

## **INTERIM REPORT**

### **RESEARCH ON 'INTERNAL ABNORMALITIES AND QUALITY OF ROCHA PEARS'.**

**September 1997 through January 1998**

**Confidential**

**A.C.R. van Schaik (project leader)**

**H.W. Peppelenbos**

**S. Robat**

**R.H. Veltman**

**M.G. Sanders**

**T.R. Lammers**

**Investigation under the authority of:**

**Frutus**

**Attn. Armando Torres Paulo**

**Estrada Nacional 366, km 4**

**2550 Cadaval, Portugal**

**Campotec S.A.**

**Attn. Paulo José de Magaride Pereira da Cruz**

**Casalinhos de Alfaiata**

**2560 Torres Vedras, Portugal**

**Cooperative Agricola dos Fruticultores do Cadaval**

**Attn. Aristides Lourenco Sécio and Pedro Nuno Nobre Nicolau da Silva**

**Estrada Nacional 115, Km 26**

**2550 Cadaval, Portugal**

**Research executed by:**

**- Agrotechnological Research Institute (ATO-DLO), Wageningen**

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## Summary

This report presents the results of the research, as conducted so far, for the cooperatives Frutus, Campotec and Agricola to improve the storage of Rocha pears to extent the storage period of these pears. Main issue is the possibility of CA-storage. An important problem of CA-storage of Rocha pears is the development of internal browning and cavities in the fruits. The main objective is the determination of safe CA-conditions (without the development of internal browning) at the condition that taste, texture, flavour and other quality aspects are maintained.

Rocha pears from two small orchards were used. They were picked in the beginning of August. The storage at ATO in CA-containers started early September. The pears from both orchards were stored at normal temperature in combination with the following gas conditions: 0.5% CO<sub>2</sub> - 3% O<sub>2</sub>, 3% CO<sub>2</sub> - 3% O<sub>2</sub>, 0.5% CO<sub>2</sub> - 1% O<sub>2</sub> and 3% CO<sub>2</sub> - 1% O<sub>2</sub>. Every 6 weeks samples were taken from the containers to measure firmness, colour, vitamin C content and internal abnormalities. This took place immediately after storage and after 4 days of shelf life simulation (at 20°C). At three sampling times the respiration and diffusion characteristics of the pears of one orchard, stored at 0.5% CO<sub>2</sub> and 3% O<sub>2</sub>, were determined.

This report describes the results from September 1997 until Januari 1998 and includes the measurements at the start and after 8 and 15 weeks of storage.

In November in 3 out of 4 CA-conditions brown and hollow pears were found. The higher the CO<sub>2</sub>-content and the lower the O<sub>2</sub>-content the more abnormalities were found. Only in 0.5% CO<sub>2</sub> - 3% O<sub>2</sub> and in normal cooling no abnormalities were found. Furthermore, an important observation was that the green ground colour of the pears stored at a low oxygen content was maintained, whereas the CO<sub>2</sub> content was of less importance. The vitamin C content was strongly influenced by the CA-conditions: the extreme CA-conditions showed a lower vitamin C content. This is possibly related with an increased degree of browning.

At this stage the optimum CA-condition can not yet be advised. This depends on the outcome of the research later in this season.

The respiration rates of Rocha pears were determined to i) relate respiration with the development of brown hearts and ii) to calculate the necessary scrubber-activity of CA-installations in practice.

Diffusion of O<sub>2</sub> and CO<sub>2</sub> and respiration are of great importance for the development of brown hearts and cavities. The measurements proved that the pulp of the Rocha pears gives little restrictions for CO<sub>2</sub> and O<sub>2</sub>. The most important diffusion barrier seems to be the skin. It also appeared that there are large differences between individual pears, which may explain the sensitivity for internal brown.

The respiration measurements showed that the respiration rate is fairly constant within the range of 21 and 4% O<sub>2</sub>. At lower oxygen contents in CA the respiration rate is strongly slowed down and will come under the supposed limit for energy production at 2% oxygen. This could indicate a critical value for the occurrence of browning at 2% O<sub>2</sub>. It is also clear that an increased CO<sub>2</sub> content strongly reduces the respiration rate.

## 1.0 Introduction

In general Rocha pears can be stored and sold from August until February using normal cold storage. At these conditions a longer storage period is not possible, because the pears become tasteless and because of the development of storage diseases such as putrefaction and superficial scald. To extend the storage period some experience with CA-storage has been gained in research and in practice. The result was that the taste of the pears stored at CA was better, less scald occurred, but the pears developed internal abnormalities. The symptoms of this were browning around the core and the development of cavities.

These problems are also known for other pear cultivars (such as Conference) and occur at pears that are picked late and stored at too high CO<sub>2</sub> and O<sub>2</sub> conditions.

Together with the cooperatives Frutus, Campotec and Agricola dos Fruticoltures and the research centre at Alcobaça, an inventory was made last year to improve the storage conditions.

Since there is ample experience with CA-storage of pears at the ATO (experience with Conference pears), it has been decided to carry out storage research with Rocha pears at the ATO for the duration of one season.

The objective of this research is to select the CA-storage conditions for Rocha pears at which no internal and external abnormalities develop and the quality (taste, texture) of the pears will be maintained.

In this report the research activities will be described as carried out from August 1997 till January 1998.

### 1.1 Relationship with research on Conference-pears

The research on Rocha pears is facilitated because of 'Europear', a European Community research project at the supervision of ATO-DLO. The general objective of the Europear project is to prevent the development of tissue disorders in pears resulting in internal browning and cavities. The causes underlying the development of the disorder are unknown, although there is a lot of correlative knowledge on the development of internal disorders. The occurrence is influenced by weather factors, orchard factors (location, nutrition), picking date, post-harvest treatments and storage conditions. However, relationships found in one country cannot simply be applied for other countries or growing seasons.

The key element in Europear is the development of technology which enables a rapid measurement and decision about the post-harvest treatments and storage conditions. Part of the research is an extensive research on gas exchange rates, diffusion resistance and pear quality throughout the storage season. This will be further explained.

### 1.2 Theoretical background gas exchange characteristics

Because pear tissue has a very low porosity, small changes in metabolic rates or diffusion rates within the tissue can lead to very low internal oxygen levels. Increased fermentation can lead to an increase of toxic metabolites like acetaldehyde (Perata and Alpi, 1991). This may reduce cell viability and induce cell death, leading to the internal disorders. Another explanation is that the combination of oxidative and fermentative processes are not sufficient to maintain cell viability (Andreev *et al*, 1991; Zhang and Greenway, 1994). The reason for a difference in occurrence of internal disorders between Northern and Southern European countries might be the influence of different climate and/or agronomical factors on pear

growth and development. This could result in different metabolic rates, resistance to gas diffusion, energy metabolism, energy needs for maintenance costs, etc. To check this hypothesis data is needed on climate and orchard conditions during the growing season and gas exchange rates, diffusion resistance and pear quality throughout the storage season, carried out in different European countries. Once a physiological explanation is found, storage conditions have to be adapted in order to prevent disorders. For this purpose predictive models are needed, which use information collected during the growth of the pear, or directly after harvest. Recently several models are developed describing gas exchange based on enzyme kinetics. The model of Peppelenbos *et al.* (1996) might function as a basis for the models that will be developed. The model uses ATP production rate as the main inhibitor of fermentative CO<sub>2</sub> production. This model enables to describe the relation between gas (O<sub>2</sub> and CO<sub>2</sub>) concentrations and O<sub>2</sub> consumption and CO<sub>2</sub> and ATP production rates.

### **1.3 Background of vitamin C measurements.**

The research with Conference Pears proved a strong relation between the occurrence of internal abnormalities (brown and cavities) and the vitamin C content. The background of this is that the vitamin C fulfils an important function in resisting browning. Initially the vitamin C prevents browning because it is a natural antioxidant. But in combination with other substances this substance can also catch so called radicals. These radicals affect the cell membranes leading to damage of the membranes. Therefore, enzymes (PPO) and polyphenols can react with each other and form a brown pigment (melanin) that finally forms the brown colour of the tissue.

The theory is, that at extreme CA-conditions less energy is available to produce vitamin C over and over again, causing a decrease of the content. When at a certain moment there is too little vitamin C available in a fruit, the radicals can no longer be caught, leading to browning. In case at specific storage conditions a significant decrease occurs during storage, this may be an indication for the development of brown hearts.

The relation with the respiration measurements is important because it gives an insight in the available energy.

## 2.0 Material and methods

### 2.1 Pears for the research

In the beginning of September the pears were transported from Portugal to the Netherlands. About the picking time (probably 2 August), the growing conditions of the pears and the period preceding the transport in Portugal no exact data are available. The pears have not been subjected to any treatment before storage to prevent putrefaction and scald. This was agreed beforehand.

The pears were transported in plastic crates and came from two different orchards, namely José Domingos dos Santos and Joáo Picarra.

At arrival at the ATO the pears were immediately stored at cooled conditions. Before the pears were put into the storage containers they were homogenized and randomized to obtain a fair distribution of pears over all the containers (storage conditions).

### 2.2 Storage facilities and storage conditions

After the pears were cooled (two days) they were placed in the CA-containers at the respective storage conditions. The containers with a content of about 600 litre were placed in a cold store room in which during the whole storage period a temperature of  $-0.5^{\circ}\text{C}$  was maintained. Measurement and control of the CA-conditions ( $\text{CO}_2$  and  $\text{O}_2$ ) are almost continuously carried out by a process computer. Per hour an air sample of the container is measured and if necessary actions are undertaken to restore the set point of the conditions. This can be: scrubbing of  $\text{CO}_2$  with potassium hydroxide, adding  $\text{CO}_2$ , ventilation or injection of nitrogen. The conditions were maintained during the whole storage period.

The pears of the two orchard were stored together in a container at the planned storage conditions.

*Storage conditions:*

$\text{CO}_2$ (carbon dioxide)	$\text{O}_2$ (oxygen)
0	21
0.5	3
0.5	1
3	3
3	1

### 2.3 Measurements of internal and external quality

Up to 1 January 1998 the pears were judged several time on quality. This took place at: start of storage, the beginning of November and half December.

The measurements were done at a sample of pears directly from the storage container concerned and at a sample of pears that was kept for 4 days at atmospheric conditions at a temperature of 19°C (shelf life simulation).

The quality measurements consisted of:

*1) Colour measurement of the skin*

Of 15 pears per storage condition and grower the colour of the skin was measured with the Minolta Chromameter. Using this method the colour path from green to yellow can be determined objectively.

*2) Measurement of firmness*

At the same 15 pears the firmness was measurement with the penetrometer. This was done with the 11 mm plunger.

*3) Visual inspection of internal and external abnormalities*

The same 15 pears were also cut into linear and cross direction. Eventual deviations were divided into 4 categories:

- 0 = no browning
- 1 = minor browning
- 2 = moderate browning
- 3 = strong browning

## **2.4 Vitamin C determinations**

The research at Conference-pears showed a strong relation between the occurrence of internal abnormalities (browning and cavities) and the vitamin C content. Vitamin C is known for its quality to combat browning (anti-oxidant effect) but can also prevent damage to the cell membranes. However, if, due to extreme storage conditions, vitamin C is no longer produced to a sufficient extent, the total vitamin C content in the pear will decrease. When at specific storage conditions a significant decrease occurs during storage, this could indicate a false storage condition.

At each judgement (start of storage, the beginning of November and half December) of all storage objects (storage conditions and origin) the vitamin C content was determined.

The measurement of vitamin C content was done to pears that came directly from the storage room. Of every 10 pears from each condition a sub-sample was taken of the tissue from the stalk to the core. This material was frozen in liquid nitrogen and subsequently stored under deep freeze conditions at respiration -80°C.

The vitamin C content in the fruit is the content of ascorbate plus de-hydro-ascorbate.

The determinations were executed with High-Performance Liquid Chromatography(HPLC).

## 2.5 Respiration and diffusion

The work focused on the determination of variation in gas exchange rates and diffusion resistance of Rocha pears. Pears were/will be measured three times during the storage period. The pears were stored at 0°C, 2% O<sub>2</sub> and 0% CO<sub>2</sub>.

Gas exchange characteristics were measured at 2°C (to test the possibility of a rapid testing method).

### *Gas exchange rates*

Fresh weight and underwater weight (Bauman and Henze, 1983) were measured. Samples from pears were taken to measure the specific weight of pear juice (used in the calculation of the porosity). Outer surface area of the pears was estimated using the length and the maximum circumference of the pear (see figure 1). Two pears were put in 1500 ml cuvettes. The cuvettes were connected to a flow through system. Gas conditions were all combinations of 0, 0.5, 1, 2.5, 6 and 21% O<sub>2</sub> with 0 and 5% CO<sub>2</sub>. Relative humidity was high (>95%) since the gas was led through water flasks. After 4 and 5 days of storage O<sub>2</sub> uptake and CO<sub>2</sub> production was determined. This was done by disconnecting the cuvettes from the flow through system, and sampling the headspace directly and after a period of 6 hours. The GC used was a Chrompack CP 2002. The measured O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> was corrected to 100% to account for possible pressure variations inside the GC. Then the concentration values (in %) were multiplied with the actual pressure inside the cuvette (in kPa). Because the volume of the cuvette and the volume of the pears is known, the gas exchange rates can be calculated in nmol/kg.s.

### *Diffusion resistance*

The method of measuring diffusion resistance as described by Peppelenbos and Jeksrud (in press) was slightly adjusted. The inert gas neon was used as well, but instead of measuring the diffusion of neon into the fruit the diffusion of neon out of the fruit was measured. First the fruit was stored in a cuvette with a high concentration of neon (5000-6000 ppm) for one night, assuming that by that time an equilibrium between the concentration inside the fruit and in the cuvette was reached. The final concentration was measured. Then the fruit was transferred to another cuvette, and after specific time intervals (seconds) the neon concentration was measured. The time intervals were selected based on the paper of Banks (1985), using the so called 'linear method'. For this 'linear' method many measurements are necessary directly after transferring the fruit. This results in a relative short period of measuring, which is an advantage when a lot of repetitions have to be carried out. Also leaks of the cuvette will have a marginal influence on the results using the linear method.

### *Gas exchange models*

Recently gas exchange models were developed (Peppelenbos and van 't Leven, 1996, Peppelenbos et al., 1996) based on Michaelis-Menten kinetics. Oxygen uptake can be described as:

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

where O<sub>2</sub> and CO<sub>2</sub> are the external or internal O<sub>2</sub> or CO<sub>2</sub> concentrations, V<sub>O<sub>2</sub></sub> = the O<sub>2</sub> uptake rate (ml.kg<sup>-1</sup>.h<sup>-1</sup>), Vm<sub>O<sub>2</sub></sub> = the maximum O<sub>2</sub> uptake rate (ml.kg<sup>-1</sup>.h<sup>-1</sup>), Km<sub>O<sub>2</sub></sub> = is the Michaelis

constant for the influence of  $O_2$  on the  $O_2$  uptake rate and  $Km_{CO_2}$  = is the Michaelis constant for the influence of  $CO_2$  on the  $O_2$  uptake rate.  $CO_2$  production can be calculated using the next equation (Peppelenbos et al., 1996):

$$V_{CO_2} = V_{O_2} * RQ_{ox} + \frac{Vm f_{CO_2}}{1 + \left( \frac{O_2}{Km f_{O_2}} \right)} \quad (2)$$

where  $RQ_{ox}$  is the RQ value for oxidative processes,  $Vmf_{CO_2}$  is the maximum fermentative  $CO_2$  production rate ( $ml \cdot kg^{-1} \cdot h^{-1}$ ) and  $Kmf_{O_2}$  the Michaelis constant for the inhibition of fermentative  $CO_2$  production by  $O_2$ .

Considering the importance of energy needs, 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). ATP produced by oxidative pathways can be calculated using equation 1.

Oxidative ATP production ( $V_{ATP(o)}$ ,  $\mu\text{mol} \cdot kg^{-1} \cdot h^{-1}$ ) can be directly derived from the  $O_2$  consumption rate ( $V_{O_2}$  in  $ml \cdot kg^{-1} \cdot h^{-1}$ ) using a conversion factor based on the ideal gas law. At  $18^\circ C$  and  $101.3 \text{ kPa}$  this conversion factor is  $41.87 \text{ } (\mu\text{mol} \cdot ml^{-1})$ , assuming that the  $ATP/O_2$  ratio is 6:

$$V_{ATP(o)} = V_{O_2} * 6 * 41.87 \quad (3)$$

Although other pathways than ethanol fermentation exist, they remain quantitatively minor (Ricard et al., 1994). During the first hours of anoxia, it is necessary to determine the sum of the main products accumulated (ethanol, acetaldehyde and lactate) to establish the fermentation rate. The determination of the rate of accumulation of 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, being the second term in equation 2:

$$V_{ATP(f)} = V_{CO_2(f)} * 41.87 \quad (4)$$

#### *Statistical analysis*

The gas exchange rates were analyzed using the nonlinear regression analysis of the statistical package Genstat. The models used within the package were derived from Peppelenbos and van 't Leven (1996) and Peppelenbos et al. (1996).

### 3.0 Results and discussion

#### 3.1 Internal disorders

The pears sampled at 3 and 7 November 1997 showed no internal abnormalities. However, at the inspection on 15 and 19 December 1997 at some storage conditions clear abnormalities were observed (see table 1).

*Table 1. Percentage of Rocha-pears with cavities and browning stored at different CA-conditions (judgement on 15 en 19 November).*

Storage condition O <sub>2</sub> - CO <sub>2</sub>	Pears from Picarra orchard				Pears from Santos orchard			
	directly		after 4 days		directly		after 4 days	
	% cav.	% brown	% cav.	% brown	% cav.	% brown	% cav.	% brown
0-21	0	0	0	0	0	0	0	0
0.5-3	0	0	0	0	0	0	0	0
0.5-1	0	33.3	0	0	13.3	26.6	0	6.6
3-3	13.7	6.7	0	0	0	0	0	6.7
3-1	46.7	73.3	40	73.3	26.6	86.7	40	86.7

Pears of both origins appeared to be sensitive to the development of abnormalities at a number of CA-conditions. Browning of the pears mainly occurred in the part from the core to the crown. In pears stored in normal cooling and stored at 0.5% CO<sub>2</sub> and 3% O<sub>2</sub> no abnormalities were found. However, when the pears were stored in 3% CO<sub>2</sub> browning as well as cavities occurred. Especially when the oxygen content was 1% the deviations clearly occurred more often. Also at storage in 0.5% CO<sub>2</sub> and 1% O<sub>2</sub> abnormalities occurred. This implies an interaction. A combination of a low oxygen content and a high CO<sub>2</sub> content intensify the problem.

At the judgement directly after storage more pears were found with abnormalities than after 4 days shelf life at 20°C.

Because judgement took place in December there is not much to say about the further developments during the storage season. Therefor we will have to wait what the best conditions for application in practice will be. As yet the combination of 0.5% CO<sub>2</sub> and 3% O<sub>2</sub> is considered safe.

#### 3.2 Firmness and colour

Figures 1 through 4 show the results of the firmness measurements. These are the average values of the pears for both orchards.

At judgement after 8 weeks as well as after 15 weeks directly from CA (see fig. 1 and 2) there were no differences between the storage conditions in firmness. There was no significant difference compared to the firmness measured at the start of the storage.

After 4 days of shelf life the firmness considerably decreased (fig. 3 and 4). There were hardly any differences between the storage conditions. However, there was a slight tendency that the pears from normal cooling were a bit firmer.

It is striking that the firmness during shelflife of Rocha pears decreases slower than that of Conference pears.

The colour of the pears is measured with a Minolta Chromameter. This objective colour meter is used as an international standard for measuring colour of fruits. This meter allows measurements of several colour spectra. For the colour range from yellow to green the a-value usually satisfies. These are also shown in graphs 5 through 8. The average observations of the 2 orchards are shown. The influence of the conditions causes the same colour development. Measurements at the pears immediately after sampling already show differences. After 8 weeks in storage (fig. 5) pears from normal cooling are a bit yellower than pears from CA. After 15 weeks (fig. 6) this difference becomes even clearer, also compared to the values at the beginning of storage.

It is striking, that the colours of pears stored in 1% O<sub>2</sub> are just as green as at the beginning of storage. The influence of a higher CO<sub>2</sub> contents (3%) seems much smaller. During a shelflife (fig. 7 and 8) of 4 days the pears obviously become more yellow, but the influence of the storage conditions remains the same as immediately after storage.

### 3.3 Vitamin C determination

The ascorbate content of the pears is shown in figures 9 and 10. The initial value at the beginning of storage is 100%. The values after 8 and 15 weeks of storage respectively are shown as a percentage of the initial values. A difference is made between the 2 orchards. For pears from Santos (fig. 9) the ascorbate percentage clearly varies per storage condition. Cold stored pears hardly show a change in ascorbate content. However, CA-storage clearly influences the vitamin C content. The more extreme the conditions (lower O<sub>2</sub> and higher CO<sub>2</sub>) the lower the vitamin C content. Especially in the combination of 3% CO<sub>2</sub> and 1% O<sub>2</sub> the vitamin C content decreases rather rapidly during storage.

The same trend but to a smaller extend is measured in pears from the Picarra origin.

Table 2 shows the relation between the vitamin C content at various storage conditions.

Table 2. Relation between browning and ascorbate content in Rocha-pears stored in different CA-conditions. (judgement after 8 and 15 weeks).

Storage condition O <sub>2</sub> - CO <sub>2</sub>	Pears from orchard Picarra				Pears from orchard Santos			
	after 8 weeks		after 15 weeks		after 8 weeks		after 15 weeks	
	% Vit. C of initial	% brown	% Vit. C of initial	% brown	% Vit. C. of initial	% brown	% Vit. C. of initial	% brown
0-21	114	0	124	0	105	0	107	0
0.5-3	102	0	97	0	96	0	95	0
0.5-1	109	0	85	33.3	103	0	70	26.6
3-3	86	0	86	6.7	70	0	85	0
3-1	102	0	84	73.3	74	0	52	86.7

At the pears of both origins there seems to be a relation between the extent of browning and the vitamin C content, corresponding with the theory mentioned before. It may be that the decrease of the vitamin C content can predict browning.

### 3.4 Respiration and diffusion

Gas exchange rates are expressed in nmoles/kg.s, following the guidelines of Banks et al.(1995). Gas exchange rates were measured at 6 different O<sub>2</sub> concentrations (0, 0.5, 1, 2.5, 6 and 21 kPa). Although the differences between these gas concentrations used are not equal, the differences in respiration rates are comparable (Fig 11). In future experiments therefore comparable gas concentrations will be used. The percentage of explained variance of the models used is reasonable for both O<sub>2</sub> uptake measurements and CO<sub>2</sub> production measurements (Table 1).

Based on oxygen uptake and carbon dioxide production estimations of respiration and fermentation rates can be made. All oxygen uptake is attributed to respiration, and the carbon dioxide production with RQ equal to the RQ at ambient air (21 kPa of oxygen) is attributed to respiration as well. All the carbon dioxide production in excess of this RQ (Rqox in the model) is considered to be the result of fermentation. Based on respiration and fermentation the total ATP production is calculated (Fig 11D). To interpret the results, also a limit value for ATP production is introduced. This limit value is estimated as the ATP production at 2 kPa of oxygen (92 nmoles ATP/kg.s), well below the advised 3 kPa for CA storage of Conference pears. Using this limit value, it can be observed in Fig 11D that an increase of carbon dioxide to 5 kPa results in a stronger decrease in ATP production than a decrease in oxygen from ambient to 2 kPa. This confirms the general idea that carbon dioxide accumulation in the storage facility should be avoided.

Diffusion resistance values varied widely between individual measurements. When measurements were compared (Fig 12A), where one measurement is on two pears, the

resistance varies between 570 and 1900 s/m. Even when respiration rates are comparable, large differences in diffusion resistance will result in a large differences in internal gas composition between individual pears. Advice on safe storage conditions should include the extent of this variation. No clear relationship between diffusion resistance and internal gas volume was found (Fig 12B). This indicates that the main contribution to the total diffusion resistance is not the flesh of the pear but something else; the skin.

## 4.0 Discussion

The observations in the period of September until January on storage and quality research on Rocha pears so far give important indications on the behaviour of pears in various storage conditions. The aim of the research is to select a storage regime in which no internal abnormalities occur and the quality of the pears is maintained for a long period of time. With quality is meant the flavour quality and the sensitivity to external storage disorders.

With special measurements it is the object to understand more of the backgrounds of the processes that lead to internal abnormalities as well as the development of the flavour quality. An important result was that at the last judgment (after 15 weeks of storage) internal abnormalities occurred in pears of both orchards. This concerned hollow pears as well as brown pears. Because in this research a so called square schedule was chosen the influence of CO<sub>2</sub> as well as O<sub>2</sub> can be judged separately as well as in coherence.

It is clear that a higher CO<sub>2</sub> content and a lower O<sub>2</sub> content increase the development of internal browning and the formation of cavities. However, the combination of 1% O<sub>2</sub> and 3% CO<sub>2</sub> leads to much more abnormalities. In normal cooling and most mild CA-combinations (3% O<sub>2</sub> and 0.5% CO<sub>2</sub>) so far no browning occurred. In the other CA-combinations with either 1% O<sub>2</sub> or 3% CO<sub>2</sub> some browning was observed.

These results lead to a preliminary indication that the CO<sub>2</sub> level should not exceed 1% and the O<sub>2</sub> level should not be below 3%. However definitive conclusions cannot be made until the whole experiment has been finished. Certainly when the results of the research in Alcobaca can be involved in this, where other conditions have been applied and also the influence of the harvesting time, better conclusions can be drawn about the optimum CA storage conditions.

Furthermore, it is possible to make the preliminary conclusion that in CA-storage the conditions that cause the most cavities and browning best guarantee colour and firmness during storage. It is remarkable that the influence of the low oxygen content to colour preservation is very strong whereas a high CO<sub>2</sub>-content hardly has an influence to yellow colouring. To maintain a green ground colour a low oxygen content seems very important. The specific measurements in relation to the development of cavities and browning are very interesting. It is obvious that the more extreme the storage conditions the more the vitamin C content drops. Especially at the combination 1% O<sub>2</sub> and 3% CO<sub>2</sub> the vitamin C content is very low. This might indicate that the vitamin C content after 8 weeks may give an indication to the occurrence of browning. It is possible that this measurement has a predictive value. It is not clear yet, whether this is influenced more by CO<sub>2</sub> than by O<sub>2</sub>.

At Conference pears vitamin C showed to be of great importance in resisting abnormalities. It appears that this also applies to Rocha pears. A follow-up conclusion could be that the processes of cavity formation and browning in Conference and Rocha pears have the same background. This opinion is also supported by the respiration measurements. If the respiration drops under a certain value, insufficient energy (ATP) can be produced to regenerate vitamin C or an other scavenger once again. Especially at a high CO<sub>2</sub> content there would be a matter of constant lack at all oxygen concentrations. However, especially when the oxygen content drops below 2% a larger energy deficit seems to develop. The further respiration measurements during the storage period will have to reveal this.

An other interesting observation is that diffusion limitation above all is accomplished by the characteristics of the skin and less by the pulp of the pears. This could indicate that, assuming the skin characteristics don't change, during the season no change of diffusion occurs. However, future research should confirm this explanation.

Fig 1 Firmness Rocha-pears after 8 weeks in storage

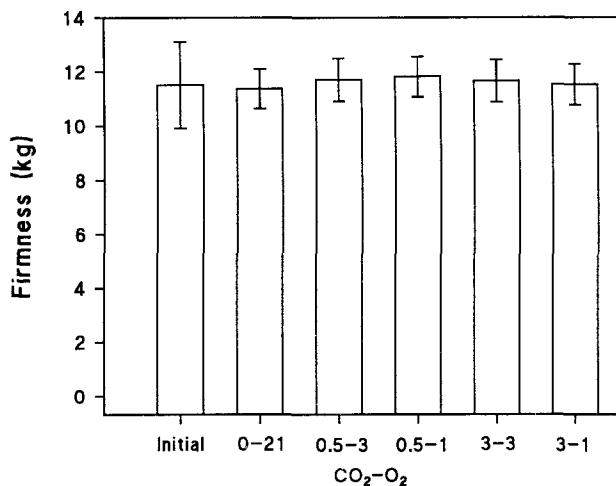


Fig 2 Firmness Rocha-pears after 15 weeks in storage

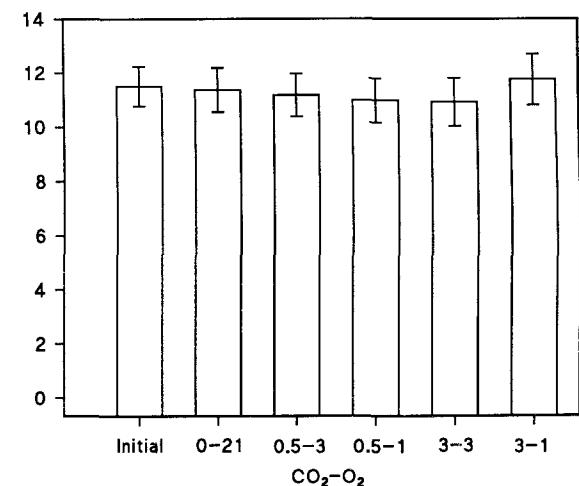


Fig 3 Firmness after 15 weeks in storage and 4 days 20°C

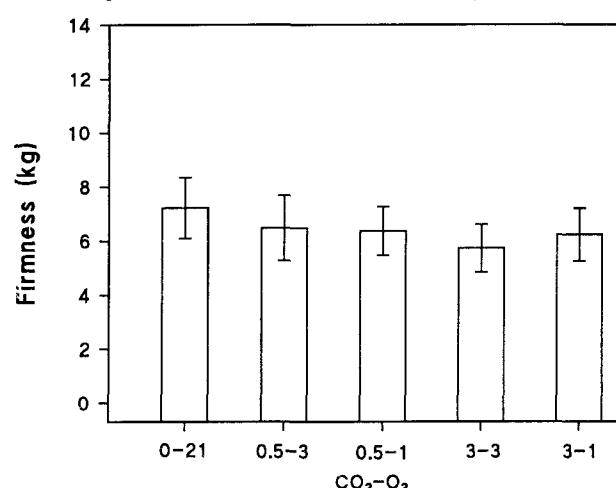


Fig 4 Firmness after 15 weeks in storage and 4 days 20°C

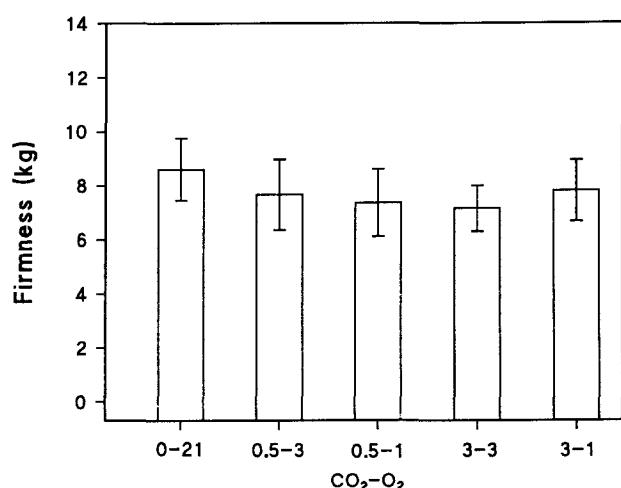


Fig 5 Colour (a-value) of Rocha-pears after 8 weeks in storage

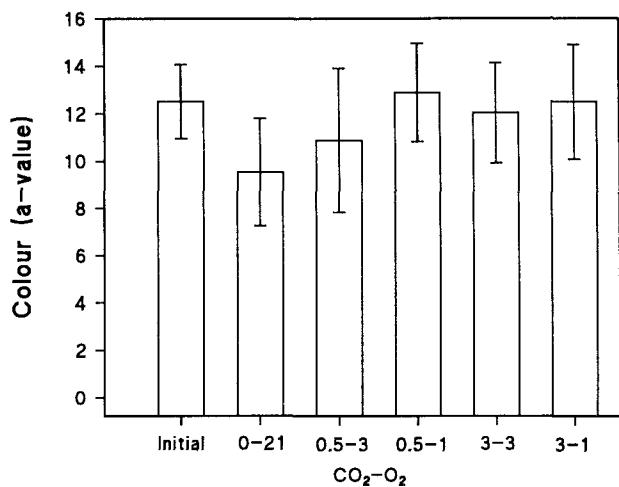


Fig 6 Colour (a-value) of Rocha-pears after 15 weeks in storage

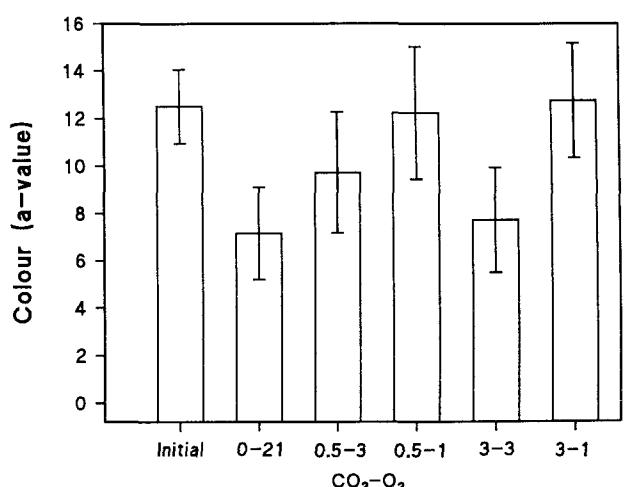


Fig 7 Colour after 8 weeks in storage and 4 days 20°C

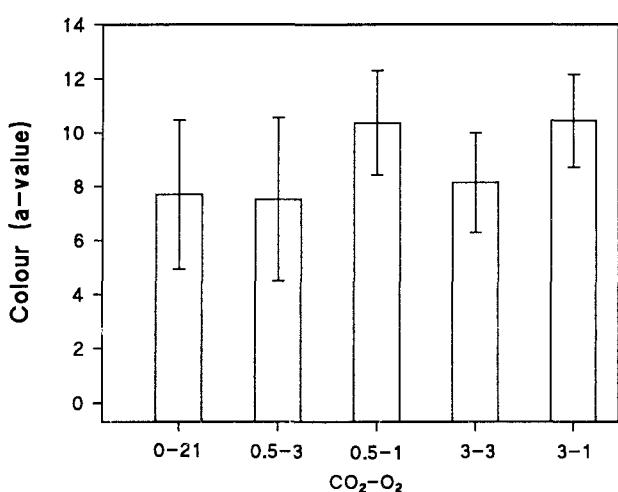


Fig 8 Colour after 15 weeks in storage and 4 days 20°C

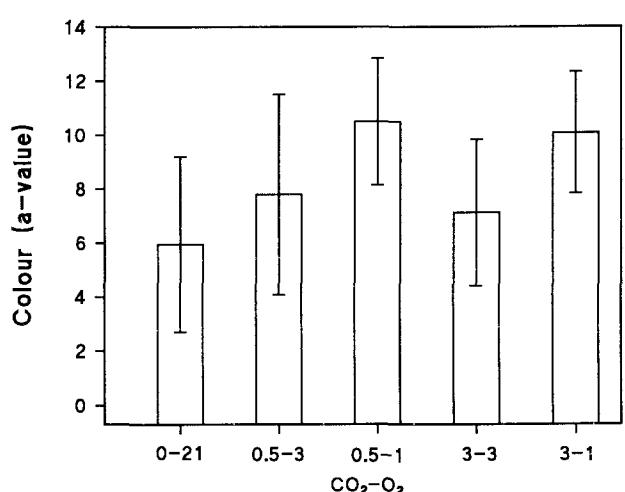


Fig. 9 Relative amount of Ascorbate (%)  
of the initial value of Santos pears.

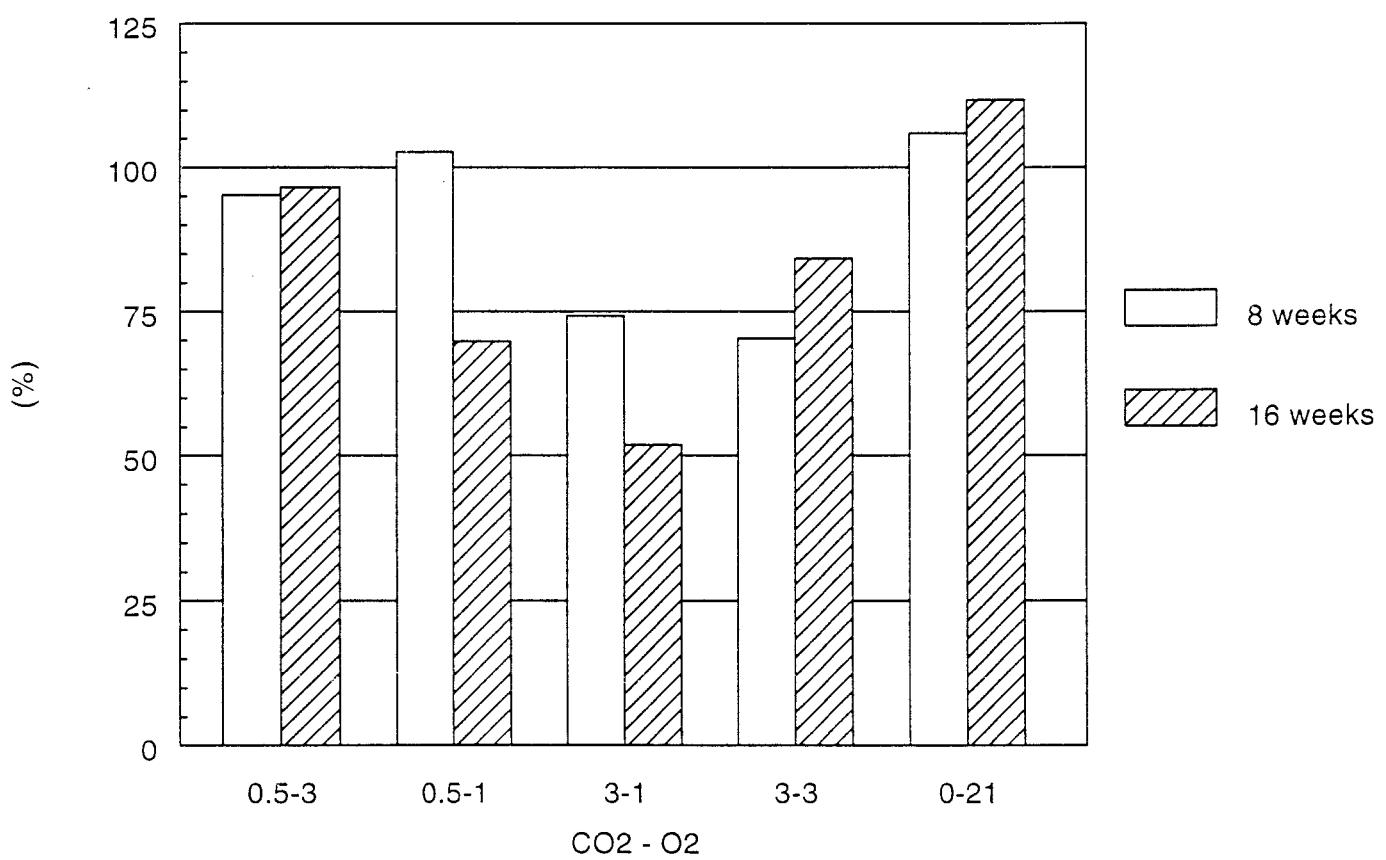
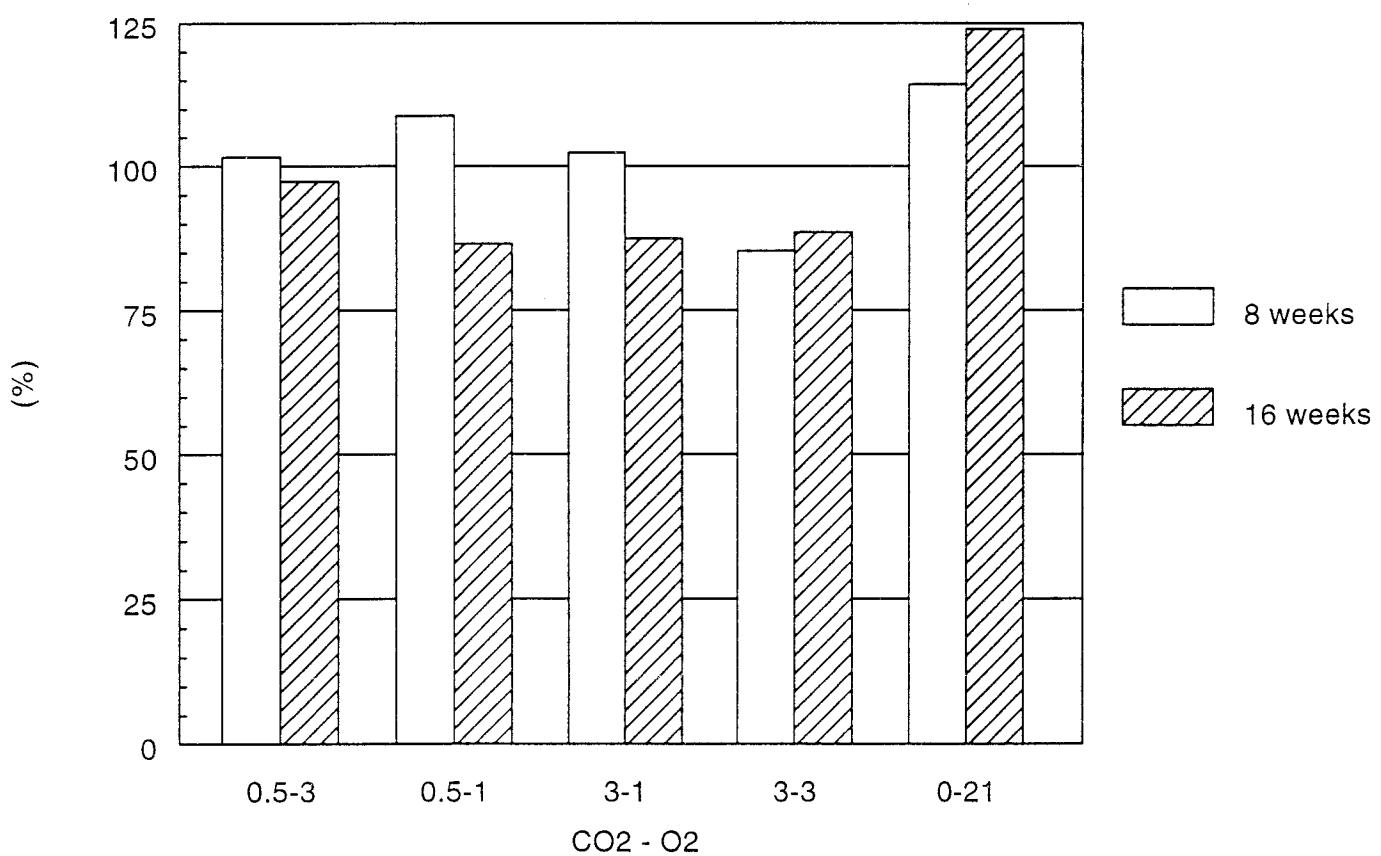


Fig. 10 Relative amount of Ascorbate (%)  
of the initial value of Picarra pears.



## Tables and Figures

*Table 3 Overview of the statistical results of fitting the gas exchange models to the data, using Genstat nonlinear regression analysis (Fit = fitted value,  $se$  = standard error,  $R^2$  = percentage of explained variance).*

tot

	Fit	<u>std</u>
$R^2$	880	19
$Vm_{O_2}$	191	11
$Km_{O_2}$	47	10
$Km_{CO_2}$	194	66
$R^2$	786	23
$Vmf_{CO_2}$	212	76
$Kmf_{O_2}$	43	26
$RQox$	95	3

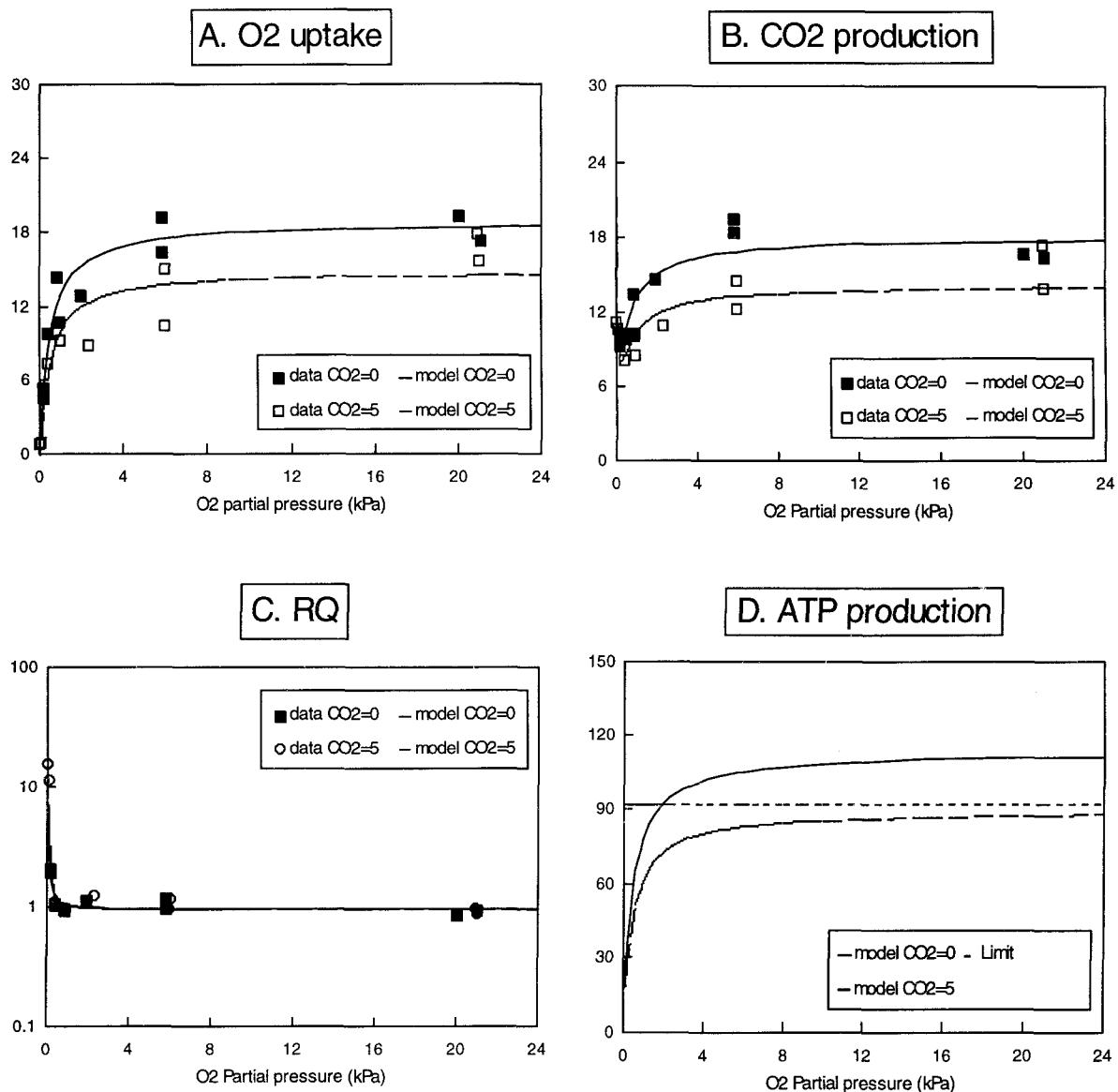


Figure 11. The  $O_2$ -uptake (11A) and  $CO_2$ -production (11B) of Rocha pears. The respiration is measured after 5 days storage at different oxygen and carbon dioxide conditions. Figure 1c shows the RQ values and model fit. Figure 11D gives the calculated ATP production.

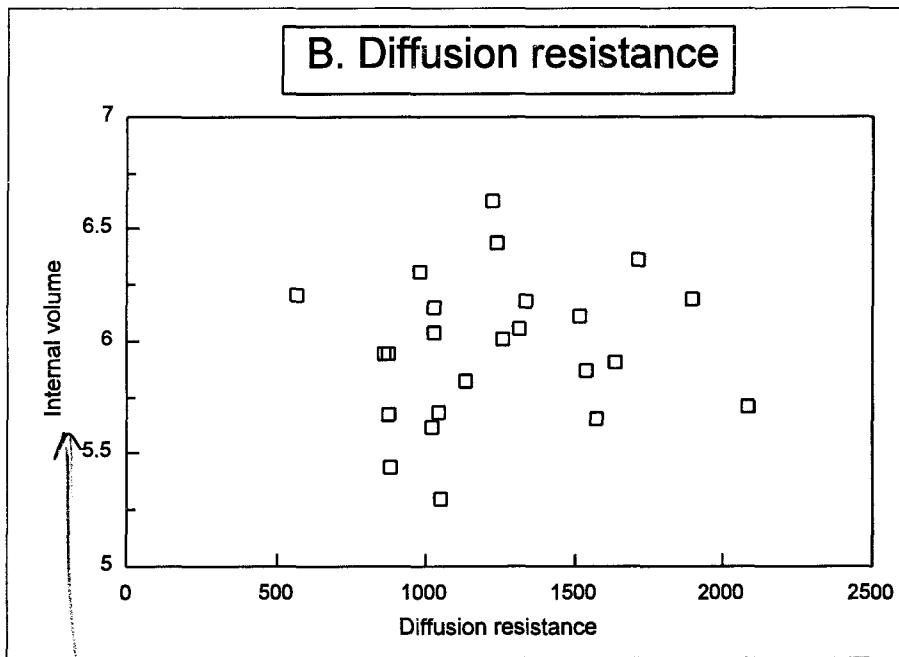
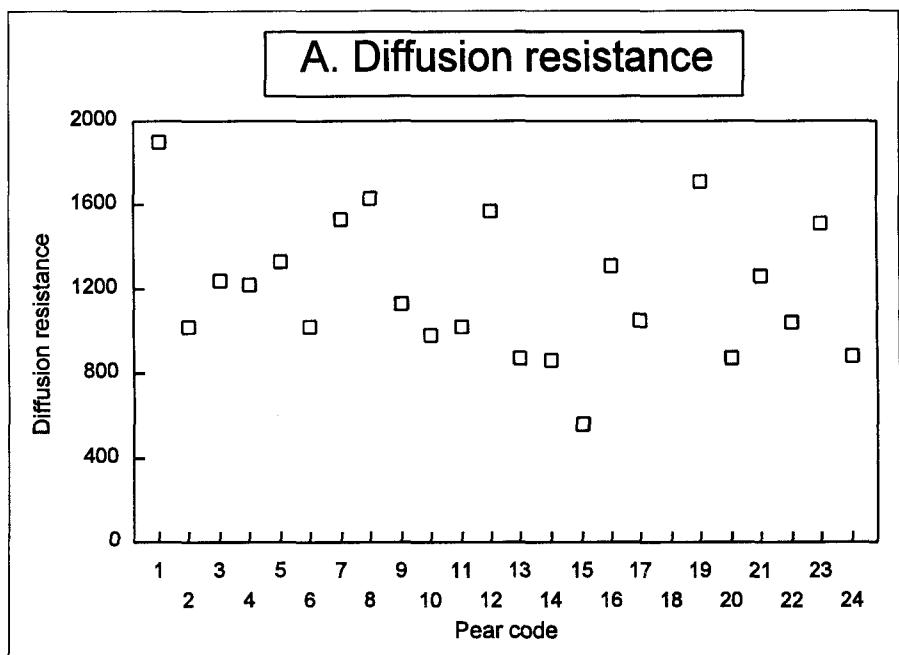


Figure 12. Diffusion resistance values per pear (Fig 12a) and as compared with internal gas volumes of the pears (Fig 12B).

porosity?

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