were connected again to the flow through system.

Table 1. Produce information.
weight = average weight of measured products (and standard deviation), volume is the measured total apple volume, internal volume is calculated.

Cultivar	Temperature °C	weight gram	apple volume ml	internal volume ml	resistance for neon $s.mm^{-1}$
Golden Delicious	19.0	168.4 ± 13.1	216.0 ± 16.9	56.7 ± 5.8	408 ± 136
Elstar	19.6	147.1 ± 10.1	186.0 ± 12.5	46.3 ± 3.3	780 ± 167
Cox's Orange Pippin	19.5	165.4 ± 13.2	199.6 ± 16.9	42.6 ± 4.9	665 ± 240

The sampled gas was led directly from the flasks to a gaschromatograph (Chrompack CP 2001). Neon, O_2 and N_2 were measured on a Molsieve A column (T = 60°C, p = 110.3 kPa), and CO_2 and ethane were measured on a Hayesep A column (T = 60°C, p = 81.4 kPa). The carrier gas was helium. For every sample 1.2 ml was taken from the flasks, so the total amount of air taken from the flasks was 18 ml, which resulted in a pressure drop of 1.38 kPa in the flasks. Concentrations were corrected for pressure loss. For calculations of the gas concentrations the second and third sample were used. Gas exchange rates were calculated using the concentration differences between measurements 1 and 4 (time difference 127 minutes). The free volume of the flasks (V_o) was calculated by subtracting the calculated apple volume the measured volumes of the ventilator and the PVC pipe from the measured flask volume. The atmospheric pressure was measured (Druck PDCR 930) to correct for changes in atmospheric pressure, and to convert gas concentration (% or ppm) to Pa.

Calculation of diffusion resistance

The process described is the diffusion of trace gas from the free volume in the flask outside the apple (V_o) to the free gas volume inside the apple (V_i). The resistance value obtained is an estimate of the overall resistance to gas diffusion of skin and flesh of an apple. Diffusion can be described with Fick's first law (Burg and Burg, 1965; Cameron and Yang, 1982):

$$\frac{ds}{dt} = \frac{(C_o^t - C_i^t) * A}{R} \quad (1)$$

where ds/dt is the rate of diffusion ($ml.s^{-1}$), R is the resistance coefficient ($s.cm^{-1}$), A is the surface area of the tissue (cm^2), C_o^t is the concentration outside the tissue ($ml.ml^{-1}$) and C_i^t the concentration inside at time t. The apple surface A was calculated from the apple volume, assuming the apple to be a perfect sphere (after Knee, 1991). The diffusion rate can also be described by (after Cameron and Yang, 1982):

$$\frac{ds}{dt} = V_o * (\frac{dC_o}{dt}) \quad (2)$$

where V_o is the free volume of the flask and dC_o the change in concentration in the free volume. When the total amount of trace gas in the flask (a closed system) is constant, and V_i is not neglected, the following is true:

$$V_i * C_i^{t=t} + V_o * C_o^{t=t} = C_o^{t=\infty} * (V_i + V_o) \quad (3)$$

where V_i = the internal volume of an apple and $C_o^{t=\infty}$ = Concentration of neon at time = ∞ (equilibrium concentration). Equations 2 and 3 can be substituted into equation 1, resulting in the next equation:

$$\frac{dC_o}{dt} = -\frac{A * (V_i + V_o)}{R * V_i * V_o} * (C_o^{t=t} - C_o^{t=\infty}) \quad (4)$$

When equation 4 is integrated from time 0 to time t, the next equation was obtained:

$$C_o^{t=t} = C_o^{t=\infty} + (C_o^{t=0} - C_o^{t=\infty}) * e^{-t * \frac{A * (V_i + V_o)}{R * V_i * V_o}} \quad (5)$$

with t = the time period after start of the experiment (s). With the concentration measurements available R can be calculated:

$$R = \frac{-t * A * (V_i + V_o)}{V_i * V_o * Ln \frac{C_o^{t=t} - C_o^{t=\infty}}{C_o^{t=0} - C_o^{t=\infty}}} \quad (6)$$

Results and discussion

The exact neon concentrations applied and the external and internal volumes measured differed per apple. The resistance values found differed widely between individual apples: the minimum and maximum values found were $1.93*10^3$ and $7.06*10^3$ s.cm^{-1} for 'Golden Delicious', $4.96*10^3$ and $11.9*10^3$ s.cm^{-1} for 'Elstar', and $2.75*10^3$ and $12.0*10^3$ s.cm^{-1} for 'Cox'. When the average resistances of the three apple cultivars were compared, the value for 'Golden Delicious' apples was lowest and for 'Elstar' apples highest (Table 1). Together with a change in neon concentration, the respiratory gases were measured. O_2 uptake rates (Fig. 1) and CO_2 production rates (not shown) were established. The highest O_2 uptake rates were found for 'Golden Delicious' apples, and the lowest for 'Elstar' apples (Fig. 1).

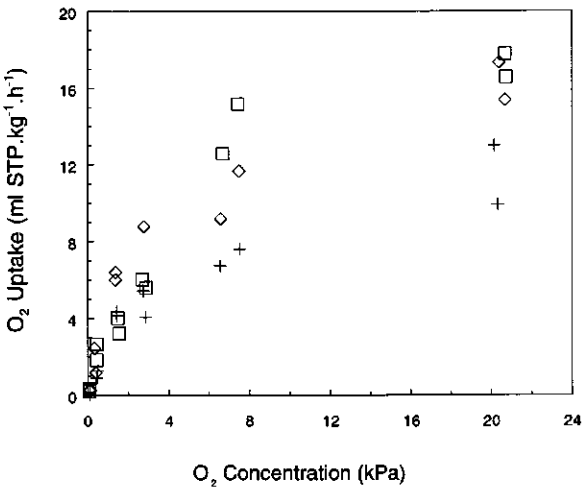

Figure 1. Oxygen uptake rates (ml STP.kg^{-1}.h^{-1}) of 'Golden Delicious' (□), 'Elstar' (+) and 'Cox's Orange Pippin' (◊) apples at several O_2 concentrations (kPa). CO_2 concentration is ± 40 Pa.

For a general view of the resistance measurements the values were averaged per apple cultivar (Table 2). The total change in neon concentration found in the experiments was about 4 Pa, which is about 4% of the initial concentration (Fig. 2). The measured accuracy of the GC on neon was 0.42 Pa at neon concentrations equivalent to 110 Pa. The total change in neon found was 12 times larger than this accuracy. For future applications of this method it will be useful to increase the initial

concentrations, or reduce the volume outside the apple (V_o). With the measured external volume (V_o), the calculated internal volume (V_i) and the measured neon concentration at the start of the experiment (Ne_{start}), the expected neon concentration at the end of the measurement (Ne_{end}) can be calculated. This calculated Ne_{end} was higher than the actually measured Ne_{end} for all three apple cultivars (Table 2). Three factors can attribute to this result: the diffusion of neon into the water phase of the apples, leakage of neon out of the flasks and/or a systematic experimental error. First diffusion of neon into the water phase was calculated.

Table 2. Summary of the measurements on neon diffusion into the products.
V_o = free volume outside the apple (ml), V_i = internal gas volume (ml), 'neon' refers to neon concentrations (Pa).

Variable	Cultivar Golden Delicious	Elstar	Cox's Orange Pippin
V_o	1309	1339	1325
V_i	56.7	46.3	42.6
$neon_{start}$ measured (a)	118.1	109.4	109.8
$neon_{end}$ measured (b)	112.4	105.4	106.0
$neon_{end}$ calculated (c)	113.2	105.7	106.4
$neon_{aq}$ (d)	0.14	0.11	0.13
c-d-b	0.66	0.19	0.27
accuracy	0.42	0.42	0.42

With the value for the solubility of neon in water of 10.5 $ml.l^{-1}$ (Greenwood and Earnshaw, 1984), the weight of the apples used and the value for the percentage of water in apples (Knee, 1991), the maximum amount of neon that can dissolve in the water phase (with an atmosphere of 100% neon around the apples) can be calculated. When this amount is related to the expected neon concentration (the calculated Ne_{end}), the decrease of the neon due to diffusion into the water phase was calculated (Ne_{aq}). This Ne_{aq} was only a minor contribution to the decrease of neon in the volume around the apple (Table 2). After correcting the calculated Ne_{end} for Ne_{aq} it still exceeded the measured Ne_{end}. For the measurements on two apple cultivars, 'Elstar' and 'Cox', this difference was less than the accuracy of the GC, and can therefore be ignored. For the experiment on 'Golden Delicious' leakage probably contributed to the concentration difference found. In equation 3 the total amount of gas in the system is assumed constant. When leakage occurs, this assumption is no longer true and the equation

cannot be used. The leakage found (calculated) in the experiments is considered too small to influence the resistance values found, and can therefore be neglected. It remains important, however, for a good functioning of equation 3, to eliminate all leakages in closed systems.

The data on gas exchange (Fig. 1) are comparable to those obtained on days preceding the current measurements (Peppelenbos and van 't Leven, 1996). This implies that the measurement of respiratory gas exchange has not been influenced by the method of measuring the neon flux. To evaluate the resistance values found, they have to be compared with data from the literature. For 'Golden Delicious' and 'Cox's Orange Pippin' apples literature data are known (Table 3).

Table 3. Comparing literature and experimental data on diffusion resistance.
Method: 1 = Ethane flux out of the product, 2 = gas sampling of a tube glued on the surface, 3 = flux of neon into the product, R is ethane and neon resistance values.

Apple cultivar	Source	Method	Age (days)	Temperature (°C)	Rel. Humidity (%)	Resistance ($s.mm^{-1}$) low	high
Golden Delicious	Banks (1985)	1	± 7	?	?	970	205
	Knee (1991)	1	?	?	?	560	760
	Data found here	3	31	19.0	>95%	193	706
Cox's Orange P.	Rajapakse et al. (1990)	2	3	20	?	594	
	Knee et al. (1990)	1	35-80	3.3	?	980	151
	Data found here	3	66	19.5	>95%	275	120

The highest resistance values found here for Golden Delicious and Cox's Orange Pippin are comparable to those found in literature. The lowest values, however, are considerably different. The significance of these differences is unclear, especially after comparing the circumstances under which the measurements were made. In earlier experiments the temperature has been lower (Knee et al., 1990) or not been given (Banks, 1985; Knee, 1991). The relative humidity, which can influence the values found for diffusion resistance (Lidster, 1990), has also not been given. Because the apples used in the experiments found in literature also have different ages, and because diffusion resistance of apples can change over time (Solomos, 1987; Park et al., 1993) it is clear that only a comparison of the order of magnitude is meaningful, which is the case here. The neon afflux method seems a good method for measuring diffusion resistances. Its advantage is that it can easily be combined with gas exchange

measurements. One has to take into account that the produce to be measured should have a considerable internal volume (V_i) compared to the volume surrounding the product (V_o) for a large enough decrease in neon concentration to be measurable.
One disadvantage of using ethane instead of neon for the measurement of diffusion resistances, in addition to a possible influence of ethylene concentrations on the ethane measurements, is that ethane production is found in ageing plant tissues. Ethane production is commonly associated with membrane damage and cell death, and is increased with increasing injury of plant tissues (Abeles et al., 1992). Particularly

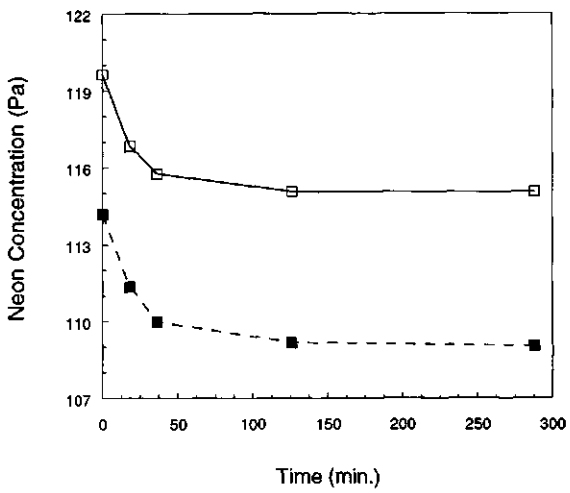

Figure 2. Typical decrease of the neon concentration in the flask in time. Measurements on the same apple of cv. Golden Delicious, at day 4 (□) and day 5 (■).

when research is focussed on a relation between changes in diffusion resistance in fruits during storage and the onset of storage disorders, this ethane production may influence the measurements.
After deriving resistance values for neon diffusion in plant tissues, and for ethane diffusion as well, it is important to know the relation to resistance values for O_2 and CO_2. This issue was not addressed by Cameron and Yang (1982) or Knee (1991). Banks (1985) suggests that ethane diffusion is probably similar to O_2 and ethylene diffusion. Using Graham's law predictions on the relationship between the diffusion of

the various gases can be made. Based on this law one would expect diffusion rates of O_2, ethane and ethylene to be comparable, but neon diffusion rate to be 18% higher and CO_2 diffusion rate to be 20% lower (Table 4). The diffusion routes, however, of neon, ethane, O_2 and CO_2 are not necessarily equal (Banks, 1985), which makes the use of only Graham's law suspect. A real comparison of resistance values is necessary, and for this purpose one might combine the method of Rajapakse et al. (1990) and the ethane (Cameron and Yang, 1982) or neon method.

Table 4. Diffusion rate of metabolic gases as compared to ethane and neon using Graham's law.

	Ethane	Neon
Carbon dioxide	0.799	0.679
Oxygen	0.937	0.796
Ethylene	0.966	0.821
Ethane	1	0.850
Neon	1.176	1

Acknowledgements

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6

Functioning of gas exchange models using external and internal gas concentrations of three apple cultivars

H.W. Peppelenbos, W.K. Jeksrud

Abstract

Based on gas exchange rates and diffusion resistance, internal gas concentrations of apple cultivars Golden Delicious, Elstar and Cox's Orange Pippin were calculated. Internal O_2 concentrations were 2.3 kPa lower at an external O_2 concentration of 20.7 kPa for Golden Delicious apples, and about 4.5 kPa lower at 20.1 and 20.4 external O_2 for Elstar and Cox's apples respectively. Internal CO_2 concentrations substantially exceeded normal external concentrations of 50 Pa. The Km values found for the three apple cultivars remained significantly different when internal instead of external concentrations were used. This indicates that the apple cultivars measured do not only show biophysical differences (resistance, porosity), but also differences at the biochemical level. For Golden Delicious apples no difference in model functioning was found when internal or external concentrations were used. In contrast, for Elstar and Cox's Orange Pippin apples the O_2 uptake and CO_2 production models showed better results (expressed as R^2) when fitted on external concentrations. It is argued that this might be explained by the experimental setup. For instance the internal O_2 concentration of Cox's Orange Pippin calculated at the optimal external O_2 concentration (1.2%) reached 0.085%. A small change of 0.1% in an external O_2 concentration close to 1% therefore can change the internal atmosphere from hypoxia to anoxia, which cannot be regarded as an equilibrium situation.

The conclusion to be drawn is that also for experimental setups, where often different temperatures are used, some precalculations using gas exchange and diffusion

resistances will help to optimize the methods. These low internal concentrations, based on data found at 18°C, also confirm the idea that optimal O_2 concentrations increase when temperature is increased.

Introduction

In studies on the storage of fruits under Controlled Atmosphere (CA) conditions often an emphasis is laid on the role of diffusion characteristics of the fruits. Dadzie et al. (1993) suggested that quality changes of a fruit stored under changed gas conditions can probably be better related to the internal then to the external concentrations. More and more it becomes clear that it is necessary to know the concentrations inside the fruit (and at the cell level) to develop physiological explanations of the mode of action of CA storage (Knee, 1991b). Due to metabolical processes in combination with diffusion resistances, the O_2 concentrations inside fruits are always lower than the O_2 concentrations surrounding them. Knowledge of O_2 diffusion is needed for studying the oxidases that may be involved in fruit respiration, but also for predicting minimum O_2 levels that can be safely used in CA storage (Solomos, 1987).

In contrast to internal O_2 concentrations, internal CO_2 exceed external concentrations. Varietal differences in susceptibility to CO_2 injury could possibly result from anatomical rather than biochemical differences (Burton, 1974). Burton (1974) was aware though that not only resistance or permeability define optimal storage conditions, but the combination of permeability, gas exchange rates and internal gas volumes. Simplified, fruits can be regarded as Modified Atmosphere (MA) packages (Dadzie, 1993), with the skin and flesh (Rajapakse et al., 1990) resembling the permeable film, and the internal gas volume resembling the free volume inside the package.

In the past years most authors describing gas exchange in relation to O_2 concentrations prefer a Michaelis-Menten type of equation, as it describes the enzymatic rate of O_2 uptake in plant tissues (Cameron et al., 1995). It was mentioned by Chevillotte (1973) that diffusion resistances can influence the type of relationship found between gas exchange rate and gas concentrations. So far, however, the equations used are based on gas concentrations outside the fruit. Yet, it is the O_2 level inside the product that limits respiration rates (Cameron et al., 1995). This paper therefore focusses on the functioning of known equations when internal instead of external concentrations are used. By combining the modelled gas exchange rates with

diffusion resistances, internal gas volumes and advised optimal (external) gas concentrations, the internal gas concentrations of three apple cultivars are compared.

Material and methods

Internal concentrations

The gas concentrations in the intercellular space can be regarded as an estimation of the intracellular gas concentrations if the resistance to gas diffusion in fruits is mainly attributed to the skin of the fruit. Although significant resistances of skin tissues have been found (Rajapakse et al., 1990), especially in fruits with high internal gas volumes, like non-climacteric apples, concentration gradients within the intercellular air space should be negligible (Kays, 1991).

To establish the intercellular gas concentrations ('internal concentrations'), the gas exchange rates and the diffusion resistance of three apple cultivars (*Malus domestica* Borkh., cv Golden Delicious, Elstar and Cox's Orange Pippin) were determined, using the method described by Peppelenbos and Jeksrud (1996). The experimental setup and the characteristics of the fruits are also given by Peppelenbos and Jeksrud (1996). With the external oxygen (O_2) concentrations, gas exchange rates and the resistance to gas diffusion, the internal O_2 concentrations can be estimated using Fick's first law:

$$[O_2]_i = [O_2]_e - \frac{V_{O2} * m * r_{O2}}{A} \qquad (1)$$

where $[O_2]_i$ = the internal O_2 concentration ($\mu mol.cm^{-3}$), $[O_2]_e$ = the external O_2 concentration ($\mu mol.cm^{-3}$), V_{O2} = the O_2 uptake rate ($\mu mol.g^{-1}.s^{-1}$), m = the mass of the fruit (g), r_{O2} = the resistance to O_2 diffusion ($s.cm^{-1}$), and A is the surface of the fruit (cm^2). Internal carbondioxide (CO_2) concentrations were derived in a comparable way:

$$[CO_2]_i = [CO_2]_e + \frac{V_{CO2} * m * r_{CO2}}{A} \qquad (2)$$

where $[CO_2]_i$ = the internal CO_2 concentration ($\mu mol.cm^{-3}$), $[CO_2]_e$ = the external CO_2 concentration ($\mu mol.cm^{-3}$), V_{CO2} = the CO_2 uptake rate ($\mu mol.g^{-1}.s^{-1}$), and r_{CO2} = the resistance to CO_2 diffusion ($s.cm^{-1}$). The resistance to O_2 and CO_2 diffusion were derived from the resistance to neon diffusion, using Graham's law to convert to O_2 and

CO_2 resistance. The internal and external O_2 concentration and the gas exchange rates were calculated from kPa or ml to µmoles using the ideal gas law. The actual temperature and pressure measured during the experiments were used for this conversion.

Gas exchange models

To test gas exchange models gas exchange rates were fitted with the external and the calculated internal concentrations. Two gas exchange models were used. O_2 uptake rates were fitted using an O_2 consumption model without inhibition by CO_2, because this inhibition was not found on the apple cultivars used (Peppelenbos and van 't Leven, 1996):

$$V_{O2} = \frac{Vm_{O2} * O_2}{Km_{O2} + O_2} \quad (3)$$

where O_2 = the external or internal O_2 concentration, V_{O2} = the O_2 uptake rate (ml.$kg^{-1}.h^{-1}$), Vm_{O2} = the maximum O_2 uptake rate (ml.$kg^{-1}.h^{-1}$) and Km_{O2} = is the Michaelis constant for the influence of O_2 on the O_2 uptake rate. CO_2 production was fitted using one of the models given by Peppelenbos et al. (1996):

$$V_{CO2} = V_{O2} * RQ_{ox} + \frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}}\right)} \quad (4)$$

where RQ_{ox} is the RQ value for oxidative processes, Vmf_{CO2} is the maximum fermentative CO_2 production rate (ml.$kg^{-1}.h^{-1}$), and Kmf_{O2} the Michaelis constant for the inhibition of fermentative CO_2 production by O_2. The data were compared with the models using the facilities for non-linear regression in the statistical package Genstat (release 5). The O_2 consumption and the CO_2 production were fitted directly without any transformation, using an iterative method to maximize the likelihood. By combining the modelled gas exchange rates with diffusion resistances, internal gas volumes and advised optimal (external) gas concentrations, the internal gas concentrations of three apple cultivars are compared. The optimal external O_2 concentration used was 1.2% for all apple cultivars (Meheriuk, 1993).

Results

After measuring gas exchange rates and diffusion resistance the internal O_2 and CO_2 concentrations per individual apple were calculated. For equal external concentrations the values were averaged, and are shown Table 1. For external CO_2 concentrations close to ambient conditions the relation between external O_2 concentrations and internal O_2 and CO_2 concentrations is shown in Fig 1. The difference between internal and external O_2 concentrations was highest at high O_2 concentrations, due to a higher respiration rate. Reduced CO_2 production rates also accounted for the lowest difference between internal and external CO_2 concentrations at O_2 concentrations close to 2%.

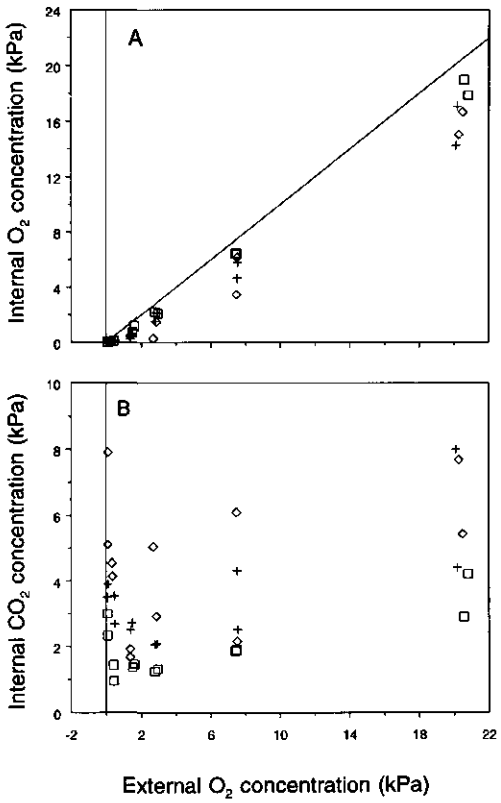

Figure 1. *Internal concentrations related to external O_2 concentrations (in kPa at 0% external CO_2, □ = Golden Delicious, + = Elstar, ◊ = Cox's Orange Pippin). A: O_2 concentrations, B: CO_2 concentrations.*

When the apple cultivars are compared, the smallest differences between external and internal concentrations were found for Golden Delicious apples (Table 1). Due to increased CO_2 production at O_2 concentrations approaching anoxia, the internal CO_2 concentrations found at these conditions were comparable to concentrations found in ambient air. The highest internal CO_2 concentrations were found for Cox's Orange Pippin apples (Table 1). For Cox's Orange Pippin apples the estimated internal O_2 concentrations approached 0 or were negative (Table 1). This indicates that the methods used become unreliable when O_2 concentrations become as low as 0.5%.

Table 1. ***External and internal O_2 and CO_2 concentrations.***
Average values of measured external (Ex) and calculated internal (In) concentrations of Golden Delicious, Elstar and Cox's Orange apples.

Golden Delicious				Elstar				Cox's Orange Pippin			
O_2 Ex	O_2 In	CO_2 Ex	CO_2 In	O_2 Ex	O_2 In	CO_2 Ex	CO_2 In	O_2 Ex	O_2 In	CO_2 Ex	CO_2 In
0.08	0.03	0.52	2.67	0.09	0.04	0.39	3.71	0.08	0.00	0.84	6.51
0.43	0.10	0.25	1.21	0.48	0.14	0.28	3.21	0.33	-0.22	0.58	4.34
1.56	0.95	0.23	1.43	1.42	0.41	0.26	2.61	1.37	0.47	0.32	1.81
2.88	2.16	0.28	1.29	2.84	1.83	0.21	2.09	2.77	0.88	0.51	3.99
7.46	6.42	0.63	1.89	7.53	5.23	0.30	3.42	7.50	4.81	0.45	4.13
20.67	18.39	0.81	3.56	20.14	15.64	0.47	6.20	20.37	15.84	0.66	6.56

The gas exchange models were fitted using data of each individual apple. For Golden Delicious apples no difference in model functioning was found when internal or external concentrations were used (Table 2). In contrast, for Elstar and Cox's Orange Pippin apples the O_2 uptake and CO_2 production models showed better results (expressed as R^2) when fitted on external concentrations instead of internal concentrations (Table 2). The change of the parameters of the O_2 model was comparable for all three apple cultivars: Vm_{O2} and Km_{O2} values decreased when internal O_2 concentrations were used. The parameters of the CO_2 production model reacted differently on using internal concentrations. The RQ_{ox} values did almost not change when internal or external concentrations were used. Kmf_{O2} values decreased for all three apple cultivars when internal concentrations were used, indicating an increase in the fermentation rate at a lower O_2 concentration. Remarkable is the high estimated value for Vmf_{CO2} of Golden Delicious when internal concentrations were used, while the Vmf_{CO2} of the other two cultivars remained almost the same. The measured gas exchange rates and the

modelling results for Golden Delicious apples are shown in Fig 2, using both external and internal concentrations. The curve representing the modelling results on O_2 uptake shifts to lower O_2 concentrations when using internal O_2 concentrations, but remains almost the same (Fig. 2A). This is not the case for CO_2 production (Fig. 2B), where the fitting procedure results in a lower minimum CO_2 production. The RQ values based on data and models also shift to lower O_2 concentrations when internal O_2 concentrations are used (Fig. 2C).

Table 2. *Modelling results.*
Results of the regression analysis for O_2 consumption and CO_2 production using internal (Intern) and external (Extern) concentrations. R^2 = Percentage variance accounted for, est = estimated values, se = standard error, Vm_{O2} = maximum O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Km_{O2} = Michaelis constant, Vmf_{CO2} = maximum fermentative CO_2 production rate ($ml.kg^{-1}.h^{-1}$), Kmf_{O2} = Michaelis constant).

			O_2 model			CO_2 model			
			R^2	Vm_{O2}	Km_{O2}	R^2	RQ_{ox}	Vmf_{CO2}	Kmf_{O2}
Golden D.	Intern	est	93.0	23.66	5.11	91.2	1.037	23.4	0.027
		se		*2.85*	*1.61*		*0.043*	*21.5*	*0.026*
	Extern	est	93.5	25.03	7.17	92.5	1.024	18.91	0.128
		se		*3.15*	*2.14*		*0.044*	*5.16*	*0.077*
Elstar	Intern	est	81.0	16.84	5.34	75.6	1.069	14.42	0.148
		se		*3.71*	*2.94*		*0.067*	*2.34*	*0.066*
	Extern	est	91.5	18.15	8.76	87.7	1.033	13.17	0.616
		se		*2.92*	*3.14*		*0.050*	*1.07*	*0.159*
Cox's O.P.	Intern	est	69.9	15.16	0.55	64.9	1.079	14.58	0.170
		se		*2.13*	*0.34*		*0.084*	*1.33*	*0.088*
	Extern	est	88.0	18.98	3.25	85.5	1.102	21.56	0.329
		se		*2.29*	*1.18*		*0.053*	*2.41*	*0.108*

After combining average values for porosity and diffusion resistances with calculated gas exchange rates at 1.2% O_2 (by using the fitted equations), internal O_2 and CO_2 were derived (Table 3). For both Golden Delicious and Elstar apples the internal O_2 concentrations were close to half the external concentration, while their internal CO_2 concentrations accumulated to three and seven times the external values. The estimated internal O_2 concentration of Cox's Orange Pippin apples was as low as 0.085% (Table 3), suggesting almost anoxia inside the apples at optimal outside O_2 concentrations. The estimated internal CO_2 concentration of Cox's apples was the highest of the three cultivars, also indicating high fermentation rates.

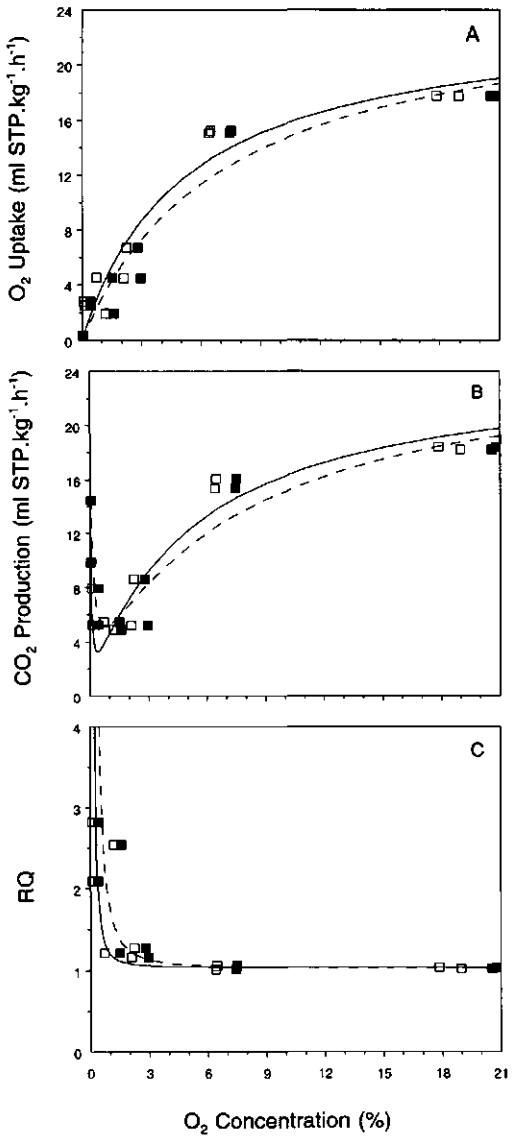

Figure 2. *Gas exchange of Golden Delicious apples. Measurements related to external concentrations (■) and to calculated internal concentrtaions (□). Models fitted using external concentrations (——) and internal concentrations (– – –). A: O_2 uptake (ml STP.kg^{-1}.h^{-1}), B: CO_2 uptake (ml STP.kg^{-1}.h^{-1}), C: RQ.*

Discussion

The calculated internal O_2 concentrations of Golden Delicious and Elstar apples stored in O_2 concentrations close to ambient air, 18.4 and 15.6 kPa respectively, correspond to found internal concentrations of other apple cultivars. Measured in ambient air the internal O_2 concentrations of King Edward VII and Sturmer Pippin ranged between 17.9 and 18.9% (at 12 °C, Kidd and West, 1949), of Stayman apples at 23 °C between 12.7% and 14.8%, of Rome Beauty, McIntosh and Gala apples between 17.2 and 19.8% (at 15 °C, Solomos, 1987). Internal O_2 concentrations of Braeburn apples measured directly under the skin was 11.7% and 10.6% at the center of the fruit (at 20 °C, Rajapakse et al., 1990). The internal O_2 concentrations of Cox's orange Pippin apples measured in ambient air (15.8 kPa) are close to values reported before. Internal O_2 concentrations found range between 16.1% (at 12 °C, Kidd and West, 1949) and 15.1% directly under the skin to 14.8% at the center of the fruit (at 20 °C, Rajapakse et al., 1990). Based on Graham's law the same value for the resistance for neon diffusion will result in different values for O_2 and CO_2 diffusion (Table 3).

Table 3. ***Estimated internal gas concentrations at an optimal external O_2 concentration of 1.2%.*** *porosity in %, resistance (R.) in $s.cm^{-1}$, concentrations in kPa, O_2 uptake and CO_2 production in ml $STP.kg^{-1}.h^{-1}$.*

Cultivar	Porosity	R. neon	R. O_2	R. CO_2	$[O_2]$ out	$[CO_2]$ out	O_2 uptake	CO_2 production	$[O_2]$ in	$[CO_2]$ in
Golden D.	26.2	3917	4920	5770	1.23	0.463	3.64	5.59	0.739	1.35
Elstar	24.9	8456	10620	12455	1.21	0.323	2.19	6.71	0.591	2.56
Cox O. P.	21.4	6265	7869	9228	1.22	0.571	5.05	10.2	0.085	3.28

The estimated internal CO_2 concentrations of Golden Delicious and Elstar apples (3.6 kPa and 6.2 kPa respectively), based on gas exchange rates and these resistance values, were also comparable to values reported before (at 21% O_2 external). Reported internal concentrations range from 1.8 to 2.5% for King Edward and Sturmer Pippin apples (Kidd and West, 1949), 0.93 to 3.25 % for Gala, McIntosh and Rome Beauty apples and 6.3 to 8.1% for Stayman apples (Solomos, 1987). The internal CO_2 concentration in ambient air found for Cox's apples was 6.6 kPa, which is higher than the 3.9% found by Kidd and West (1949). These authors, however, conducted their experiment at a lower temperature (12°C). This likely resulted in a lower respiration

rate and consequently a lower internal CO_2 concentration. Based on these values for internal concentrations it can be concluded that the method used, based on a combination of neon diffusion and Graham's law, results in comparable results as other methods. Elstar and Cox's Orange Pippin apples showed the largest difference between external and internal concentrations (Table 3), but this was to be expected since both cultivars show higher resistance values in combination with lower porosity than Golden Delicious apples.

Table 2 and 3 show high standard errors and deviations, indicating large variance between individual apples. Also Knee (1991b), in a survey of Cox's Orange Pippin apples from different orchards, found that all possible combinations of high and low respiration and high and low resistance can occur. This variability of plant material prevents precise control of intercellular atmosphere; it seems that recommended atmospheres can be designed only to avoid completely anaerobic conditions and a harmful level of CO_2 in the center of the least permeable individual fruit (Burton, 1974).

The gas exchange models currently known, like equations 3 and 4, are based on a description of enzyme kinetics. One simplification of the models is that the calculated gas exchange rates are related to O_2 concentrations in the air around a fruit, while the actual O_2 concentrations at the (modelled) enzyme level are much lower. This difference resulted in lower Km values for O_2 uptake and CO_2 production for all three apple cultivars when internal concentrations were used. For this reason Chevillotte (1973) suggested to use the term Km_{ex} when external concentrations were used, to show that the Km values cannot be attributed to enzyme functioning directly.

The Km values found for the three apple cultivars remained significantly different when internal instead of external concentrations were used. This indicates that the apple cultivars measured do not only show biophysical differences (resistance, porosity), but also differences at the biochemical level. Therefore the statement of Burton (1974) that differences between apple varieties in susceptibility to CO_2 injury could possibly result from anatomical rather than biochemical differences' has to be reconsidered.

Chevillotte (1973) concluded that the single enzyme model is the only representation which leads to a satisfactory study at the cytochrome oxidase-oxygen reaction level *in vivo*, when possible changes brought about by diffusion are taken into account. The lower Km values found when internal concentrations were used can indeed be attributed to diffusion limitations. A worse functioning of the models when internal concentrations of Elstar and Cox's Orange Pippin apples are used, is more difficult to

understand. The first thing to argue is the correctness of the resistance values. As stated before, these values are based on Fick's law, assuming one main barrier for diffusion: the fruit surface (skin). When also the fruit tissue has a considerable resistance to gas diffusion, it invalidates such an approach (Knee, 1991b). From research by Rajapakse et al. (1990) it is known that diffusivity in flesh tissues must be taken into consideration. Nevertheless the percentage of the total O_2 gradient between the external atmosphere and the internal core cavity caused by flesh in apples was relatively small: 4.5% in Cox's Orange Pippin and 11% in Braeburn apples (Rajapakse, 1990). This indicates that the values found for the three apple cultivars in chapter 5 are likely to be close to the overall diffusion resistance to gas diffusion. For products other than apples the contribution of flesh resistance can add up to 30% in Asian pears and 56% in nectarines (Rajapakse et al., 1990). This indicates that both the ethane and the neon method cannot be simply applied to all types of commodities. Another explanation for the worse model behaviour, and the unreliable internal O_2 concentrations of Cox's apples (Table 1), might be the experimental setup. Using headspace techniques to quantify gas exchange rates, the assumption was that gas exchange rates were in equilibrium with gas concentrations. With gas concentrations changing 0.3% at ambient air and 0.1% maximally at O_2 concentrations lower than 5%, this assumption seems correct. When, however, the internal O_2 concentrations were calculated at optimal external O_2 concentrations (Table 3), the internal O_2 concentration of Cox's Orange Pippin apples was 0%. A small change of 0.1% in an external O_2 concentration close to 1% therefore could change the internal atmosphere from hypoxia to anoxia. That surely cannot be regarded as an equilibrium situation. The conclusion to be drawn is that also for experimental setups, where often different temperatures are used, some precalculations using gas exchange and diffusion resistances will help to optimize the methods.

With respect to the low internal concentrations as given in Table 3, it has to be noted that the optimal (external) concentration used (Meheriuk, 1993) was given in combination with low storage temperatures, whereas the current experiment used 18°C. This confirms the idea that optimal O_2 concentrations increase when temperature is increased, as reported by Beaudry et al. (1992) for blueberry and Joles et al. (1994) for raspberry fruit.

7

Respiratory characteristics and calculated ATP production of apple fruit in relation to tolerance to low O_2-concentrations.

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1996, J. Hort. Sci., 71 (5): (in press)

Abstract

The applicability of respiratory characteristics to determine optimal O_2 concentrations for the storage of apples was tested. A comparison was made between gas exchange rates of apples directly after harvest and after a period of storage. Apples of three harvest dates were used. Optimal O_2 concentrations were based on gas exchange data and gas exchange models fitted on the data, using the Anaerobic Compensation Point (ACP) and the Respiratory Quotient Breakpoint (RQB). A third way was comparing total ATP production with estimated maintenance energy requirements, revealing the Maintenance Oxygen Concentration (MOC). ATP production was calculated using gas exchange models. MOC was defined as the oxygen concentration with the minimal ATP production rate necessary for maintaining cell viability. The optimal O_2 concentrations as established by ACP, RQB and MOC differed considerably. Because ACP values differed from normally advised values, the ACP was unsuitable for a quick determination of the optimal O_2 concentration of the apples used. The RQB, however, might be suitable, but than the limit used to establish the RQB should be more than 0.5 units higher than the RQ measured in ambient air. The ACP and the RQB were decreased to lower O_2 concentrations after storage, suggesting optimal concentrations to decrease during storage. In contrast the MOC was increased after storage, which is in agreement with results found in practice.

Model calculations indicated the lowest optimal O_2 concentration for the second (optimal) harvest using the ACP, the RQB and the MOC. It is suggested that research on the relationship between Maintenance Energy Requirements and cell injury will clarify an important part of the changes in optimal O_2 concentrations (or the tolerance to low O_2 concentrations) during ageing or maturation of harvested plant tissues.

Introduction

In the Netherlands the majority of the picked apples are stored under Controlled Atmosphere (CA) conditions. For apples, and other harvested plant products, it is found that lowering the O_2 concentration in the surrounding atmosphere leads to a slower maturation and senescence (Kader et al., 1989). When O_2 concentrations become to low, disorders like browning and necrosis of tissues are found. Therefore the main focus in the research of the storage of apples, and other harvested plant products, is the establishment of the tolerance to low O_2 concentrations. The 'optimal O_2 concentration' is considered the concentration where maturation is reduced the most without the occurrence of disorders which can be related to low O_2 concentrations. These optimal O_2 concentrations differ between species, cultivars and varieties (Kader et al., 1989), and can even differ between years for the same variety (Stow, 1989). Experiments to establish optimal O_2 concentrations are based on trial and error and are time consuming (Wollin et al., 1985; Gran and Beaudry, 1993). Conclusions can be drawn after the storage period, revealing the optimal concentrations of the past year. The question then is whether these conditions are also valid in the years to come. A quick method has potential to determine optimal O_2 concentrations before storage of the product. Wollin et al. (1985) suggested the use of gas exchange characteristics for establishing these concentrations. They hypothised that minimal 'respiration' (CO_2 production) could correspond to maximum storage life. This was also suggested by Boersig et al. (1988), who introduced the term Anaerobic Compensation Point (ACP), defined as the O_2 concentration where CO_2 production is minimal. Later also the Respiratory Quotient Breakpoint (RQB) was introduced, defined as the O_2 concentration where the RQ is increasing rapidly (Gran and Beaudry, 1993). Both the ACP and the RQB are thought to be useful for the establishment of optimal O_2 concentrations. Boersig et al. (1988) found a change in the ACP when pear fruit was ageing. At the moment it is unknown whether a change in the ACP or the RQB can be related to a change in optimal O_2 concentrations.

Both the ACP and the RQB are based on the premiss that fermentation is directly related to disorders found in plant products stored at low O_2 concentrations. Often an O_2 concentration is considered optimal when O_2 consumption is minimized without the development of fermentation (Banks et al., 1993; Gran and Beaudry, 1993). On the other hand fermentation is also believed to occur so plant cells can meet their requirements for ATP (Good and Muench, 1993; Fox et al., 1994). The ATP required to keep anaerobic tissues alive is generated in fermentation processes (Pfister-Sieber and Brändle, 1994). For flooded plants it is found that a minimum ADH activity is required to survive even short periods of anoxia (Kennedy et al., 1992). Recently the main injuries in plant tissues occurring during anoxia are related to changes in energy metabolism (Pfister-Sieber and Brändle, 1994). Although fermentation is highly inefficient compared to respiration, fermentation can be regarded as 'just' another source for ATP. Instead of focusing on the O_2 concentration where fermentation is ceasing, the total ATP production of both respiration and fermentation might also be a measure for the tolerance to low O_2 concentrations. Based on this concept, it is unimportant which metabolic route (oxidative or fermentative) is providing the energy, as long as the sum of both routes covers Maintenance Energy Requirements (MER). MER is defined here as the minimal ATP production to maintain cell viability.
Optimal concentrations based on actual fruit characteristics could probably help to avoid storage losses. For this purpose the usability of respiratory characteristics, and models describing these characteristics, were tested. Gas exchange characteristics of Cox Orange Pippin apples of three harvest dates, carried out directly after harvest, were examined. Optimal gas concentrations were estimated using the ACP and the RQB. These values were compared with an estimated total ATP production, based on models describing O_2 consumption and CO_2 production. Gas exchange rates were also measured after a period of storage, to examine possible changes in ACP, RQB and ATP production during storage.

Materials and methods

Gas exchange measurements
Gas exchange measurements were carried out on apples (*Malus domestica* Borkh., cv. Cox's Orange Pippin) directly after harvest and after a period of storage under CA conditions. Apples of three harvest dates were used to compare apples with different

maturity. The selection of the harvest dates was based on the method of Streif (1983), resulting in an early harvest (9 September 1994), a harvest with apples of a maturity considered to be optimal for storage (16 September 1994) and a late harvest (23 September 1994). At the days of harvest part of the apples was put in CA storage. Storage conditions were standard Dutch CA conditions (1°C, 1.2% O_2, 2% CO_2). Another part of the apples was used for gas exchange measurements. These latter apples were placed in flasks connected to a flow through system and were subjected to 0, 0.5, 1, 2, 5 or 21% O_2 in combination with 0.1% CO_2. Per gas condition four apples were individually measured. The temperature used for measuring was 18°C, and the relative humidity was close to saturation (97-99%). After two days at these conditions gas exchange rates were measured twice per day, and the values were averaged. O_2 consumption rates and CO_2 production rates were measured by the method described by Peppelenbos and van 't Leven (1996). After 140 days of storage gas exchange measurements were repeated on the CA stored apples, using the same gas concentrations.

Gas exchange models

Oxygen uptake was calculated using a model without inhibition of oxygen uptake by carbon dioxide (Chevillotte, 1973):

$$V_{O2} = \frac{Vm_{O2} * O_2}{Km_{O2} + O_2} \quad (1)$$

where V_{O2} is the O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), Vm_{O2} is the maximum O_2 consumption rate ($ml.kg^{-1}.h^{-1}$), O_2 is the O_2 concentration (%) and Km_{O2} is the Michaelis constant for the influence of O_2on the O_2 uptake rate (% O_2). CO_2 production was calculated using an adapted version of the model given by Peppelenbos et al. (1993), which combines CO_2 produced by oxidation and fermentation processes:

$$V_{CO2} = V_{O2} * RQ_{ox} + \frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}}\right)} \quad (2)$$

where V_{CO2} is the total CO_2 production rate ($ml.kg^{-1}.h^{-1}$), RQ_{ox} is the RQ value for oxidative processes, Vmf_{CO2} is the maximum fermentative CO_2 production rate (ml.

$kg^{-1}.h^{-1}$), and Kmf_{O2} the Michaelis constant for the inhibition of fermentative CO_2 production by O_2. The RQ, necessary for establishing the RQ Breakpoint, was calculated by dividing the fit found for the CO_2 production model by the fit found for the O_2 uptake model. Both gas exchange models were fitted on the data using the facilities for non-linear regression in the statistical package Genstat.

Determination of optimal O_2 concentrations

To assess optimal O_2 concentrations both measured and modelled gas exchange rates of apples of the three harvest dates were used. Optimal O_2 concentrations were estimated using the ACP (the O_2 concentration where CO_2 production is minimal) and the RQB (the O_2 concentration where RQ is increasing rapidly). Because the definition of the RQB is inadequate for a model showing a gradual increase of the RQ with decreasing O_2 concentrations (equation 2), the definition was more specified. In this paper the RQB is considered the O_2 concentration where the RQ exceeded a specific limit: 0.5 units higher than the RQ measured in ambient air. This limit value was chosen because a RQ as high as 1.5 is still regarded as being safe for storage (Wollin et al., 1985). By using gas exchange rates the total ATP production at various O_2 concentrations can be estimated (Andrich et al., 1994). The oxidative ATP production ($V_{ATP(o)}$, $\mu mol.kg^{-1}.h^{-1}$) was derived from the O_2 consumption rate (equation 1, V_{O2} in $ml.kg^{-1}.h^{-1}$) using a conversion factor based on the ideal gas law:

$$V_{ATP(o)} = V_{O2} * 6 * 41.87 \qquad (3)$$

where 41.87 ($\mu mol.ml^{-1}$) is the conversion factor (at 18°C and 101.3 kPa) and 6 is the ATP/O_2 ratio. It is assumed that the production of ethanol and CO_2 is the main fermentation route (Perata and Alpi, 1993; Ricard et al., 1995). Therefore the fermentative ATP ($V_{ATP(f)}$) production was derived from the fermentative CO_2 production (second term in equation 2):

$$V_{ATP(f)} = \left(\frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}} \right)} \right) * 41.87 \qquad (4)$$

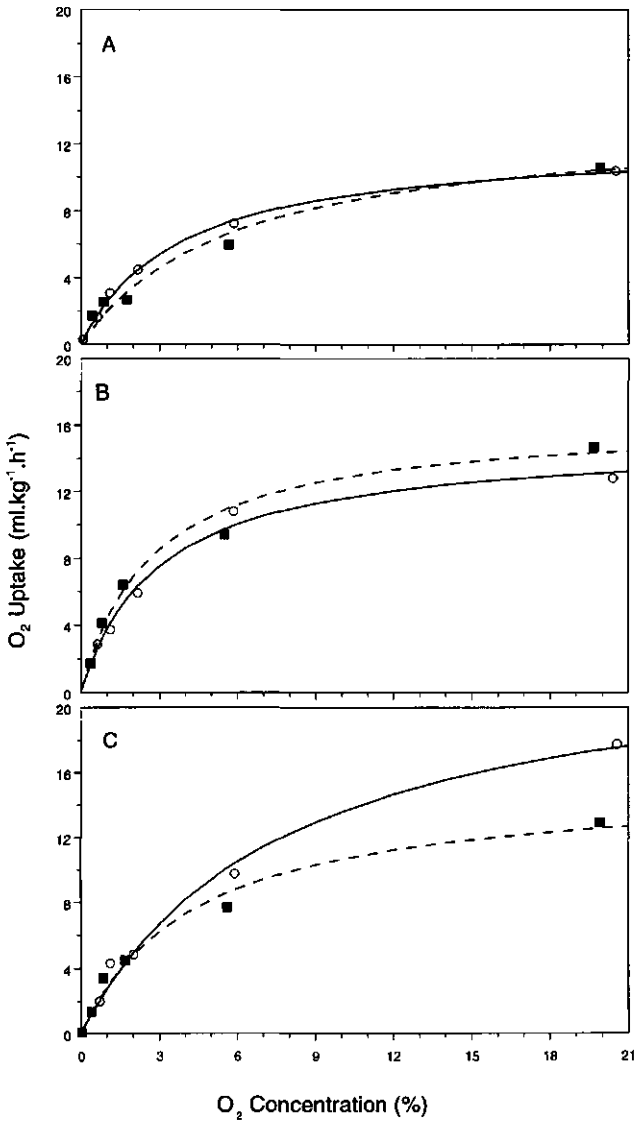

Figure 1. *The O_2 consumption (ml.kg^{-1}.h^{-1}) data (○ = after harvest, ■ = after storage) and fitted models (——— = after harvest, – – – = after storage). A: first (early) harvest, B: second (optimal) harvest, C: third (late) harvest.*

Processes involved for maintaining cell viability are often referred to as maintenance (Thornley, 1970), and the energy needed for maintenance as maintenance energy (Pirt, 1965) or maintenance requirement for ATP (Zhang and Greenway, 1994). Maintenance energy can be regarded as the lowest ATP production rate necessary to maintain cell viability. The O_2 concentration where all energy production is used for maintenance is referred to as the Maintenance Oxygen Concentration (MOC). The estimated total ATP production, the sum of the calculated oxidative and fermentative ATP production, was compared with a specific limit value (Maintenance Energy Requirements). The O_2 concentration where the ATP production equalled the value for MER was considered to be the MOC and an estimate for the optimal O_2 concentration. At the moment no value for MER is known. Therefore the ATP production rate at 1.2% O_2 (the advised optimal storage condition for Dutch apples, Meheriuk, 1993) at the optimal harvest data (harvest 2) was used as a reasonable estimation of MER.

Results

The results of the gas exchange measurements and the fitting of the models are shown in Fig. 1 (O_2 consumption), Fig. 2 (CO_2 production) and Fig. 3 (RQ). The percentage of the variation accounted for (R^2) of the O_2 consumption model ranged between 91.5 and 97.8% (table 1).

Table 1. ***Regression analysis of O_2 consumption and CO_2 production data.***
R^2 = Percentage variance accounted for (indication for the goodness of fit) and $_{adj}$ = adjusted for the number of parameters. est = estimated values, se = standard error.

	Harvest 1				Harvest 2				Harvest 3			
	start		storage		start		storage		start		storage	
	est	se	est	se	est	se	est	se	est	se	est	se
O_2 Consumption												
R^2	93.9		96.4		97.8		91.5		94.7		94.4	
Vm_{O2}	12.2	1.1	13.5	1.1	15.1	0.71	16.3	1.6	24.3	2.8	15.3	1.4
Km_{O2}	3.78	0.89	5.83	1.33	3.00	0.40	2.74	0.81	7.85	2.07	4.37	1.09
CO_2 Production												
R^2	69.7		80.4		84.4		96.4		68.9		93.5	
RQ_{ox}	0.860	0.099	1.08	0.09	1.01	0.06	1.01	0.03	0.945	0.120	1.00	0.05
Vmf_{CO2}	15.6	1.5	13.2	2.4	21.4	1.4	11.8	0.7	26.2	2.8	18.6	1.4
Kmf_{O2}	0.722	0.217	0.136	0.073	0.503	0.098	0.273	0.048	0.712	0.222	0.152	0.035

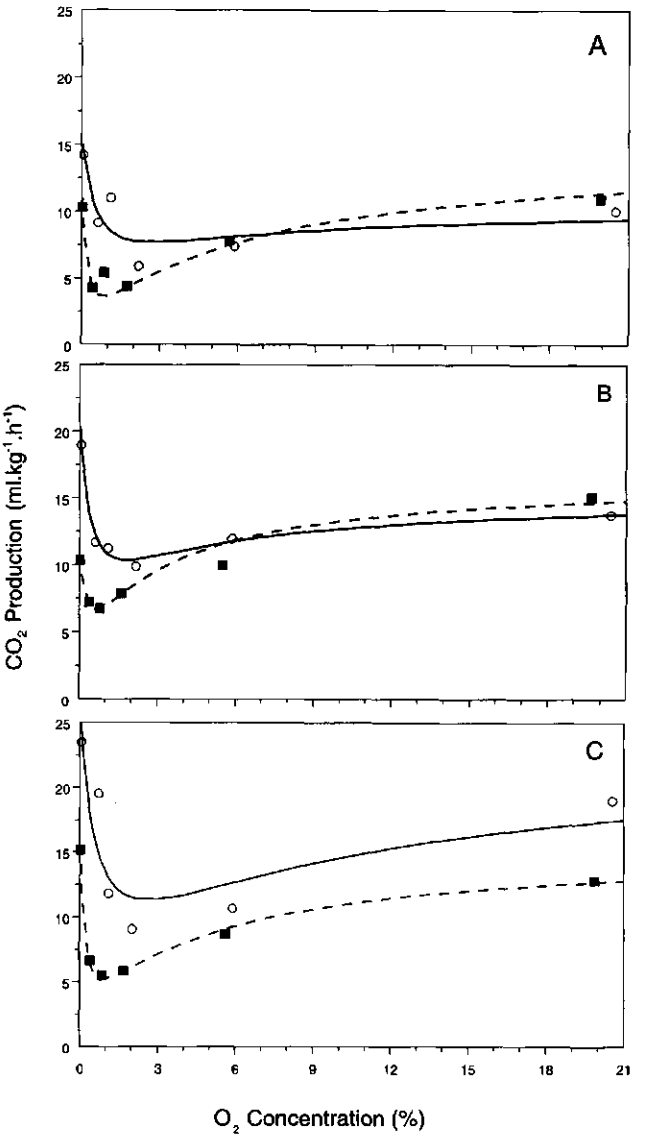

Figure 2. *The CO_2 production (ml.kg^{-1}.h^{-1}) data (O = after harvest, ■ = after storage) and fitted models (——— = after harvest, – – – = after storage). A: first (early) harvest, B: second (optimal) harvest, C: third (late) harvest.*

The modelling results for the CO_2 production model were less, specially for the measurements directly after harvest, with R^2 ranging between 68.9 and 96.4 (table 1). Different values for the model parameters were found for the different harvest dates and storage periods (table 1). The O_2 consumption rate measured directly after harvest in ambient air (21% O_2) increased with harvest date (Fig. 1). The O_2 consumption rate measured directly after harvest was comparable to the rate measured after the storage period for both the early and the 'optimal' harvest (Fig. 1a, 1b). For the late harvest the O_2 consumption rate measured in ambient air was decreased after storage (Fig. 1c). The Km values for O_2 consumption (Km_{O2}) were lowest for the optimal harvest (table 1). This was found directly after harvest as well as after the storage period.

The measurement of the CO_2 production always showed a typical lowest rate at a low O_2 concentration, enabling the establishment of the ACP (Fig. 2). For apples of all three harvest dates the maximum fermentative CO_2 production rate (Vmf_{CO2}), established using the CO_2 production rate at 0% O_2, was decreased after the storage period (table 1). This was also the case for Kmf_{O2}, the O_2 concentration where fermentative CO_2 production reached 50% of its maximum rate. All the RQ_{ox} values found were close to 1, except for the apples of the first harvest directly after harvest where RQ_{ox} was 0.86 (table 1). Based on gas exchange data the values found for the ACP and the RQB were almost equal (table 2). Model calculations almost always resulted in considerably higher O_2 concentrations for the RQB than the ACP (table 2). Interestingly the ACP and the RQB, based on data as well as the models, almost always decreased to lower O_2 concentrations after storage, suggesting optimal

Table 2. ***Estimated optimal O_2 concentrations for storage (in %).***
ACP = Anaerobic Compensation Point, RQB = Respiratory Quotient Breakpoint, MOC = Maintenance Oxygen Concentration. Data: values derived from gas exchange data, Model: values derived from model calculations.

		ACP		RQB		MOC	
		start	storage	start	storage	start	storage
Harvest 1	data	2.18	0.41	1.65	1.76		
	model	2.76	0.82	2.71	1.45	2.40	3.74
Harvest 2	data	2.17	0.80	2.17	0.80		
	model	1.86	0.62	2.61	1.11	1.19	1.20
Harvest 3	data	2.02	0.86	2.02	0.86		
	model	2.68	0.94	3.65	1.38	1.53	2.21

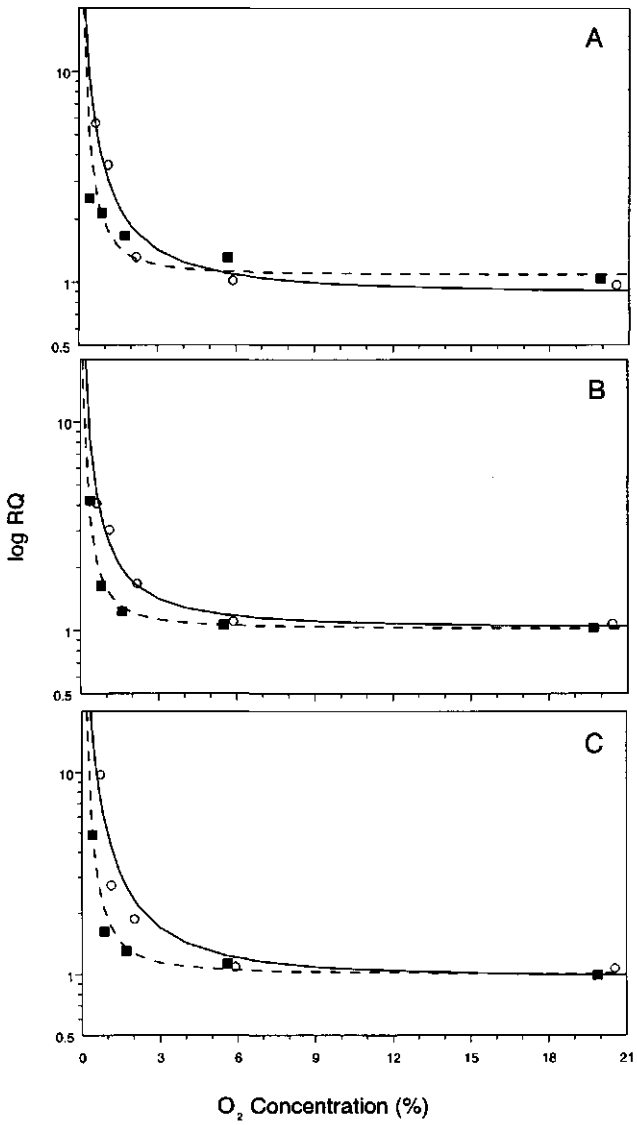

Figure 3. *The RQ data (○ = after harvest, ■ = after storage) and fitted models (——— = after harvest, – – – = after storage). A: first (early) harvest, B: second (optimal) harvest, C: third (late) harvest.*

concentrations to decrease during storage (Figs 2 and 3). In contrast the MOC increased after storage for apples of the first and the third harvest date (Fig. 4, table 2). Only for the second harvest date the MOC remained equal (table 2).
When the three harvest dates were compared, optimal oxygen concentrations as derived with the ACP and the RQB are almost equal (based on the gas exchange data). The model calculations, however, indicated a lower optimal O_2 concentration for the second harvest using the ACP, the RQB and the MOC. This expected higher tolerance of apples of the second harvest to low O_2 was found directly after harvest and after storage.

Discussion

The O_2 concentrations supposed not to be injurious differ considerably when derived by the ACP, the RQB or the MOC. Assuming one O_2 concentration to be injurious for apples of the same harvest, a conclusion is that these concepts are not valid simultaneously. To have more clarity on the validity of the concepts they are examined more closely. Two practical aspects of using respiratory characteristics are considered: a. the use of gas exchange measurements directly after harvest as an indicator for (fixed) gas concentrations during storage, b. the use of gas exchange measurements during storage as a tool to change storage concentrations.

Meheriuk gives an optimal O_2 concentration of 1.2% for the storage of Cox Orange Pippin apples grown in the Netherlands. Apples picked in 1994 and stored commercially at this O_2 concentration showed good quality after storage. Therefore 1.2% can be considered a good storage concentration for apples of this particular year. The optimal concentrations derived from the measurements directly after harvest, however, are always higher than 1.2% O_2 (table 2). Only the MOC of apples of harvest 2 is equal, but this is due to definition used (see material and methods). By adjusting limit values for the RQB and the MOC lower O_2 concentrations can be derived. The limit used to establish the RQB should be more than 0.5 units higher than the RQ measured in ambient air. Based on Fig 3. the limit would approximately be 3.6 for the first harvest, 2.8 for the second harvest and 3 for the third harvest. These RQ values are considerably higher than the RQ's of Gran and Beaudry (1993), who used RQ values between 1.5 and 2, dependent on the number of data available. Remarkably the RQ values found by Gran and Beaudry (1993) at high O_2 concentrations (10-15

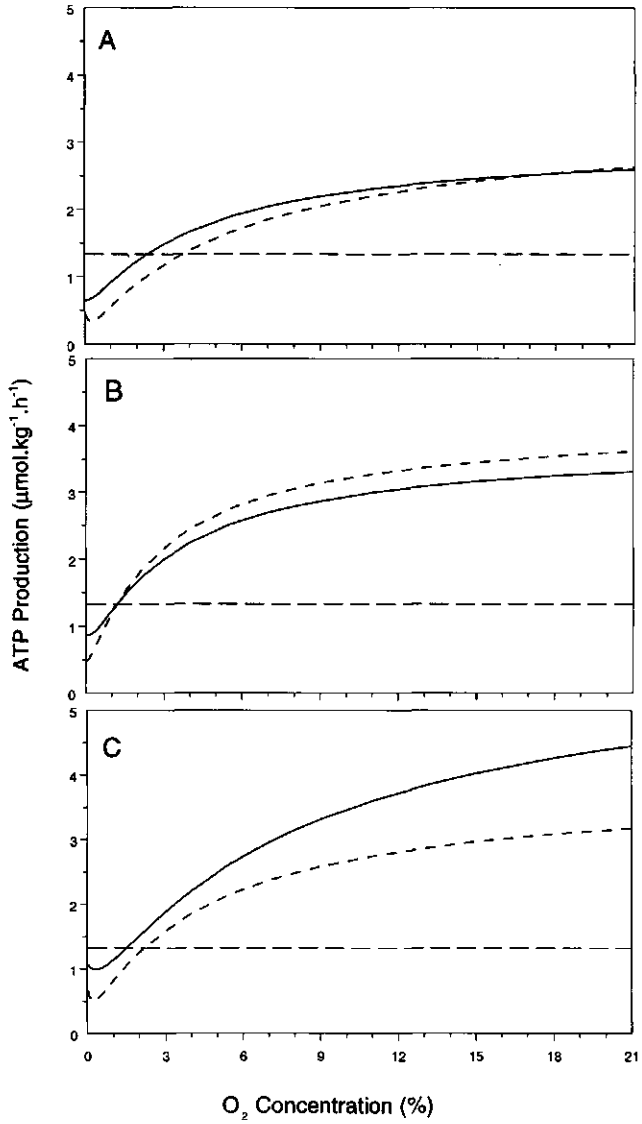

Figure 4. *The ATP production (mmoles.kg^{-1}.h^{-1}) derived from the models (——— = after harvest, - - - - = after storage, — — — = limit / MER). A: first (early) harvest, B: second (optimal) harvest. C: third (late) harvest.*

kPa) are also close to 1.5, which differs from the data found in the current measurements. The different temperature during the gas exchange measurements (1°C for Gran and Beaudry, 1993) might be the cause of the differences found.
The RQB seems useful for a quick determination of the optimal O_2 concentration, but a more clear definition of the RQB is required. The MOC was set at 1.2% O_2 by definition. Therefore the use of the MOC can only be judged when measurements of several years result in comparable values (and storage results). The ACP is directly derived from a graphical representation, and cannot be modified by adjusting limit values. CO_2 production can only be lowest at one O_2 concentration. The ACP therefore seems unsuitable for a quick determination of the optimal O_2 concentration of the apples used.
It is known that apples of different harvest date or maturity react differently to the same O_2 concentrations (North et al., 1977, Blanpied and Silsby, 1989; Curry, 1989). After storing Cox Orange Pippin apples of three harvest dates at 1.25% O_2, North et al. first noticed internal disorders (brown tissue) in the late picked apples. Based on current gas exchange characteristics different optimal O_2 concentrations were found between the different harvest dates. In general the established optimal O_2 concentration of the second (optimal) harvest is the lowest (table 2), suggesting the highest tolerance to low O_2 concentrations. This result underlines the possible use of gas exchange characteristics in establishing optimal O_2 concentrations.
Optimal O_2 concentrations, as derived by ACP, RQB and MOC, seem to change in time. One practical aspect of changing optimal O_2 concentrations is that a fixed O_2 concentration for storage will not be the best procedure. One solution could be dynamic control of storage concentrations (Wollin et al., 1985). When respiratory characteristics are to be used for this purpose, changes in the derived optimal concentrations should correspond to changes in optimal concentrations found in practice. The optimal O_2 concentrations established by ACP and RQB were found to change to lower values after storage. The MOC, however, changed to higher O_2 concentrations, indicating a decreased tolerance to low O_2 after storage. Only this latter result agrees with results found in practice. Lidster et al. (1985) found that the storage of McIntosh apples for three months at 1% O_2 and subsequently in 3% O_2 resulted in lower low oxygen injury and senescent disorder levels then apples stored continuously at 1% O_2 (more injury) or 3% O_2 (more disorders). Comparable results were found by Little and Peggie (1987). Kader et al. (1989) state that the limit of tolerance to low O_2

would be higher than advised if storage duration is increased. This suggests a decrease of the tolerance to low O_2 concentrations and an increase of optimal O_2 conditions.

Wollin et al. (1985) assumed that if the product is kept at the O_2 concentration with minimal CO_2 production (equal to the ACP), no low O_2 injury was to be found. The O_2 concentration should be continuously monitored and adjusted if necessary. Considering the data found the ACP is changing indeed, but to other O_2 concentrations than are expected to be beneficial. Using the ACP or the RQB for dynamic control could lead to severe problems. A combination of gas exchange rates and models which enable the calculation of ATP production might reduce that risk.

According to Pfister-Sieber and Brändle (1994) changes in tolerance of plant tissues to low O_2 concentrations is probably a matter of minor changes in metabolic rates. Based on maintenance energy requirements (MER) two hypothetic explanations can be given for this change. The first explanation is that metabolic rates decrease, especially the fermentative rate, leading to a lower ATP production rate at a low O_2 concentration. The second explanation is that MER is not constant, but possibly increases during storage, leading to a shortage of ATP at low O_2 concentrations. In both cases the storage of fruit at a constant low O_2 concentration leads to an increase of injury levels. The decrease in fermentative rate after storage (Fig. 2), leading to a decrease in ATP production at low O_2 concentrations (Fig. 4), supports the first hypothesis if a constant value for MER is assumed. Consequently an increased fermentation rate (or glycolytic rate; Pasteur effect) would increase ATP production and tolerance to low O_2. This latter phenomenon is observed by Waters et al. (1991), studying root apices of wheat. In this paper Maintenance Energy Requirements are assumed to be constant at varying O_2 concentrations (Fig. 4). Zhang and Greenway (1994) calculated that maintenance energy requirements under anoxia are lower than in air. They assumed, however, that the plant tissue they studied used all the energy produced for maintenance, irrespective of gas conditions. It is unclear, however, if the division of energy use between growth or maintenance in this case is sufficient. Apples after harvest do not show 'growth', but do show processes related to ripening (maturation) which require energy. In fact the basis of the storage under low oxygen concentrations of fruits and vegetables is that ripening is reduced (Kader et al., 1989). A situation without growth is not necessarily a situation with only maintenance.

Therefore it is proposed that total ATP production (V_{ATP}) is described as:

$$V_{ATP} = V_{ATP(g)} + V_{ATP(r)} + V_{ATP(m)} \quad (5)$$

where $V_{ATP(g)}$= ATP production used for growth, $V_{ATP(r)}$= ATP production used for processes related to ripening, $V_{ATP(m)}$= ATP production used for maintenance of cell viability. The MER level used in this paper, is based on $V_{ATP(m)}$ only, and can be referred to as the minimum ATP production necessary to maintain cell viability. We hypothise that more research on the relationship between maintenance energy requirements and cell injury will clarify an important part of the changes in tolerance to low O_2 concentrations (or optimal O_2 concentrations) during ageing or maturation of harvested plant tissues.

8

Alcoholic fermentation of apple fruits at various oxygen concentrations. Model prediction and photoacoustic detection.

H.W. Peppelenbos, H. Zuckermann, S.A. Robat

Abstract

Apples (*Malus domestica* Borkh., cv. Elstar) were stored at various O_2 concentrations, ranging between normoxia and anoxia. Gas exchange rates and the production of acetaldehyde and ethanol were measured. A gas exchange model, which distinguishes oxidative from fermentative CO_2 production, was fitted to the data. The results indicate alcoholic fermentation to be active at all the O_2 concentrations used, and increasing in importance when O_2 concentrations are lowered. After calculating the amount of metabolites in the apple tissue from the data measured in air, a close relationship was found between model predictions of alcoholic fermentation rates and measured metabolite production in normoxia and anoxia. In hypoxia, however, the model predicted higher CO_2 production rates in comparison to the metabolites actually found. Because the model was fitted to CO_2 production data, this indicates another source for CO_2 in hypoxia than alcoholic fermentation.

Introduction

Higher plants are aerobic organisms and need oxygen to survive (Perata and Alpi, 1993). When plant tissues are exposed to low oxygen concentrations also anaerobic processes are found. The duration of survival of plant organs under anoxia varies from a few hours to several days (Ricard et al., 1994). The ATP required to keep anaerobic tissues alive is generated in fermentation (Pfister-Sieber and Brändle, 1994). Active fermentation is thought to be restricted to hypoxia and anoxia only. Therefore fermentation is often described as an on/off process: the absence of oxygen results in a *switch* from respiration to fermentation (Perata and Alpi, 1993), fermentation usually *starts* with lactic acid formation (Pfister-Sieber and Brändle, 1994), and a change in pH is triggering the *switch* from lactic acid production into ethanolic fermentation (Fox et al., 1995).

Despite some debate on the role of lactic acid in this pH shift, ethanol is almost always found in plant tissues under hypoxia or anoxia (Perata and Alpi, 1993). Sometimes ethanol is also detected in plant tissues kept in air. In the headspace ethanol was found for muskmelon seeds (Pesis and Ng, 1984) and tobacco leave cultures (Bucher et al., 1994; Yang et al., 1995). Also acetaldehyde, the precursor of ethanol, is detected around fruits, seeds and leaves kept in air (Thomas, 1925; Pesis and Ng, 1984; Yang et al., 1995). Ricard et al. (1994) explain this phenomenon by (partial) hypoxia within these tissues, because oxygen diffusion was hampered by a water layer (leave culture), impermeable coats (germinating seeds) or bulky tissues (fruits). Nevertheless it is not clear yet whether fermentation is functional under aerobic conditions, or at what level of hypoxia it becomes so (Ricard et al., 1994).

When Peppelenbos et al. (1996) developed models to calculate CO_2 production of harvested plant tissues stored under various O_2 concentrations, they included a term for alcoholic fermentation. The models used fitted well to experimental gas exchange data, but also suggested that fermentation is always active, albeit at low rates at high O_2 concentrations. Because this seems in contrast with current knowledge, it is unclear whether these models are actually describing alcoholic fermentation.

The evidence for ethanol or acetaldehyde production in ambient air greatly depends on the sensitivity of the detection method (Ricard et al., 1994). Measured in the head space by GC techniques the lower concentration found by Kimmerer and MacDonald (1987) for ethanol and acetaldehyde was 0.05 $ng.ml^{-1}$, while the lowest detectable production found by Pesis and Ng (1984) was 1 $\mu mol.g^{-1}$ for ethanol and 13 $nmol.g^{-1}$

for acetaldehyde, and 2 nmol.g^{-1} for ethanol and acetaldehyde by Bucher et al. (1994). Recently, in addition to GC techniques, also laser driven techniques were used to measure volatiles in air (Woltering et al. 1988; Harren et al., 1990). The use of these latter techniques enabled a much lower detection limit for ethylene.
The focus of this paper was to apply this latter technique for the measurement of ethanol and acetaldehyde of apples stored under various gas conditions. The data found were compared with one of the models describing oxidative and fermentative CO_2 production (Peppelenbos et al., 1996).

Material and methods

Gas exchange measurements
The product selected for the experiment was apple (*Malus domestica* Borkh., cv. Elstar). The harvest of the (preclimacteric) apples took place in september 1995 in the Netherlands. After three months of storage under Controlled Atmosphere conditions (1°C, 1.2% O_2 and 0.5% CO_2) the apples were placed in air tight flasks. The flasks were connected to a flow through system.
Six different O_2 concentrations, in 3 replicates, were provided to the apples: 0, 0.5, 1, 2, 6 and 21% supplemented with N_2. The gas mixtures applied contained no argon. The CO_2 concentration was kept below 500 ppm. The gas was led through a water flask, resulting in a relative humidity close to saturation (above 98% r.h.). The flasks were kept in a temperature controlled room, with a temperature of 20°C. After three, four and five days under the mentioned conditions gas exchange measurements were carried out as described in Peppelenbos and Van 't Leven (1996). In the method used the flasks are closed, and relative small changes in O_2 and CO_2 are monitored. After the gas exchange measurements (lasting 2 hours) the flasks were connected again to the flow through system. After 4 and 5 days of storage 6 flasks were kept in closed condition after the gas exchange measurements, in order to carry out head space analysis of ethanol and acetaldehyde. These 6 flasks were no longer used for gas exchange measurements, resulting 12 flasks measured at day five. The gas exchange measurements were repeated with 18 other apples one week after the first measurements.

Acetaldehyde and ethanol measurement

Ethanol and acetaldehyde were detected using a photoacoustic (PA) detection technique. Details on the technique are described by Harren et al. (1990) and Bijnen (1995). The main device used is a CO-laser, which enables a conversion of radiation into acoustic energy. The laser is line tunable over a large infrared frequency range (350 lines between 1200 to 2100 cm^{-1}), where many gasses show a specific 'fingerprint' absorption. Via collisional relaxation, the excited molecules transfer their vibrational energy to translational energy, which results in a pressure increase. The laser radiation is modulated at a frequency of about 1 kHz by a chopper so that the gas absorption is generating a periodic pressure modulation: a sound. The absorption cell (PA cell) is built as a acoustic resonator to optimally sustain this periodic pressure modulation. A condensor microphone mounted at the anti-node of the resonator detects the sound. The PA cell is placed inside the CO-laser cavity to profit from the one order of magnitude increase in laser power as compared to an extracavity position. A multi component analysis of the gas in the headspace of the apple cuvette was performed by tuning the grating to 5 specific laser lines and measuring the PA signals which are proportional to the absorption (after normalization to the intracavity laser power). The absorption coefficients of acetaldehyde, ethanol and water vapour at these laser lines were determined beforehand. The concentrations of these gasses were calculated using the mathematical formalism of Meyer and Sigrist (1990), involving matrix manipulation. Concentrations were corrected for background concentrations found at the start of every measurement. A full cycle of positioning the grating and measuring on 5 lines took about 5 minutes.

Head space gas of the flasks containing the apples was brought into the PA detector by flushing it with pure N_2 of 0.5 liter/hour. To reduce the influence of water vapour on the measurements a cooling trap and teflon tubing (FEP) were used for the gas flow. Stable readings were found after 0.4 hours (24 minutes) of flushing. With an average free volume in the flasks of 1423 ml this means a dilution of the O_2 concentrations with about 12%. Since the readings remained stable between 0.4 and 0.7 hours after flushing, we assume that this diluting influence on the rate of acetaldehyde and ethanol production was negligible. The measurements were carried out in combination with the gas exchange measurements. Due to technical problems, however, the first series of measurements resulted in unreliable results (not shown). Therefore only the data collected in the second series were used.

Gas exchange models

The models used to relate gas exchange rates to gas conditions are based on simple Michaelis-Menten type of equations. The O_2 uptake rates measured were fitted to equation 1 (Chevillotte, 1973):

$$VO_2 = \frac{Vm_{O2} * O_2}{(Km_{O2} + O_2)} \quad (1)$$

where Vm_{O2} is the maximum O_2 uptake rate ($ml.kg^{-1}.h^{-1}$) and Km_{O2} the Michaelis constant for O_2 uptake. Because CO_2 can be produced by oxidative and fermentative processes, these processes were described separately. Oxidative CO_2 production is calculated as:

$$VCO_2ox = VO_2 * RQ_{ox} \quad (2)$$

where VCO_2ox is the oxidative CO_2 production rate ($ml.kg^{-1}.h^{-1}$) and RQ_{ox} the RQ of the oxidative processes. Fermentative CO_2 production is calculated using model 2 of Peppelenbos et al. (1996):

$$VCO_2f = \frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}}\right)} \quad (3)$$

where Vmf_{CO2} is the maximum fermentative CO_2 production rate ($ml.kg^{-1}.h^{-1}$) and Kmf_{O2} the Michaelis constant for the inhibition of fermentative CO_2 production. Total CO_2 production, therefore, is the combination of equations 2 and 3:

$$VCO_2 = VO_2 * RQ_{ox} + \frac{Vmf_{CO2}}{1 + \left(\frac{O_2}{Kmf_{O2}}\right)} \quad (4)$$

Statistical analysis

Gas exchange data were compared with equations 1 and 4 using non-linear regression in the statistical package GENSTAT (Rothamstead, U.K.). In all cases the non-linear equations were fitted directly without any transformation, using an iterative method to

maximize the likelihood. At low concentrations of ethanol and acetaldehyde a linear relationship is assumed between levels measured in the gas phase and levels in the tissue (after Kimmerer and MacDonald, 1987). Therefore, to clarify the relationship between fermentation rates as measured by ethanol and acetaldehyde concentrations in the headspace and the modelled fermentative CO_2 production, tissue concentrations of ethanol and acetaldehyde were estimated using GENSTAT and a simple linear equation:

$$CO_2\ f = a * Acetaldehyde + b * Ethanol \quad (5)$$

with CO_2f, ethanol and acetaldehyde in mmoles.$kg^{-1}.h^{-1}$ and a and b empirical constants.

Results

The response of the gas exchange rates of the apples measured to various oxygen concentrations is shown in figures 1A (O_2 uptake) and 1B (CO_2 production). In figure 1B the contribution of both oxidative and fermentative processes to the total CO_2 production, as fitted with the equations used, is shown. Already at O_2 concentrations close to normoxia the equations used predict fermentative CO_2 production, albeit small. When O_2 concentrations are lowered, fermentative CO_2 production increases in importance, until at an O_2 concentration of about 1.1% it equals oxidative CO_2 production (Fig 1B). After fitting equation 1 to the O_2 uptake data a Km_{O2} value of 5.7 was found (Table 1), meaning that at an O_2 concentration of 0.5% O_2 uptake rates were decreased to 50% of its maximum rate. For fermentative CO_2 production a Kmf_{O2}

Table 1. *Regression analysis of gas exchange data on O_2 consumption and CO_2 production models. R^2 = Percentage variance accounted for (indication for the goodness of fit), $_{adj}$ = adjusted for the number of parameters, est = estimated values, se = standard error.*

Model	R^2_{adj}	variate	est	se
O_2 Consumption	93.4	Vm_{O2}	9.44	0.60
		Km_{O2}	5.72	0.95
CO_2 Production	61.1	RQ_{ox}	0.956	0.043
		Vmf_{CO2}	6.67	0.57
		Kmf_{O2}	0.506	0.142

of 0.51 was found, indicating that at an O_2 concentration of 0.5% the fermentative CO_2 production reached 50% of its maximum rate. The RQ of the oxidative processes was estimated as 0.956 (Table 1). Acetaldehyde and ethanol were detected at all O_2 concentrations applied, including concentrations close to normoxia (Table 3). The lowest acetaldehyde and ethanol concentrations found were 0.3 ppb and 30 ppb respectively.

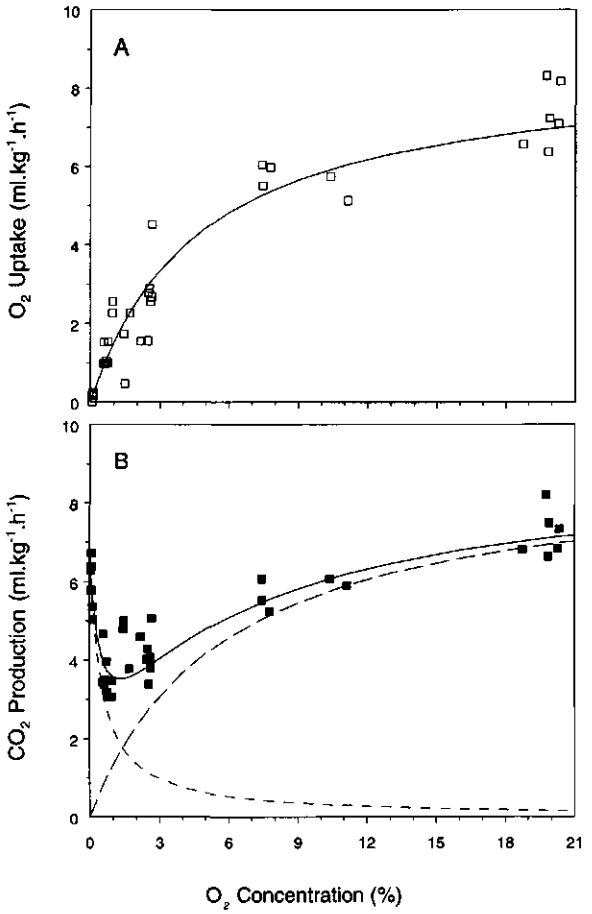

Figure 1. *Gas exchange rates of Cox Orange Pippin apples stored at various O_2 concentrations. A: O_2 uptake: measurements (□) and model fit (———). B: CO_2 production: measurements (■) and model fit; total CO_2 production (———), oxidative CO_2 production (— — —) and fermentative CO_2 production (– – – –).*

A typical detection curve is shown in figure 2, where two special features appear due to the flushing of the headspace around the apples to the sample cell. First a background concentration is found, which had to be corrected for (measurements after 18-24 minutes). The second feature is that stable concentrations are reached after 42 minutes. Measurements after this time period were used to estimate acetaldehyde and ethanol concentrations. The concentrations were used to calculate production rates. Because gas exchange rates were derived at concentrations changing 0.2% in O_2 maximally, it is assumed that metabolic rates are in equilibrium with external gas conditions. Therefore the CO_2 production found (and fitted) can be considered as the actual CO_2 production inside apple tissues. Acetaldehyde and ethanol, however, were measured in the gas phase surrounding the apples. It is known that concentrations of acetaldehyde and ethanol are higher within tissues than in the gas phase. For a good comparison between measurements on metabolites and the predicted fermentation rates the internal metabolite concentrations were calculated. The conversion factors used were estimated by equation 5, revealing 267 for acetaldehyde and 377 for ethanol (meaning internal ethanol concentrations to be 377 times higher inside than outside; Table 2).

Table 2. ***Ratio between ethanol and acetaldehyde in tissue and in air.***
(data of North and Cockburn (1975) were transformed into umol.g^{-1}.h^{-1} before calculating the ratio, and the equation given by Kimmerer et al. (1987) was used for air concentrations below 100 ppm).

Source	tissue	Acetaldehyde	Ethanol
North, Cockburn (1975)	whole apples (cv. Golden Delicious)		226-303
	whole apples (cv. H. Wonder with Grenadier)		718-857
Kimmerer et al. (1987)	leaves of woody plants	334	5001
CO_2 model fitted to data	whole apples	267	377

When predicted fermentative CO_2 production was compared with the summation of (calculated) internal acetaldehyde and ethanol concentrations a good relation was found at the lowest O_2 concentrations used, but the relation was less at higher O_2 concentrations (Table 3). Especially at hypoxia the predicted fermentative CO_2 production exceeds the ethanol and acetaldehyde production considerably (fig. 3).

Table 3. ***Ethanol and acetaldehyde production and predicted fermentative CO_2 production.***
O_2: O_2 concentrations actually measured during the storage period, headspace: acetaldehyde and ethanol production derived from headspace measurements, tissue: calculated production using headspace production and conversion factors of table 2, fermentative CO_2: calculated using equation 3.

day	O_2 (%)	Headspace Acetaldehyde nmol.kg^{-1}.h^{-1}	Headspace Ethanol nmol.kg^{-1}.h^{-1}	Tissue Acetaldehyde µmol.kg^{-1}.h^{-1}	Tissue Ethanol µmol.kg^{-1}.h^{-1}	Tissue Total µmol.kg^{-1}.h^{-1}	Model fermentative CO_2 µmol.kg^{-1}.h^{-1}
4	0.067	422.5	316.9	112.8	119.5	232.3	248.2
5	0.068	339.6	445.9	90.7	168.2	258.9	247.6
4	0.073	340.3	434.5	90.9	163.9	254.7	245.5
5	0.619	32.0	251.7	8.53	94.9	103.5	126.5
4	1.463	0.437	30.2	0.117	11.4	11.5	72.3
5	2.174	0.013	8.83	0.004	3.33	3.33	53.1
5	2.465	0.011	1.09	0.003	0.413	0.416	47.9
4	2.593	0.438	32.8	0.117	12.4	12.5	45.9
4	7.752	0.283	20.2	0.076	7.62	7.70	17.2
5	10.40	0.243	20.8	0.065	7.86	7.93	13.0
5	18.76	0.010	1.01	0.003	0.383	0.385	7.38
4	20.28	0.256	0.00	0.068	0.000	0.068	6.84

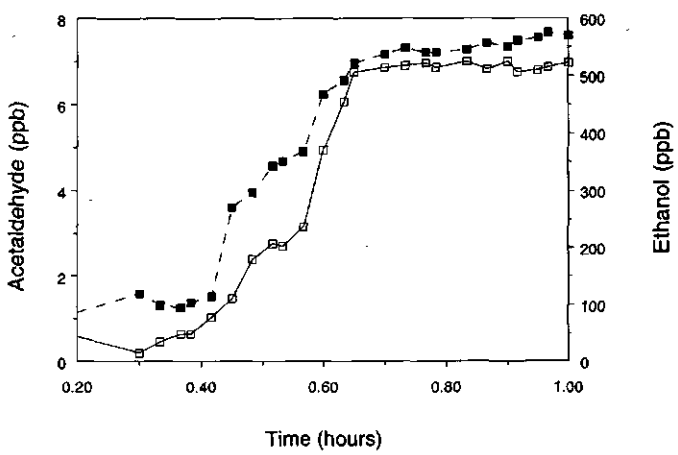

Figure 2. *Typical detection curve of ethanol (■) and acetaldehyde (□) as measured with the PA technique and the methods described (apple in 7.6-7.4% O2).*

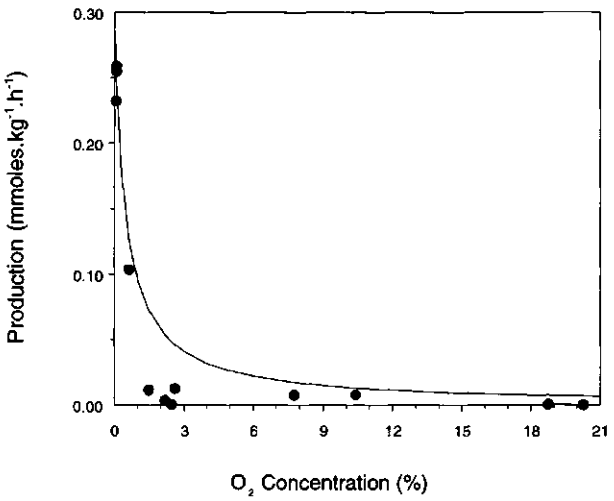

Figure 3. *Total ethanol and acetaldehyde production (●), of Elstar apples stored at various O_2 concentrations, compared with the modelled fermentative CO_2 production (——).*

Discussion

The lowest acetaldehyde and ethanol concentrations found (0.3 ppb and 30 ppb respectively) are remarkably lower than the lowest concentrations detected by Kimmerer and MacDonald (1987): 25.5 ppm and 24.4 ppm respectively. Also much lower production rates could be detected: 0.01 $pmol.g^{-1}.h^{-1}$ of acetaldehyde and 1 $pmol.g^{-1}.h^{-1}$ of ethanol (Table 3), whereas Bucher et al. (1994) found 2 $nmol.g^{-1}$ for ethanol and acetaldehyde. The data found suggest alcoholic fermentation always to be active, even at higher O_2 concentrations. Already in 1933 Fidler also reported 40 µg of ethanol per gram of apple tissue stored in air. More authors found fermentative metabolites in harvested plant tissues kept in air (Colelli et al., 1991; Nanos et al., 1992, Mateos et al., 1993, Larsen and Watkins, 1995). At these conditions the metabolites were always detected in the tissue, but never in the gas phase surrounding the tissue. Kimmerer and Kozlowski, for instance, found no ethanol or acetaldehyde in the gas phase surrounding 11 different higher plants. It seems that fermentation is

active under all O_2 concentrations, although ethanol is not necessarily to be found. This can be due to remetabolization (Bucher et al., 1994) or to the limits of the detection methods used.

The main argument to explain fermentation in ambient air is the existence of O_2 gradients inside bulky plant tissues such as apples. Although the O_2 concentration surrounding the apples equals normoxia, part of the internal gas phase could become hypoxic or even anoxic (Brändle, 1968). This would explain the ethanol production found in ambient air (Knee, 1991b). Nevertheless one has to consider that this was found for apples at the climacteric peak, when gas exchange rates reach maximum levels. For preclimacteric apples, like the apples used in this experiment, internal concentrations found range between 16.1% to 18.9% (Burton, 1974), 12.7% to 14.8% (Solomos, 1987) or 11.7% to 15.1% directly under the skin and 10.6% to 14.8% at the center of the fruit (Rajapakse et al., 1990). Although these internal O_2 concentrations are considerably lower than the external ones, they cannot be considered to resemble hypoxia. This would mean that fermentation is a constitutive process in plant tissues, but increasing in importance when O_2 concentrations are lowered. This hypothesis is in line with data on ADH activity (Andrews et al., 1993), where active ADH is found at O_2 concentrations as high as 40%. The enzyme, however, regarded as limiting for ethanolic fermentation is not ADH but Pyruvate DeCarboxylase or PDC (Kennedy et al., 1992). Although Perata and Alpi (1993) state that PDC is not active in normoxia, other authors suggest a constitutive PDC in leaves and other plant tissues (Kimmerer and MacDonald, 1987; Kennedy et al., 1992). If acetaldehyde, detected in air, was actually derived from pyruvate, this would indicate that fermentative pathways are not fully turned off under aerobic conditions (Ricard et al., 1994). Present data suggest that this hypothesis is correct.

The gas exchange model used to describe fermentative CO_2 production predicts fermentation always to be active, albeit at very low rates when O_2 concentrations are as high as normoxia (Fig 1B). Although qualitatively this seems in accordance with the ethanol and acetaldehyde measurements, it is also important whether the actual production rates are comparable. Therefore modelled fermentative CO_2 production was compared with the sum of estimated internal concentrations of ethanol and acetaldehyde. This revealed that, although model predictions seemed accurate at anoxia, at higher O_2 concentrations the model clearly overestimates fermentative CO_2 production, especially at hypoxia. Since the model was fitted to actual CO_2 production

data, it would suggest another source for CO_2 in addition to fermentation and respiration.

An excess of CO_2 production over ethanol production has been reported before. Leshuk and Saltveit (1991) for instance found increased CO_2 production 7 minutes after exposing carrot discs to anoxia, while increased ethanol production was found after 30 minutes. A possible explanation is that in the first period of anoxia around the exposed tissue the actual O_2 concentrations inside the tissue experiences hypoxia. One metabolic route possibly being active in hypoxia is the operation of a partial TCA cycle (Ricard et al., 1994). It is suggested that reactions occurring in helminths also take place in higher plants, where malate is converted to fumarate and then succinate (Kennedy et al., 1992). Another source for excess CO_2 during anoxia might be the conversion from glutamate to (gamma) amino butyric acid (Bertani and Reggiani, 1991). Both reactions generate CO_2 without ethanol. Good and Muench (1993), however, argue that for root tissues succinate and malate are products of anaerobic fermentation in the short term (1-2 days), but not in the long term. Because for the apples the gas exchange data were derived after 3 to 5 days of storage, it seems that in apples an additional CO_2 producing process is not only temporarily active. Also Yearsly et al. (1996) find for Cox's orange Pippin apples a discrepancy between RQ increase and increase in ethanol production at low O_2 concentrations. When different pathways are involved in the release of CO_2, it questions the use of simple Michaelis-Menten kinetics for the description of CO_2 production.

The ratio between ethanol levels in air and in the apple tissue, as estimated by the fitting procedure using modelling results, is remarkably close to values found by North and Cockburn (1975) for other apple cultivars (Table 2). The value found by Kimmerer et al. (1987) after measurements on woody plants, however, is much higher. Nevertheless the ratio found for acetaldehyde, also found by these authors, is comparable (Table 2). This result indicates that the estimated internal concentrations are dependable, but also underline the close relationship between model predictions and metabolite measurements.

The assumption, based on the CO_2 production model, that fermentation is active at higher O_2 concentrations, seems correct. The model used, however, does not seem to describe fermentation only. When total CO_2 production rates have to be estimated, for applications such as Modified Atmosphere packaging of fruits and vegetables, this is not causing problems (Peppelenbos et al., 1996). When the model is used to

estimate other processes, one has to be cautious. The discrepancy between model predictions and measurements on metabolites clearly stresses the importance of a good description of the processes occurring in hypoxia. The question is whether the CO_2 production model, based on simple Michaelis-Menten type of kinetics, can be used to incorporate additional processes.

Acknowledgements

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9

General discussion.
The role of gas exchange characteristics and models in storing and packaging fruits and vegetables

State of the art

In 1989 Banks et al. opened their paper, presented at the main conference dealing with CA storage and MA packaging, with the complaint that 'despite several decades of MA research, our knowledge of the physiology of MA effects remains sketchy and empirically based'. Establishment of the lower O_2 limit for stored fruit has normally been accomplished by trial and error (Gran and Beaudry, 1993), by keeping fruits or vegetables under various gas conditions for a certain period, revealing a condition as the best to maintain specific quality aspects of the commodity. Because there are so many fruits and vegetables, with different characteristics for every cultivar, location where it was grown and even the year when it was grown, this type of research has the potential of endless continuation.

Banks et al. (1989), however, came with another approach: modelling. A model is no more than a simplified representation of the system studied (Rabbinge and de Wit, 1989). By simulating reality with the model, the properties of the models are studied and compared to those of the systems they represent. If unacceptable discrepancies between model and system are observed, it is possible to judge which aspects are then to be studied experimentally (Rabbinge and de Wit, 1989). 'Even inaccurate models can provide valuable improvements in understanding, and can be used to identify critical parameters regulating the system under study, and thereby assist research programme management' (Banks et al., 1989). Modelling is widely used to gain insight into the functioning of repeatable systems and has proved to be very productive (Rabbinge and de Wit, 1989).

The research carried out in the past 4 years, as described in the previous chapters, was focused on modelling the main processes involved in CA storage and MA packaging: respiration and fermentation. The goal was to enable the calculation and prediction of the response in gas exchange behaviour of a range of products to various gas conditions. This chapter evaluates whether some progress has been made, if practical applications can be derived, if the modelling was linked to physiological processes actually occurring, and if the methods used throughout the experiments are correct.

Respiration and quality changes

In 1995 Brash et al. published a paper entitled 'shelf-life of stored asparagus is strongly related to postharvest respiratory activity'. Generally, the effect of reduced O_2 and/or elevated CO_2 on reducing the respiration rate has been assumed to be the primary reason for the beneficial effects of CA and MA on fresh produce (Kader et al., 1989). Several authors, including Kader himself, have severe critics on this approach. It was well understood by early workers as Kidd and West that the correlation between a reduction in the respiration rate and an extension of storage life was probably an over-simplification (Burton, 1974). Knee (1980) stated: 'increased knowledge of respiratory metabolism does not suggest an obvious mechanism for CO_2 effects or O_2 effects'. For Laties (1995) 'it challenges the imagination to contemplate a time when fruit respiration was the cynosure of ripening and postharvest studies without regard of the role of ethylene'. He continues to say that 'the preoccupation with fruit respiration understandably stemmed from the perception that respiration was a measure of metabolic health and integrity'. In recent years also questions arose on the role of ethylene, since not all fruits exhibit an ethylene-induced climacteric on ripening, and ethylene can also elicit a respiratory response without concomitant ripening. This makes Laties (1995) admit that 'the course and nature of respiration in ripening fruit, whether in or out of cold storage, remains of paramount interest'.

Modelling gas exchange

Traditionally in studies on quality changes of fruits and vegetables under changed gas conditions the focus was laid on respiration. The focus of the research described within this thesis, however, was more and more directed towards the role of fermentation, and its relation with respiration. Questions arose like 'when does fermentation occur?',

'does it start at a specific O_2 concentration as is often thought?', 'what happens in the energy balance when both processes are active?'. The only way to get anywhere was to quantify fluxes. To estimate fermentation rates, first respiration rates had to be accurately calculated: how much oxygen was consumed, how much carbon dioxide produced, at which concentrations. Then models were needed to integrate the data and interpolate between gas concentrations without gas exchange measurements. Once having the gas exchange models, it became possible to estimate energy fluxes, and relate these fluxes to storage behaviour.

Michaelis-Menten kinetics

Although Chevillotte used Michaelis-Menten kinetics already in 1973 to describe respiration rates in relation to O_2 concentrations, only recently the advantages of this type of equation have been recognized (Banks et al., 1989; Andrich et al., 1991; Lee et al., 1991; Peppelenbos et al., 1993). This approach seems more logical because it describes the enzymatic rate of O_2 uptake in plant tissues (Cameron et al., 1995). 'It should be remembered, however, that this is a single enzyme/single substrate model and will only be appropriate to whole plant tissues and organs when a response is dependent upon a single critical reactant acting at a single enzyme site' (Knee, 1980). This is often thought about the simple models as used throughout the thesis. Chevillotte (1973), however, already mentioned that although the kinetics seem to be that of the terminal enzyme, the maximum rate of the reaction is limited by the other components of the respiratory chain (cushioning effect). Which means that the calculated 'overall' rates are related to the total chain of all enzyme reactions involved.

The inhibitory effect of CO_2

Lee et al. (1991) modelled the inhibitory effect of CO_2 on O_2 uptake by an extension of the Michaelis-Menten equation. Cameron et al. (1995) find this a logical application of the inhibitor concept, but find hardly any data in the literature to support a universal inhibition of CO_2 on O_2 uptake. 'Thus, a negative effect of CO_2 partial pressure on O_2 uptake cannot be assumed without adequate confirmation' (Cameron et al., 1995). Using the Michaelis-Menten model, Dadzie et al. (1993) also made the simplifying assumption that variation in carbon dioxide concentrations in the model systems has no effect on the respiration rate. The experiments described in Peppelenbos and van 't Leven (1996; chapter 2), however, did show an inhibitory effect of increased CO_2 on

O_2 uptake of broccoli, asparagus, mungbean and chicory. After comparing the different possible mechanisms to describe this inhibition, the so-called non-competitive type was preferred, confirming the choice of Lee et al. (1991). For the different products, however, the inhibitory action of CO_2 seemed to be different. This seems to confirm the statement of Knee (1980) that 'increased knowledge of respiratory metabolism does not suggest an obvious mechanism for CO_2 effects. Very likely not just one mechanism is involved. This was accommodated by combining both the competitive and uncompetitive type of inhibition into one model, the so called 'combined inhibition'. This latter model showed equal results as the best models using only one type of inhibition. Although the inhibitory effect of CO_2 seems to be well described by an extension of the enzyme kinetics model, it is not clear yet whether only a Michaelis-Menten type of kinetics is involved. Research on the enzymes in the respiratory chain should reveal this information.

Temperature and model functioning

When Controlled Atmosphere (CA) is applied to store products, a fixed temperature is used. Modified Atmosphere (MA) packages, however, can be subjected to changes in temperature during the distribution from packing houses to retailers. These changes can influence the gas composition within the packages (Dadzie et al., 1993; Cameron et al., 1994). Therefore the relation between temperature and gas exchange rates must be known. For every product studied the metabolic rates increase with temperature. Dadzie et al. (1993) and Joles et al. (1994) modelled this influence of temperature by multiplying the maximum uptake rate (Vm_{O2}) as measured at 0°C with a specific Q10 value. Gas exchange data of several authors, on blueberry and raspberry fruits, show the Km_{O2} to increase with temperature (Beaudry et al., 1992; Cameron et al., 1994). Peppelenbos et al. (1993), however, found a decrease in the Km with increasing temperature for mushrooms. The Michaelis-Menten approach requires an accurate description of the Km (Km_{O2}), preferably over a range of conditions (Cameron et al., 1995).

Cameron et al. (1994) modelled both Vm_{O2} as well as Km_{O2} as exponential functions of the temperature. This approach needs per variable (like Vm_{O2}) two additional variables to estimate the influence of the temperature (Cameron et al., 1994). To describe gas exchange rates with the models described within this thesis, using maximally 7 variables (Vm_{O2}, Km_{O2}, Km_{CO2}, RQ_{ox}, Vmf_{CO2}, Kmf_{O2} and Kmf_{CO2}), it could

mean 14 additional variables to estimate. This seems like a re-introduction of extensive empirical research. The risk is that 'something that started as an explanatory model degenerates progressively into a cumbersome descriptive model' (Rabbinge and de Wit, 1989). Therefore a more fundamental approach seems necessary. Since models are used based on enzyme kinetics, the focus should be on the relation between temperature and enzyme functioning. Also the influence of temperature on diffusion characteristics might contribute to the changes observed in experiments.

Modelling fermentative CO_2 production

In chapter 3 several models, based on enzyme kinetics, were tested on describing oxidative and non-oxidative CO_2 production. After statistical analysis two models appeared to function well. Based on physiological considerations no choice in favor of one of them could be made. Nevertheless in the other chapters of this thesis (4, 5, 6, 7 and 8) only one model is used; the one which uses O_2 as an inhibitor of alcoholic fermentation. This was only based on the fact that this model was slightly simpler and easier to test than the one using oxidative ATP production as an inhibitor of alcoholic fermentation.

Anaerobic metabolism in plants appears much less diverse than it did 10 years ago. Although other pathways than ethanol fermentation exist, they remain quantitatively minor (Ricard et al., 1994). Therefore calculating CO_2 from ethanolic fermentation seems to cover most of the non-respiratory CO_2 production. During the first hours of anoxia, it is necessary to determine the sum of the main products accumulated (ethanol, lactate and alanine), and this gives a mean fermentation rate for the period considered. The determination of the rate of accumulation of ethanol alone may be acceptable for later times of anoxia (Ricard et al., 1994). The assumption, based on the CO_2 production model, that ethanolic fermentation is active at higher O_2 concentrations, seems correct. The model used, however, does not seem to describe fermentation only. Results from chapter 8 suggest an additional CO_2 source, especially at hypoxia. An excess of CO_2 production over ethanol production has been reported before (Leshuk and Saltveit, 1991; Ricard et al., 1994; Kennedy et al., 1992; Bertani and Reggiani, 1991). Although several theories are posed to explain this phenomenon, the physiology remains unclear. When total CO_2 production rates have to be estimated, for applications such as Modified Atmosphere packaging of fruits and vegetables, there appear to be no problems since the model fits to total CO_2 production including the

additional source (chapter 8). When the model is used to estimate other processes than gas exchange, one has to be cautious. In chapter 7 for instance, fermentative rates were used to estimate ATP production. Fermentative CO_2 production was estimated from the increase in CO_2 production relative to O_2 uptake. If part of this CO_2 production cannot be attributed to fermentation, the estimated contribution of fermentation to total ATP production is overestimated. To calculate the energy status, also the contribution of the additional proces(ses) have to be quantified. Ricard et al. (1994), however, state that in higher plants the synthesis of succinate is a quantitatively minor pathway of anaerobic metabolism in terms of ATP production. Also other processes than succinate synthesis, like amino butyric acid synthesis (Bertani and Reggiani, 1991), could occur during hypoxia contributing to ATP production. If energy status is actually related to disorders occurring during CA storage, as was hypothised in chapter 7, a better insight in and quantification of these processes occurring during hypoxia are needed.

Diffusion resistances and gas exchange rates

So far most gas exchange models have used the level of O_2 outside the fruit as a value of substrate availability; yet, it is the level of O_2 inside the product that limits O_2 uptake by enzyme systems (Cameron et al., 1995). Fruits themselves can be compared with modified atmosphere packages within which their tissues reside (Dadzie et al., 1993). This concept considers the fruit skin as the major barrier for gas diffusion. Cameron and Yang (1982) developed an elegant method of measuring this specific diffusion resistance by using ethane: 'We chose ethane for preloading because it is neither produced or metabolized to a significant degree by the tissue, it should have properties similar to ethylene, and it can be quickly and accurately determined at low concentrations by gas chromatography'. Nowadays ethane production is commonly associated with membrane damage and cell death, and is increasing with increasing injury of plant tissues (Abeles et al., 1992). Specially when research is focused on a relation between changes in diffusion resistance of fruits during storage and the onset of storage disorders, this ethane production might influence the measurements. Another disadvantage of ethane or other hydrocarbon gases in tissues with high fat content is their high solubility in lipids. For instance, avocados give unrealistically high intercellular volume when ethane is used for measurement (Solomos, 1987). These two arguments question the use of ethane. Neon seems a good alternative, since it is

not being produced in any metabolic process. The solubility, however, still has to be tested to prevent the same mistakes as mentioned by Solomos (1987).
After deriving resistance values for neon diffusion in plant tissues, and for ethane diffusion as well, it is important to know the relation with resistance values for O_2 and CO_2. This issue was not addressed by Cameron and Yang (1982) or Knee (1991a). Banks (1985) suggests that ethane diffusion is probably similar to O_2 and ethylene diffusion. Using Graham's law, predictions on the relationship between the diffusion of the various gasses can be made, which is done in chapter 6. Based on this law the diffusion rates of O_2, ethane and ethylene should be comparable, but the diffusion rate of neon should be 18% higher and of CO_2 20% lower. The diffusion routes, however, of neon, ethane, O_2 and CO_2 are not necessarily equal. The movement of O_2 is thought to be restricted to stomata or lenticels, but CO_2 may also diffuse directly through the cuticle of fruits (Banks, 1985). Also biochemical processes are involved when for instance CO_2 from the gas phase dissolves into the water phase of the fruit cells (Brown, 1985). This makes the overall use of Graham's law questionable. A real comparison of resistance values is necessary, and for this purpose one might combine the method of Rajapakse et al. (1990) and the ethane (Cameron and Yang, 1982) or the neon method.

Another possible drawback of the method of loading or preloading with an inert gas, is that the approach uses Fick's law to calculate diffusion. This, however, can only be done if the skin is the major barrier for diffusion (Knee, 1991a,b), and if the rate of CO_2 production is uniform throughout the tissue (Solomos, 1987). From research by Rajapakse et al. (1990) it is known that for some products diffusivity in flesh tissues must be taken into consideration. In apples, however, the percentage of the total O_2 gradient between the external atmosphere and the internal core cavity caused by flesh was found to be only 4.5% in Cox's Orange Pippin and 11% in Braeburn apples (Rajapakse, 1990). This indicates that the values found for the three apple cultivars in chapter 5 are very likely close to the overall diffusion resistance to gas diffusion. For products other than apple the contribution of flesh resistance can add up to 30% in Asian pears and 56% in nectarines (Rajapakse et al., 1990). Therefore both the ethane and the neon method cannot be simply applied to all types of commodities. For apple, the most important product for CA storage, the methods seem appropriate.

The experimental setup

Chevillotte (1973) concluded that the single enzyme model is the only representation which leads to a satisfactory study at the cytochrome oxidase-oxygen reaction level *in vivo*, when possible changes brought about by diffusion are taken into account. When the current results are analyzed, differences in Km values can be attributed to diffusion limitations. The result that the models show a worse functioning in the case of Elstar and Cox's Orange Pippin apples is more difficult to understand. If the resistance values obtained are correct, the main reason for the worse model behaviour might be due to the experimental setup. Before this can be explained, it has to be noted that Elstar and Cox's Orange Pippin apples showed higher resistance values, lower porosity than Golden Delicious apples, in combination with equal gas exchange rates in the case of Cox's Orange Pippin. Using headspace techniques to quantify gas exchange rates, the assumption was that gas exchange rates were in equilibrium with gas concentrations. With gas concentrations changing 0.3% at ambient air and 0.1% maximally at O_2 concentrations lower than 5%, this assumption seems correct. When, however, the internal O_2 concentrations were calculated at optimal external O_2 concentrations (Table 3), the internal O_2 concentration of Cox's Orange Pippin apples was 0%. A small change of 0.1% in an external O_2 concentration close to 1% therefore can change the internal atmosphere from hypoxia to anoxia. That surely cannot be regarded as a stable equilibrium situation. It also confirms the idea posed by Pfister-Sieber and Brändle (1994), that changes in tolerance of plant tissues to low O_2 concentrations is probably a matter of minor changes in metabolic rates. The conclusion to be drawn is that also for experimental setups, where often different temperatures are used, some precalculations using gas exchange and diffusion resistances will help to optimize the methods.

Gas exchange characteristics and tolerance to low oxygen concentrations

During oxygen limitation in higher plants, energy metabolism switches from respiration to fermentation (Bucher et al., 1994). Although postharvest studies often regard fermentation as a process to be avoided (see later), other studies see fermentation as another source for ATP, necessary to survive periods of anoxia (Good and Muench, 1993; Bucher et al., 1994; Fox et al., 1994). Nevertheless there is also no simple relationship between survival and the activity of fermentative metabolism (Ricard et al., 1994). This becomes strikingly clear when the minimum ATP need, necessary for plant

tissues to stay alive, is unknown. In fact the energy balance and energy use in non-growing tissues - the so-called maintenance energy requirement - are often calculated, but in terms of cell biology are poorly understood (Brady, 1987). In theory in one tissue a high fermentation rate still does not cover these maintenance needs, while in another tissue showing low fermentation rates the ATP production is sufficient. Ricard et al. (1994) do offer one observation in favor of the role of ATP: the decrease in ATP was suggested to be the main cause for the rapid acidification of the cytosol found after transfer of tissues to anoxia. The remark of Brady also addresses another issue: energy use in non-growing tissues (like most harvested fruits and vegetables) is often completely regarded as for maintenance. Also Zhang and Greenway (1994) assumed that the plant tissue they studied used all the energy produced for maintenance, irrespective of gas conditions. It is unclear, however, if the division of energy use between growth or maintenance in this case is sufficient. Apples after harvest do not show 'growth', but do show processes related to ripening (maturation) which require energy. In fact the basis of the storage under low oxygen concentrations of fruits and vegetables is that ripening is reduced (Kader et al., 1989). A situation without growth is not necessarily a situation with only maintenance. Therefore it is proposed that total ATP production is divided between growth, ripening and maintenance processes. The Maintenance Energy Requirements level used in this thesis, can be referred to as the minimum ATP production necessary to maintain cell viability.

Optimum concentrations for storage

Throughout the years optimum O_2 concentrations for storage were thought to be related to specific points; the Extinction Point (EP), the Anaerobic Compensation point (ACP) or the Respiration Quotient Breakpoint (RQB). The EP was defined by Blackman (1928) as the highest O_2 concentration with no anaerobic metabolism, measured as ethanol or acetaldehyde production. In 1933, however, Fidler measured 40 µg of ethanol per gram of apple tissue stored in air. Nevertheless the EP theory is still being used, for instance by Knee (1991b) who states that 'the minimum safe concentration for oxygen can be checked by measuring the concentration at which ethanol is first detected as oxygen is lowered. Ethanol is now detected as a normal constituent of apples and many other fruits held under aerobic conditions, and the EP is, therefore, an untenable concept based on archaic analytical methods (Boersig et al., 1988). For products such as apples and pears the CO_2 production was observed

to be minimal at the EP, and therefore CO_2 production was also used for the determination of the EP. However, products with low fermentation rates will show a minimal CO_2 production at a lower O_2 concentration than the EP, sometimes resulting in a minimal CO_2 production at 0% O_2 (see broccoli, mungbean in chapter 3). In 1951 Turner therefore stressed that the EP should be determined by anaerobic products like ethanol and lactate and not by CO_2 production. Nevertheless in 1988 another concept was proposed, the Anaerobic Compensation Point (ACP), defined as the O_2 concentration at which CO_2 production is minimal (Boersig et al., 1988). The ACP can be explained as the O_2 concentration where an increase in anaerobic CO_2 production compensates for the decrease in aerobic CO_2 production. Although not mentioned by the authors, this implies anaerobic metabolism at higher O_2 concentrations than the ACP, and a gradual shift from aerobic to anaerobic metabolism. But as for the EP the ACP can only be used for products forming ethanol. The RQB is, like the EP, also based on a sudden start of anaerobic metabolism. It is described as the O_2 concentration where the RQ is starting to increase when O_2 is further lowered. Also the RQB is thought to be useful for establishing the lowest O_2 concentration for storage (Gran and Beaudry, 1993). Based on chapter 7 this assumption seems justified when one measurement is carried out before storage, and a better definition is used to establish the RQ limit above which the RQB should rise. The ACP, however, does not show a relationship with optimal storage conditions of three apple cultivars. Since apple is the product stored in highest quantities under CA, this is a strong practical disqualification of the ACP concept. It also raises questions on the hypothesis of Wollin et al. (1985), who assumed that if the product is kept at the O_2 concentration with minimal CO_2 production (equal to the ACP), no low O_2 injury was to be found. They suggested that this specific O_2 concentration should be continuously monitored and adjusted if necessary. Considering the data found the ACP is changing indeed, but to other O_2 concentrations than are expected to be beneficial. Using the ACP or the RQB for dynamic control could lead to severe problems. A combination of gas exchange rates and models which enable the calculation of ATP production might reduce that risk. This confirms the conclusions of Chervin et al. (1996), that there is a need for more fundamental studies of energy metabolism in horticultural products subjected to CA conditions. Energy metabolism is more related to cell functioning than gas exchange rates, and it is expected that research on energy metabolism will help to clarify the occurrence of disorders in produce subjected to extreme gas conditions.

Fermentation in normoxia

Although ethanol and acetaldehyde are often measured in plant tissues in normoxia, it was never measured in the gas phase surrounding the tissues. The two products may be immediately re-metabolized (Bucher et al., 1994). This leaves unanswered whether fermentation actually exists at normoxia. The main argument to explain fermentation in ambient air is the existence of O_2 gradients inside bulky plant tissues such as apples. Ricard et al. (1994) explain this phenomenon by (partial) hypoxia within these tissues, because oxygen diffusion was hampered by a bulky tissue. Although the O_2 concentration surrounding the apples equals normoxia, part of the internal gas phase could become hypoxic or even anoxic (Brändle, 1968). It would explain the ethanol production found in ambient air (Knee, 1991b). This diffusion aspect slightly modified the thinking on fermentation as optimal oxygen concentration is then defined as the *internal* O_2 concentration where the respiration is minimized without development of fermentation (Banks et al., 1993). Nevertheless one has to consider that this was found for apples at the climacteric peak, when gas exchange rates reach maximum levels. For preclimacteric apples, like the apples used in chapter 8, internal concentrations found range between 16.1% to 18.9% (Burton, 1974), 12.7% to 14.8% (Solomos, 1987) or 11.7% to 15.1% directly under the skin and 10.6% to 14.8% at the center of the fruit (Rajapakse et al., 1990). Also the research described in chapter 6 does not find internal O_2 concentrations lower than 16.5% in apples stored in ambient air. Although these internal O_2 concentrations are considerably lower than the external ones, they cannot be considered to resemble hypoxia. With acetaldehyde and ethanol production measured at these conditions it would mean that fermentation is a constitutive process in plant tissues, but increasing in importance when O_2 concentrations are lowered.

Fermentation in postharvest studies

When harvested plant products are stored at very low O_2 or very high CO_2 concentrations, increased fermentation rates are found together with necrotic and discolored tissues (Kader et al., 1989). Therefore it is often assumed that a direct relation exists between fermentation and the occurrence of disorders. Kader (1986) for instance stated that the decarboxylation of pyruvate to form acetaldehyde, CO_2 and ultimately ethanol, results in the development of off-flavours and tissue breakdown. In this line of thinking research on Controlled Atmosphere (CA) storage was always

focused on avoiding fermentation. With increasing accuracy of detection techniques, and ethanol and acetaldehyde being detected in ambient air (chapter 8), a complete avoidance of fermentation seems impossible. Also the possible relation between increased ethanol levels and decreased cell viability was not found by Perata and Alpi (1991). The only influence of ethanol on quality seems therefore the induction of off-taste and off-odours. This changes the view upon ethanolic fermentation during the storage of harvested plant tissues. Instead of avoiding fermentation, the optimization of storage conditions could become the balancing between possible beneficial aspects of increased fermentation (in terms of energy production) and detrimental aspects (off-odours and off-taste). This implies the introduction of dynamic optimal gas concentrations instead of static ones, which are used now. Storage facilities could be improved by the incorporation of monitoring and feed-back systems that respond to the actual physiology of the stored product. The gas exchange models described within this thesis could be used as a basis for the calculation of energy production (chapter 7), while ethanol, the main cause for off-odours and off-taste, can be detected directly in the atmosphere surrounding the stored produce. In doing this, two important criteria have two be known: the minimum energy production necessary for maintenance, and the ethanol level that should be avoided.

Major conclusions

The influence of CO_2 on O_2 uptake was investigated, and the known Michaelis-Menten equation given by Chevillotte (1973) was extended with the type of inhibition adequately describing this influence (chapter 2). Models describing fermentative CO_2 production were developed and combined with oxidative CO_2 production. Although the mechanism inhibiting alcoholic fermentation remains unclear, CO_2 production of various products can be described under a range of combinations of O_2 and CO_2 (chapter 3). Two parameters of the models, Kmf_{O2} and Vmf_{CO2}, could only be determined if 0% O_2 was one of the conditions used to measure gas exchange. This unusual gas condition, not optimal for the storage of any product known, is being used since a few years in every gas exchange experiment conducted at ATO-DLO and by institutes cooperating in several EU financed research programmes. Although gas exchange of mungbean and microbial growth on it could not be distinguished, a model was developed describing the total gas exchange of mungbean and microbial growth (chapter 4), making it possible to estimate mungbean respiration in MA packages. A method was

derived enabling the simultaneous measurement of metabolic gas exchange and the resistance to gas diffusion (chapter 5). Results of these measurements showed limitations to experimental setups using headspace techniques, and indicated that optimal O_2 concentrations are very likely limited to a specific temperature (chapter 6). Although other CO_2 sources are likely to exist in hypoxia, the models can also be used to estimate other metabolic aspects, such as ATP fluxes. In chapter 7 it was shown that ATP fluxes, in combination with maintenance requirements, very likely help to understand tolerance of plant tissues to low oxygen conditions. Finally research was carried out to investigate whether predictions of the models describing fermentative CO_2 production, that fermentation is not limited to low O_2 concentrations, was correct. Measurements on acetaldehyde and ethanol seem to confirm this prediction (chapter 8). If correct, these results mean that the traditional view upon fermentation used in postharvest studies is incorrect. Not the avoidance of fermentation should than become the main focus of research, but the possible function of fermentation. It was hypothised that one role of fermentation during storage is the contribution to the energy status of the products subjected to CA conditions.

Future outlook

Models will become increasingly important to guide future research in postharvest studies, instead of fitting data after all measurements have been carried out. This confirms the ideas of Banks et al. (1989). Based on results and ideas within this thesis, future research seems necessary on several aspects.

a. Before carrying out experiments on gas exchange, some precalculations using gas exchange and diffusion resistances will help to optimize the methods.
b. Because changes in temperature during the distribution from packing houses to retailers can influence the gas composition within MA packages, the relation between temperature and gas exchange rates must be known. Because the models used are based on enzyme kinetics, the focus should be on the relation between temperature and enzyme activity.
c. It is expected that more research on the relationship between maintenance energy requirements and cell injury will clarify an important part of the changes in tolerance to low O_2 concentrations (or optimal O_2 concentrations) during ageing or maturation of harvested plant tissues. Studies on cell cultures could help to gain more knowledge on fundamental aspects as energy metabolism in relation to

storage needs (Zhang and Greenway, 1994; Chervin et al., 1996). Cell studies might also help to avoid the variance between individual products caused by diffusion resistances.

d. The possible relation between energy status and storage disorders in products subjected to CA conditions, also raises questions on their pretreatments. Andrews et al. (1994) showed that the extent to which the mRNAs for PDC and ADH increased in maize seedlings subjected to anoxia depended on whether the seedlings had been previously acclimated to hypoxia or anoxia. Pretreatments might improve storability of certain products by increasing their fermentative ATP production capacity.

e. Optimal gas conditions for storage of produce are not fixed values but change with temperature and, more important, also during the storage period. Using fixed gas conditions for long term storage, which is the common procedure, could lead to problems and loss of stored produce. Interactive storage facilities, responding to physiology of the stored material, could help to reduce the risk of storage losses. Processes that should be quantified are energy metabolism and fermentation rates. Parameters related to these processes are gas exchange rates and acetaldehyde and ethanol production. For the calculation of energy production the gas exchange models described within this thesis could be used (chapter 7), while ethanol, the main cause for off-odours and off taste, can be detected directly in the atmosphere surrounding the stored produce. In doing this, two important criteria, presently unknown, have two be established: the minimum energy production necessary for maintenance, and the ethanol level that should be avoided. The first criterium can be obtained in cell studies, when energy metabolism and cell viability under various gas conditions is measured. The second criterium can be obtained by sensoric analysis. This type of knowledge, in combination with sensors and adequate software, will help to improve static CA facilities to dynamic storage systems.

10

Summary

Oxygen consumption as influenced by carbon dioxide

High carbon dioxide (CO_2) concentrations can reduce the oxygen (O_2) consumption rate of a number of fruits and vegetables. This reduction can be modelled by incorporating an inhibition term in an Michaelis-Menten type of model, describing the overall respiration process as a single enzyme reaction. Four types of inhibition can be distinguished: 1. the competitive type, 2. the uncompetitive type, 3. a combination of both previous types and 4. the non-competitive type (after Chang, 1981). Using the inhibition terms a good estimation of O_2 consumption could be obtained. This supports the use of Michaelis-Menten kinetics for modelling O_2 consumption.

Depending on the product the statistical analysis gave good results for the competitive and the uncompetitive type of inhibition. Based on gas exchange data only, no distinction between the competitive and uncompetitive type of inhibition could be made. The data suggest the simultaneous existence of both types of inhibition. However, for reasons of simplicity the non-competitive type of inhibition is preferred, giving good results for all the products tested. This non-competitive inhibition term, evaluated in chapter 2, is used in other chapters whenever an influence of CO_2 on respiration was found.

Oxidative and fermentative carbon dioxide production

Because the main metabolic sources for CO_2 emission by higher plants are respiration and fermentation, both processes have to be incorporated in a model describing total CO_2 production. For this purpose one existing model (Peppelenbos et al., 1993) was adjusted and, based on different theories, two models were developed. The adjusted model used O_2 as an inhibitor of fermentative CO_2 production, whereas the two new models used the ATP production rate, representing ATP concentration. The difference

between the latter two models is that in the first one ATP production is calculated by using only oxidative processes, while in the second one ATP production is calculated by a combination of oxidative and fermentative processes. All models allow for increased CO_2 production at low O_2 concentrations, as is often found for several products. The best performance was found for the adjusted model and the new one which used oxidative ATP. The results do not clarify whether increased fermentation rates can be attributed to decreased O_2 levels or decreased energy fluxes. The approach used, however, enables the calculation of CO_2 production rates of different types of commodities stored under various gas conditions. This facilitates a better prediction of CO_2 conditions inside storage rooms and MA packages.

Alcoholic fermentation as influenced by carbon dioxide

Not only respiration can be influenced by high CO_2 concentrations. In several products this influence is also found on fermentation. This influence was incorporated in the CO_2 production model based on the inhibition of alcoholic fermentation by O_2 (the 'adjusted' model). Gas exchange rates of mungbean sprouts under various O_2 and CO_2 concentrations were used to validate the model. With the modification applied, CO_2 production rates were described better. Although CO_2 production at low O_2 concentrations was reduced by high CO_2 concentrations, the data showed no influence on ethanol and acetaldehyde levels.

The data obtained indicate large differences between gas exchange rates of different batches of mungbean sprouts. It is suggested that microbial metabolism attributes substantially to total CO_2 production rates found, and might explain these differences.

The simultaneous measurement of gas exchange and diffusion resistance

A method was developed to measure metabolic gas exchange rates and gas diffusion resistance of apples simultaneously, under various gas conditions. For this purpose the trace gas neon was selected. After closing a flask containing an apple already kept at a specific gas condition, the neon partial pressure was brought to 110 Pa. Changes in oxygen and carbondioxide concentration in the flask were used to calculate gas exchange, and the decrease in neon concentration was used to calculate gas diffusion resistance. The calculated resistance values were compared with data obtained from literature, and estimations of O_2 and CO_2 resistance values were made. The method worked well on apples, but this will not necessarily be the case when products are

measured with small internal gas volumes.

Functioning of gas exchange models using internal and external concentrations
Based on gas exchange rates and diffusion resistance, internal gas concentrations of apple cultivars Golden Delicious, Elstar and Cox's Orange Pippin were calculated. Internal O_2 concentrations were 2.3 kPa lower at an external O_2 concentration of 20.7 kPa for Golden Delicious apples, and about 4.5 kPa lower at 20.1 and 20.4 external O_2 for Elstar and Cox's apples respectively. Internal CO_2 concentrations substantially exceeded normal external concentrations of 50 Pa. The Km values found for the three apple cultivars remained significantly different when internal instead of external concentrations were used. This indicates that the apple cultivars measured do not only show biophysical differences (resistance, porosity), but also differences at the biochemical level.
For Golden Delicious apples no difference in model functioning was found when internal or external concentrations were used. In contrast, for Elstar and Cox's Orange Pippin apples the O_2 uptake and CO_2 production models showed better results (expressed as R^2) when fitted on external concentrations. It is argued that this might be explained by the experimental setup. For instance the internal O_2 concentration of Cox's Orange Pippin calculated at the optimal external O_2 concentration (1.2%) reached 0.01%. A small change of 0.1% in an external O_2 concentration close to 1% therefore can change the internal atmosphere from hypoxia to anoxia, which cannot be regarded as an equilibrium situation. The conclusion to be drawn is that also for experimental setups some precalculations using gas exchange rates and diffusion resistances will help to optimize the methods.

Gas exchange characteristics and prediction of optimal gas conditions for CA storage
The applicability of respiratory characteristics to determine optimal O_2 concentrations for the storage of apples was tested. A comparison was made between gas exchange rates of apples directly after harvest and after a period of storage. Optimal O_2 concentrations were based on gas exchange data and gas exchange models fitted on the data, using the Anaerobic Compensation Point (ACP) and the Respiratory Quotient Breakpoint (RQB). A third to establish optimal gas concentrations way was comparing total ATP production with estimated maintenance energy requirements, revealing the

Maintenance Oxygen Concentration (MOC). ATP production was calculated using gas exchange models. MOC was defined as the oxygen concentration with the minimal ATP production rate necessary for maintaining cell viability. The optimal O_2 concentrations as established by ACP, RQB and MOC differed considerably. Because ACP values differed from normally advised values, the ACP was unsuitable for a quick determination of the optimal O_2 concentration of the apples used. The RQB, however, might be suitable, but than the limit used to establish the RQB should be more than 0.5 units higher than the RQ measured in ambient air. The ACP and the RQB were decreased to lower O_2 concentrations after storage, suggesting that the optimal concentrations decrease during storage. In contrast the MOC was increased after storage, which was in agreement with data as found in the literature. Model calculations indicated the lowest optimal O_2 concentration for the second (optimal) harvest using the ACP, the RQB and the MOC. It is suggested that research on the relationship between Maintenance Energy Requirements and cell injury will clarify an important part of the changes in optimal O_2 concentrations (or the tolerance to low O_2 concentrations) during ageing or maturation of harvested plant tissues.

Fermentation at high oxygen concentrations

Apples were stored at various O_2 concentrations, ranging between normoxia and anoxia. Gas exchange rates and the production of acetaldehyde and ethanol was measured. A gas exchange model, which distinguishes oxidative from fermentative CO_2 production, was fitted to the data. The results indicate alcoholic fermentation to be active at all the O_2 concentrations used, and increasing in importance when O_2 concentrations are lowered. After calculating the amount of metabolites in the apple tissue from the data measured in air, a close relationship was found between model predictions of alcoholic fermentation rates and measured metabolite production in normoxia and anoxia. In hypoxia, however, the model predicted higher CO_2 production rates in comparison to the metabolites actually found. Because the model was fitted to CO_2 production data, this indicates another source or CO_2 in hypoxia than alcoholic fermentation.

Conclusions

The influence of CO_2 on O_2 uptake was investigated, and the known Michaelis-Menten equation given by Chevillotte (1973) was extended with the type of inhibition

adequately describing this influence. Models describing fermentative CO_2 production were developed and combined with oxidative CO_2 production, enabling the calculation of CO_2 production of various products under a range of combinations of O_2 and CO_2. Although gas exchange of mungbean and microbial growth on it could not be distinguished, a model was developed describing the total gas exchange of mungbean and microbial growth, enabling the calculation of mungbean gas exchange in MA packages. A method was derived enabling the simultaneous measurement of metabolic gas exchange and the resistance to gas diffusion. Results of these measurements showed limitations to experimental setups using headspace techniques, and indicated that optimal O_2 concentrations are very likely limited to a specific temperature. Measurements on acetaldehyde and ethanol confirm the prediction of the models describing fermentative CO_2 production, and show that fermentation is not limited to low O_2 concentrations.

Optimal gas conditions for storage of produce are not fixed values but change with temperature and, more important, also during the storage period. Using fixed gas conditions for long term storage, this could lead to problems and the loss of the stored produce. Interactive storage facilities, responding to physiology of the stored material, will help to reduce this risk. Processes that should be quantified are energy metabolism and fermentation rates. Parameters related to these processes are gas exchange rates and acetaldehyde and ethanol production. For the calculation of energy production the gas exchange models described within this thesis could be used. ATP fluxes, in combination with maintenance requirements, very likely help to understand the tolerance of plant tissues to low oxygen conditions.

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Samenvatting

De invloed van kooldioxide op zuurstofconsumptie

In een omgeving met een verlaagde zuurstofconcentratie neemt de ademhalingssnelheid van groenten en fruit af. De relatie tussen zuurstofconcentratie en zuurstofopname kan worden beschreven in een model gebaseerd op de Michaelis-Menten kinetiek, waarbij het totale ademhalingsproces beschreven wordt als een enkele enzymreactie. Ook een hoge kooldioxideconcentratie kan bij een aantal produkten de ademhalingssnelheid verlagen. Deze verlaging kan worden beschreven worden door een zogenaamde inhibitieterm in te bouwen in het genoemde model. Er kunnen vier soorten inhibitie onderscheiden worden (naar Chang, 1981): 1. het 'competitive' type; 2. het 'uncompetitive' type; 3. het 'non-competitive' type, dat een combinatie van type 1 en 2 is waarbij beide typen een even sterke invloed hebben, en 4. een nieuwe beschrijving van de combinatie van type 1 en 2 waarbij deze typen in verschillende mate een rol spelen. Door gebruik te maken van inhibitietermen kon het zuurstofverbruik goed beschreven worden. Dit ondersteunt het gebruik van de Michaelis-Menten-kinetiek voor het modelleren van de ademhaling. Afhankelijk van het product geeft de statistische analyse de beste resultaten voor het 'competitive', het 'uncompetitive' type of de nieuwe combinatie (type 4). Gebaseerd op gegevens over alleen gasuitwisseling is het niet mogelijk een voorkeur voor één van deze inhibitietypen aan te geven. Het suggereert dat beide typen inhibitie gelijktijdig naast elkaar voorkomen, zoals beschreven in type 3 en 4. Om redenen van vereenvoudiging wordt voor algemeen gebruik toch de voorkeur gegeven aan het 'non-competitive' type, dat voor alle geteste producten goede resultaten gaf.

Oxidatieve en fermentatieve kooldioxideproductie

Ademhaling en fermentatie zijn de belangrijkste metabolische bronnen voor CO_2-

emissies door hogere planten. Daarom moeten in een model voor de totale CO_2-productie beide processen worden opgenomen. Er bestond echter geen model met parameters die voor deze processen relevant zijn. Voor dit doel zijn daarom verschillende modellen ontwikkeld en getest. Allereerst is een bestaand model aangepast, dat zuurstof gebruikt als remmer van fermentatieve CO_2-productie. Daarnaast zijn er twee modellen ontwikkeld die de ATP-productiesnelheid gebruiken als remmer van fermentatieve CO_2-productie. Deze twee 'ATP-modellen' verschillen in de berekeningswijze van de ATP-productie: één model gebruikt alleen oxidatieve processen, terwijl het andere model een combinatie van oxidatieve en fermentatieve processen gebruikt.
De drie modellen zijn in staat de toename van de CO_2-productie bij lage O_2-concentraties te beschrijven, zoals vaak gevonden wordt bij producten zoals appels. Het aangepaste model en het ATP-model met alleen oxidatieve processen komen het meest overeen met de metingen. De resultaten maken echter niet duidelijk of een toename in fermentatiesnelheid het gevolg is van verlaagde O_2-concentraties of van verlaagde energiefluxen. De gebruikte benadering maakt het in elk geval mogelijk om CO_2-productiesnelheden van diverse producten te berekenen bij bewaring onder verschillende gascondities. Dit maakt een betere voorspelling van CO_2-condities in bewaarcellen en MA-verpakkingen mogelijk.

Alcoholische fermentatie onder invloed van kooldioxide
Niet alleen de ademhaling kan beïnvloed worden door hoge CO_2-concentraties. In verschillende producten is ook een invloed van CO_2 op de fermentatie gevonden. Deze invloed is ingebouwd in het CO_2-productiemodel gebaseerd op remming van alcoholische fermentatie door O_2. Gegevens over gasuitwisseling bij taugé onder verschillende O_2- en CO_2-condities zijn gebruikt om het model te valideren. Met deze aanpassing worden de CO_2-productiesnelheden beter beschreven. Ofschoon de CO_2-productie bij lage O_2-concentraties gereduceerd werd, wat te verklaren is door een verlaagde fermentatiesnelheid, is er geen verlaging van de ethanol- en aceetaldehydeconcentraties gevonden. Verder zijn er bij de verschillende experimenten grote verschillen gevonden in gasuitwisselingssnelheid. Gezien de grote maar wisselende hoeveelheid micro-organismen op taugé, zou een verklaring voor de verschillen kunnen zijn dat het microbiële metabolisme een aanzienlijke bijdrage levert aan de totale CO_2-productiesnelheid.

Gelijktijdige meting van gasuitwisseling en diffusieweerstand

Een methode is ontwikkeld om gelijktijdig gasuitwisselingssnelheden als gevolg van metabole processen en de gasdiffusieweerstand in appels onder verschillende gascondities te meten. Voor dit doel is het spore-gas neon gebruikt. Na afsluiting van een cuvet, met daarin een onder specifieke gascondities bewaarde appel, werd aan de cuvet neon toegevoegd tot een partiële druk van 110 Pa. De veranderingen in O_2 en CO_2 in de cuvet werden gebruikt om de gasuitwisseling te berekenen, terwijl de afname in neonconcentratie werd gebruikt om de gasdiffusieweerstand te berekenen. De berekende waarden voor de weerstand zijn vergeleken met literatuurgegevens en met de resultaten zijn de diffusieweerstanden voor O_2 en CO_2 geschat. Deze methode werkte goed bij appels. Het is echter van belang op te merken dat dit waarschijnlijk niet het geval zal zijn bij producten met een klein intern gasvolume.

Het functioneren van gasuitwisselingsmodellen bij gebruik van externe en interne gasconcentraties

Interne gasconcentraties van Golden Delicious, Elstar en Cox's Orange Pippin appels werden berekend op basis van gasuitwisselingssnelheden en diffusieweerstanden. Hieruit volgde dat voor Golden Delicious de interne zuurstofconcentratie 2.3 kPa lager was dan de externe concentratie van 20.7 kPa. Bij Elstar en Cox werden interne zuurstofconcentraties berekend die 4.5 kPa lager waren dan de omgevingsconcentratie (gemiddeld 20.1 kPa en 20.4 kPa respectievelijk). Interne kooldioxideconcentraties waren aanmerkelijk hoger dan 50 Pa zoals in gewone lucht wordt gevonden. Als naar de modellen wordt gekeken, dan blijkt dat de Km waarden significant verschillen tussen de drie appel cultivars, ook als er interne concentraties worden gebruikt. Dit suggereert dat er tussen de drie gemeten appelcultivars niet allen biofysische, maar ook biochemische verschillen bestaan. Voor Golden Delicious functioneerden de modellen even goed als er interne in plaats van externe gasconcentraties werden gebruikt. Daarentegen werden er voor Elstar en Cox's Orange Pippin betere resultaten gevonden (uitgedrukt in R^2) als de externe concentraties gebruikt werden. Als verklaring wordt aangevoerd dat dit waarschijnlijk te wijten is aan de proefopzet. Als bijvoorbeeld de interne zuurstofconcentratie van Cox-appels wordt berekend bij de optimale externe concentratie (1.2%), dan wordt een waarde van 0.01% gevonden. Een verandering in de externe concentratie van 0.1%, zoals wordt gebruikt voor de gasuitwisselingsmetingen, kan dan betekenen dat de interne atmosfeer verandert van

hypoxia naar anoxia. Dit komt niet overeen met een evenwichtssituatie. De conclusie is dan ook dat proefopzetten verbeterd kunnen worden met behulp van enkele berekeningen vooraf, waarbij schattingen van gasuitwisselingssnelheden en diffusieweerstanden gebruikt worden.

Voorspelling van optimale gascondities met behulp van gasuitwisseling

Onderzocht is in hoeverre ademhalingskarakteristieken toepasbaar zijn om optimale O_2-concentraties voor het bewaren van appels te bepalen. Hiertoe zijn metingen van gasuitwisseling van appels van drie oogsttijdstippen, direct na de oogst en na een bewaarperiode van 4 maanden, vergeleken. Optimale O_2-concentraties zijn bepaald op basis van deze metingen en op basis van modellen gefit op de metingen. Hierbij is gebruik gemaakt van drie karakteristieken; het 'Anaerobic Compensation Point of ACP, de zuurstofconcentratie waar de kooldioxideproductie minimaal is, en het Respiration Quotiënt Breakpoint of RQB, de zuurstofconcentratie waar de RQ snel in waarde stijgt. Daarnaast zijn optimale O_2-concentraties bepaald door de totale ATP-productie te vergelijken met de geschatte energiebehoefte voor onderhoud, wat de Maintenance Oxygen Concentration of MOC opleverde. De ATP-productie is berekend met behulp van eerder beschreven gasuitwisselingsmodellen. De MOC is gedefinieerd als de zuurstofconcentratie met de minimale ATP-productiesnelheid die nodig is om een cel in leven te houden. De gevonden optimale O_2-concentraties op basis van de drie berekeningswijzen (ACP, RQB of MOC) verschillen aanzienlijk. Omdat de ACP-waarden afwijken van de in het algemeen geadviseerde waarden, lijken deze ongeschikt voor een snelle bepaling van de optimale O_2-concentratie van de gebruikte appels. Het RQB lijkt meer geschikt, mits de limiet die gebruikt wordt om het RQB vast te stellen meer dan 0.5 eenheid hoger is dan het RQ zoals in normale lucht gemeten wordt. Het ACP en het RQB daalden naar lagere O_2-concentraties na bewaring, wat zou wijzen op een verlaging van de optimale concentraties tijdens de bewaarperiode. De MOC daarentegen was toegenomen na bewaring, wat overeenkomt met resultaten die in de praktijk gevonden worden. Modelberekeningen laten de laagste optimale zuurstofconcentraties zien bij de tweede (optimale) oogst, zowel bij gebruik van het ACP en het RQB als van de MOC. Verwacht wordt dat onderzoek naar de relatie tussen de energiebehoefte voor onderhoud en celschade voor een belangrijk deel de veranderingen in optimale zuurstofconcentraties (of de tolerantie voor lage zuurstofconcentraties) gedurende veroudering of rijping van geoogste plantenweefsels

zal kunnen verklaren.

Fermentatie bij hoge zuurstofconcentraties

In een experiment zijn appels bewaard bij verschillende zuurstofconcentraties, variërend van normoxia tot anoxia. Hierbij zijn gasuitwisselingssnelheden en de productie van aceetaldehyde en ethanol gemeten. Een gasuitwisselingsmodel dat oxidatieve CO_2-productie onderscheidt van fermentatieve CO_2-productie is gefit op de data. De resultaten tonen alcoholische fermentatie aan bij alle gebruikte zuurstofconcentraties, toenemend in belang bij dalende zuurstofconcentraties. Tevens zijn de hoeveelheden metabolieten in het appelweefsel berekend met behulp van de gegevens gemeten in lucht. Er blijkt een duidelijke relatie te bestaan tussen de modelvoorspellingen van alcoholische fermentatiesnelheden en de gemeten metabolietproductie bij normoxia en anoxia. Bij hypoxia echter voorspelt het model hogere CO_2-productiesnelheden dan op basis van de werkelijk gevonden metabolieten verwacht kan worden. Omdat het model gefit is op gegevens over de CO_2-productie, geeft het voorgaande aan dat er in hypoxia een andere CO_2-bron is naast ademhaling en alcoholische fermentatie.

Conclusies

De invloed van CO_2 op de opname van O_2 is onderzocht. De bekende Michaelis-Menten-vergelijking zoals gegeven door Chevillotte (1973) is uitgebreid met het type inhibitie dat deze invloed goed beschrijft.

Er zijn enkele modellen ontwikkeld die fermentatieve CO_2-productie beschrijven, die gecombineerd zijn met oxidatieve CO_2-productie. Dit maakte het mogelijk de CO_2-productie van verschillende producten te berekenen binnen een serie van combinaties van O_2 en CO_2.

Alhoewel gasuitwisseling van taugé en microbiële groei op taugé niet onderscheiden kon worden, is een model ontwikkeld voor de totale gasuitwisseling van taugé en microbiële groei. Hierdoor is het mogelijk gemaakt de ademhaling van taugé in MA-verpakkingen te schatten.

Er is een methode ontwikkeld die het mogelijk maakt gelijktijdig metabolische gasuitwisseling en de weerstand voor gasdiffusie te meten. Resultaten van deze metingen tonen beperkingen aan experimentele opzetten waarbij gebruik gemaakt wordt van zogenaamde head-space technieken. Tevens geven de resultaten aan dat

optimale O_2-concentraties zeer waarschijnlijk beperkt zijn tot een specifieke temperatuur.

De ontwikkelde modellen kunnen gebruikt worden om andere metabolische aspecten zoals ATP-fluxen te berekenen. ATP-fluxen gecombineerd met de energiebehoefte voor onderhoud helpen zeer waarschijnlijk om de tolerantie van plantweefsel voor lage zuurstofcondities te begrijpen. Metingen aan aceetaldehyde en ethanol lijken te bevestigen dat fermentatie niet beperkt is tot lage O_2-concentraties, zoals de modellen die fermentatieve CO_2-productie beschrijven voorspellen.

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Nawoord

Een onderdeel van het boekje dat een promoveren begeleidt is het duidelijk maken aan de lezer dat de promotie, het boekje of het onderzoek onmogelijk was geweest zonder hulp. Het is het moment bij uitstek om aan te geven dat de bijdrage van de genoemde mensen wel degelijk gewaardeerd wordt. Bijkomend voordeel is bovendien dat het de prestatie van de promovendus wat relativeert.

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theorieën, teveel om op te noemen. Als ik weer veel tijd moest stoppen in proefvoorstellen, offertes, subsidieregelingen, potentiële opdrachtgevers, vergaderingen, telefoontjes, rondleidingen en meer van dat productieve werk, ging hij onverstoorbaar door met het produceren van prachtige data over gasuitwisseling. Even langslopen bij hem in Q4 en ik kon er weer even tegen.
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Herman Peppelenbos
Ede, 4 oktober 1996

Curriculum vitae

Herman (Willem) Peppelenbos werd geboren op 24 mei 1963 te Raalte, Overijssel. In 1981 deed hij eindexamen Atheneum-B aan het Florens Radewijnsz. College te Raalte, waarna hij met de studie Biologie begon aan de toenmalige Landbouw Hogeschool te Wageningen. In 1988 studeerde hij af, met als doctoraalvakken Hydrobiologie, Luchthygiëne en -verontreiniging en Informatica. Een stageperiode werd doorgebracht op het 'Aquatic Weed Lab' van de University of California in Davis. Tot juni 1990 vervulde hij zijn vervangende dienst bij de n.v. KEMA te Arnhem. Vanaf juli 1990 werkt hij bij het instituut voor AgroTechnologisch Onderzoek (ATO) te Wageningen. In de eerste jaren verrichtte hij onderzoek dat gericht was op de invloed van gascondities op de kwaliteit van groente, fruit, champignons en leliebollen. In 1992 werd dit werk uitgebreid met onderzoek naar de beïnvloeding van ademhaling en fermentatie, en de modellering daarvan. Dit proefschrift is een weergave van dat laatste.