A THEORETICAL APPROACH ON THE ROLE OF FERMENTATION IN HARVESTED PLANT PRODUCTS

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1. Introduction

Most research on controlled atmosphere storage (CA) and modified atmosphere packaging (MA) places emphasis on optimal gas concentrations, defined as the concentrations where quality change of a product almost ceases without introduction of tissue disorders. They depend on the tolerance of products to low O_2 concentrations [O_2], often, in the range of 1 - 4% O_2. Tolerance to these conditions appears to depend on both morphological and metabolic adaptations that are both species and tissue specific (Ratcliffe, 1995). During O_2 limitation, energy metabolism switches from respiration to fermentation leading to disorders like necrotic and discoloured tissues, off odours and off tastes (Kader et al., 1989), suggesting that they have a direct relationship. Kader (1986) stated that decarboxylation of pyruvate to acetaldehyde through to ethanol results in the development of off-flavours and tissue breakdown. In fact [O_2] is often considered to be optimal when respiration rates are reduced without development of fermentation (Banks et al., 1993). Therefore, traditionally, research on CA has been focused on avoiding fermentation. There have been three concepts; the Extinction Point (EP, Blackman, 1928), the Anaerobic Compensation point (ACP, Boersig et al., 1988) and the Respiration Quotient Breakpoint (RQB, Gran and Beaudry, 1993). All are related to fermentation rates. The EP is the highest [O_2] with no anaerobic metabolism, measured as ethanol or acetaldehyde production. However, ethanol is now detected as a normal constituent of apples and many other fruits held under aerobic conditions (Boersig et al., 1988; Ke et al., 1990, 1993; Colelli et al., 1991, Nanos et al., 1992), so that the EP is therefore an untenable concept (Boersig et al., 1988). The ACP, the [O_2] at which CO_2 production is minimal, can be explained as the [O_2] where an increase in anaerobic CO_2 production compensates for the decrease in aerobic CO_2 production. This implies anaerobic metabolism at higher [O_2] than the ACP. The RQB is the [O_2] where the RQ increases when [O_2] is further lowered. The ACP and the RQB are not directly related to fermentation rates but are derived from gas exchange rates. Both the ACP and the RQB accept a certain increase in fermentation rate, measured as an increased CO_2 production or RQ. With fermentation active at supposed optimal concentrations, and even in normal air, it is unclear whether there is an [O_2] which precludes fermentation. Therefore occurrence of fermentation itself cannot be a criterion for selecting optimal storage concentrations. Throughout the years two possible explanations arose for the correlation between increased fermentation and tissue disorders: (1), a toxic effect of fermentative metabolites; (2), insufficient energy production to cover energy demands. We will discuss the physiological relevance and implications for storage procedures.

Toxic effect of fermentative metabolites:

Fermentative metabolites are often considered to be the cause of storage disorders with which they are often associated. Many metabolic studies of the survival of plant tissues in the absence of oxygen are focused on the possible toxicity of the main fermentation end-products, lactic acid, acetaldehyde and ethanol (Perata and Alpi, 1993; Ricard et al., 1994).

Lactic acid - In many plants, formation of lactate precedes ethanol production. Davies et al. (1974) therefore proposed a regulatory role for lactate during the shift from oxidative to fermentative pathways, through decreasing cell pH and thereby increasing ADH activity. Different researchers, however, have found that the production of lactate is not well matched with the initial fall in the cytoplasmatic pH (Ratcliffe, 1995). Also the occurrence of lactic fermentation prior to the induction of alcoholic fermentation is not universally present in plants (Perata and Alpi, 1993). Nonetheless, the regulation of cytosolic pH is considered to be the

major determinant of plant tissue survival in anoxia (Ricard et al., 1984); and attempts to understand the time dependence of cytoplasmatic pH in an anoxic tissue need to take into account the full range of events that potentially could influence pH (Ratcliffe, 1995). Lactic acid is sometimes found in harvested fruits and vegetables (Andreev and Vartapetian, 1992; Ke et al., 1993), but the concentrations found are always lower than ethanol concentrations. Lactate is thought to be toxic at lower concentrations than ethanol (Perata and Alpi, 1993), so that lactate accumulation may still be responsible for the damage occurring under prolonged anoxia (Ricard et al., 1994). As lactate production is a factor causing acidosis, a low LDH activity may contribute to the survival under anoxia (Perata and Alpi, 1993).

Ethanol - Alcoholic fermentation is always found in plant tissues exposed to anoxia (Perata and Alpi, 1993) and ethanol is often its most abundant product (Pfister-Sieber and Brändle, 1994; Ricard et al., 1994). Ethanol is a normal trace constituent of apples (Wilkinson and Fidler, 1973). These authors relate increased ethanol concentrations only to quality problems such as off taste and off odour. Other authors, however, suggest there is also a direct relation between ethanol and tissue disorders (Kader, 1986). In fields of research other than postharvest biology this question has been extensively investigated. Crawford (1967) proposed that flood tolerance of plants depended on decreased ethanol production, reducing its presumed toxic effects. Later ethanol was found to be toxic indeed, but only at very high concentrations that are never found in plant tissues (Perata and Alpi, 1993). Many plant tissues can accommodate ethanol concentrations much higher than those found in nature (Kennedy et al, 1992). In contrast, research on ADH null mutants demonstrated that ADH activity is essential for extended survival of maize during anoxia (Kennedy et al., 1992). Therefore the assumption that ethanol is toxic under certain conditions is not generally accepted (Pfister-Sieber and Brändle, 1994), making unlikely a direct relationship between concentrations of ethanol and storage disorders of fruits and vegetables.

Acetaldehyde - Acetaldehyde accumulation has emerged as a central mechanism (Jones, 1989). Already in 1928 acetaldehyde was shown to be more toxic than ethanol (Thomas, 1928). In a simple but convincing experiment, Perata and Alpi (1991) found that toxic effects of exogenously added ethanol in carrot cells were not observed when ethanol oxidation to acetaldehyde was prevented by also adding an ADH inhibitor. Acetaldehyde appears to affect the secondary metabolism and developmental processes of plant tissues (Ricard et al., 1994). Aldehydes in general are known to be reactive towards proteins with uncharged amino acids (Chervin et al., 1996), so interfering with the functioning of essential enzymes. Unclear is whether acetaldehyde can normally accumulate in high enough quantities to cause damage. It is interesting to note that for yeasts and bacteria the strains most tolerant to ethanol have an excess of ADH, such that acetaldehyde is not allowed to accumulate (Jones, 1989).

Energy metabolism and maintenance

Maintenance - One function of fermentation is the recycling of NADH in order to facilitate an increased glycolysis, enabling a relatively inefficient production of ATP. Under very low [O_2], the glycolytic pathway replaces the Krebs cycle as the main source of the energy needed (Kader, 1986). Fermentation is increased in low [O_2] situations where the oxidative pathway is not capable of covering ATP demand (Ke et al., 1990; Nanos et al., 1992) or when mitochondrial activity is already maximal (Romieu et al., 1992). Although increased fermentation is believed to occur so that the cell can supply needed ATP (Good and Muench, 1993; Bucher et al., 1994; Fox et al., 1994), there is no simple relationship between survival and the rate of fermentative metabolism (Ricard et al., 1994). In theory a tissue with a high fermentation rate not covering these maintenance needs can exist, and also tissues showing low fermentation rates with sufficient ATP production. It is obvious that living cells need a continuous supply of energy (ATP) to maintain essential processes such as protein turnover, support of ion gradients across cell membranes, and membrane repair (Penning de Vries, 1975). These are often referred to as maintenance processes (Pirt, 1965; Thornley, 1970). Energy use in non-growing tissues (like most harvested products) is often completely regarded as for maintenance (Zhang and Greenway, 1994), irrespective of gas conditions. It is unclear if such a division of energy use between growth and maintenance is sufficient. Apples after

harvest show no growth, but do have processes that require energy (ripening, maturation). In fact the basis of fruit and vegetable storage under low [O_2] is that ripening is reduced (Kader et al., 1989). A situation without growth is not necessarily a situation with only maintenance. Total ATP production should thus be divided between growth, ripening and maintenance processes (Peppelenbos and Rabbinge, 1996).

Energy status and disorders - Pfister-Sieber and Brändle (1994) state that the main injuries related to anoxia are eventually due to changes in energy metabolism. Results of Roberts et al. (1984) and Saint-Ges et al. (1991) also suggest a relation between cell problems and energy status. They found a close relation between a decrease in nucleoside triphosphates and pH changes. This acidification may result from both inhibition of proton pumping at low ATP concentrations and proton release through ATP hydrolysis (Ricard et al., 1994). Data of Trippi et al. (1989) show a close relation between ATP concentration and membrane permeability. Energy status can be described by expressing ATP, ADP and AMP, contents as concentration ratios (like ATP/ADP) or the adenylate energy charge (Pradet and Raymond, 1983). However concentrations are the result of an equilibrium of opposite fluxes, and in theory ATP concentrations could remain constant while ATP production and consumption rates are changing dramatically. This calls into question the use of concentrations only to describe energy status. ATP production in combination with knowledge on maintenance needs might be the alternative. Disorders might develop when the gas conditions supplied result in lower energy production than maintenance energy needs (disorder a; Figure 1). Also a decrease in metabolic rates or an increase of maintenance needs during storage (disorder b; Figure 1) could lead to tissue damage.

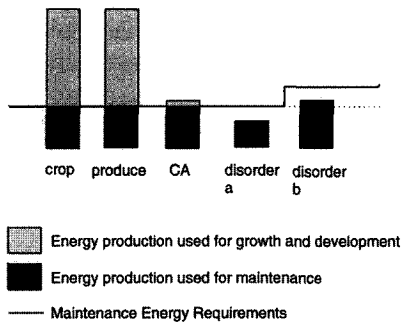

Figure 1. Schematic representation of energy production and consumption.

Calculating ATP production using gas exchange models - It is important to be able to calculate energy production under various gas concentrations. By using gas exchange models, ATP production can be estimated by combining oxidative and fermentative ATP production (after Andrich et al., 1993). Oxidative ATP production can be calculated using an O_2 uptake model (Chevillotte, 1973):

$$V_O2 = \frac{Vm_O2 * O_2}{Km_O2 + O_2} \quad [1]$$

where V_{O2} = the O_2 uptake rate ($ml \cdot kg^{-1} \cdot h^{-1}$), O_2 and CO_2 are the external or internal O_2 or CO_2 concentrations, Vm_{O2} = the maximum O_2 uptake rate ($ml \cdot kg^{-1} \cdot h^{-1}$) and Km_{O2} = Michaelis constant for the influence of O_2 on the O_2 uptake rate. Oxidative ATP production ($V_{ATP(o)}$, $1mol \cdot kg^{-1} \cdot h^{-1}$) is directly derived from O_2 consumption rate (V_{O2} in $ml \cdot kg^{-1} \cdot h^{-1}$) using a conversion factor based on the ideal gas law, 41.87 $1mol \cdot ml^{-1}$, at 18°C and 101.3 kPa, assuming that the ATP/O_2 ratio is 6;

$$V_{ATP(o)} = V_O2 * 6 * 41.87 \quad [2]$$

Fermentative CO_2 production can be calculated using the second term of a model given by Peppelenbos et al. (1996):

$$V_{CO2} = V_O2 * RQ_{ox} + \frac{Vmf_{CO}2}{1 + \left(\frac{O_2}{Kmf_O2}\right)} \quad [3]$$

where RQ_{ox} is the RQ value for oxidative processes, Vmf_{CO2} is the maximum fermentative CO_2 production rate ($ml \cdot kg^{-1} \cdot h^{-1}$) and Kmf_{O2} the Michaelis constant for inhibition of fermentative CO_2 production by O_2. Using Equ. [1], [2] fitted on data of Peppelenbos and Rabbinge (1996), O_2 uptake and CO_2 production are calculated for various [O_2] values (Figure 2).

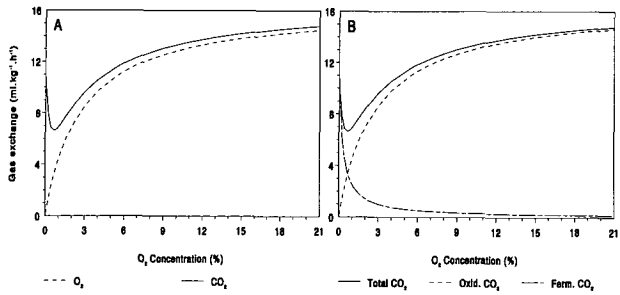

Figure 2. Gas exchange models fitted to gas exchange rates of 'Cox's Orange Pippin' apples. A.: O_2 consumption and CO_2 production, B.: Oxidative and fermentative CO_2 production.

Although other pathways than ethanol fermentation exist, they are minor (Ricard et al., 1994). During the first hours of anoxia, it is necessary to determine the sum of the main products accumulated (ethanol, lactate and alanine) to establish the fermentation rate. The values for ethanol alone may be acceptable for later times of anoxia (Ricard et al., 1994). Fermentative ATP ($V_{ATP(f)}$) production is therefore derived from the fermentative (ethanolic) CO_2 production:

$$V_{ATP(f)} = V_{CO2(f)} * 41.87 \quad [4]$$

The calculated total ATP production can be compared with a specific limit value, the Maintenance Energy Requirement (MER). The [O_2] where ATP production falls below the MER can be used as an estimate for the optimal concentrations for storage (Peppelenbos and Rabbinge, 1996). This is shown for 'Cox's Orange Pippin' apples (Fig. 3), when the energy production at 1.2% O_2 (the advised optimal [O_2] in the Netherlands) is assumed to be the limit value. Though the contribution of fermentation to total ATP production seems small (Fig. 3A), at [O_2] values used for long term apple storage it could play an important role (Fig. 3B).

Possible implications for storage procedures

Fermentative metabolites - The role of lactic acid seems somewhat ambivalent. On the one hand its accumulation can cause damage in plant tissues, but it also seems necessary in some tissues to increase ADH activity and enable increased glycolytic ATP production. It is therefore unclear whether regulation of LDH activity will result in less problems in the stored plant tissues. There seems to be no relationship between ethanol levels in plant tissues and tissue disorders. Nevertheless high ethanol concentrations can result in off-odours and off-taste, and so ethanol concentrations should be kept below specific levels. Because odour and taste depend on many different compounds, the limit levels for ethanol will probably differ for

different cultivars and species. During storage not only ethanol but also acetaldehyde levels can increase (Folchi et al., 1995). Because acetaldehyde is known to be toxic for plant tissues (Perata and Alpi, 1991), it might be wise to monitor acetaldehyde levels during CA storage. There also seems to be a CA risk in not being able to control [O_2] within narrow margins. A continuous variation in [O_2] might cause increased acetaldehyde concentrations. The main risk, however, for high acetaldehyde levels occurs directly after CA or MA. When products, containing high concentrations of ethanol, are suddenly exposed to high [O_2], the ethanol is re-metabolized, leading to an increase in acetaldehyde (Nanos et al., 1992; Mateos et al., 1993; Pfister-Sieber and Brändle, 1995). An important observation is that ADH is not the only enzyme capable of converting ethanol to acetaldehyde. In many eukaryotic cells a cytochrome P-450 acts in this manner. Acetaldehyde production by this enzyme may occur even in the presence of ADH inhibitors (Jones, 1989).

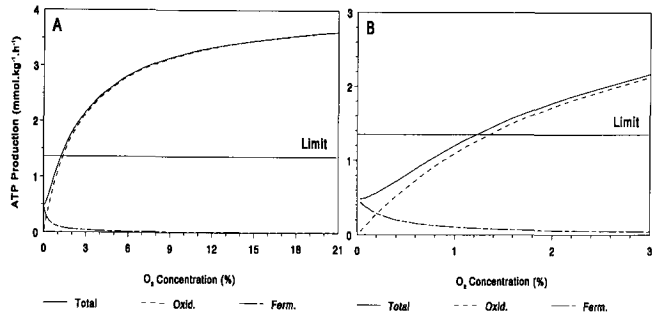

Figure 3. Estimated ATP production based on gas exchange models. A.: Broad range of [O_2] values; B.: Detail at low [O_2].

Pretreatments - The possible relation between energy status and storage disorders in products subjected to CA conditions, also raises questions on pretreatments of these products. Xia and Saglio (1992), for instance, showed that acclimation to anoxia can be induced in maize root tips by a hypoxic pretreatment, which raises the level of ATP but does not increase glycolytic flux. Andrews et al. (1993) showed that the extent to which mRNAs for PDC and ADH increased in maize seedlings subjected to anoxia depended on whether the seedlings had previously been acclimated to hypoxia or anoxia. Pretreatments might improve storability of certain products by increasing their fermentative ATP production capacity. Increased ADH levels might also reduce the risk of toxic effects by acetaldehyde (Jones, 1989).

Control of storage conditions - Optimal gas conditions for storage of produce are not fixed values but can change during storage (Lidster et al., 1985; Kader et al., 1989). This could result from changes in energy demands or production (Peppelenbos and Rabbinge, 1996). Using fixed gas conditions for long term storage could lead to problems and increased losses. Interactive storage facilities, responding to the stored material, could help to reduce such risks. Then the primary consideration in optimizing gas concentrations will be whether total energy production at those conditions is sufficient for maintaining cell viability. Two important criteria have to be known: the minimum energy production necessary for maintenance, and the ethanol level that should be avoided.

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