ATO



# Packaging Minimally Processed Fruits and Vegetables

## Final Report

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Confidential

Filipa Margarida de Souto Martins

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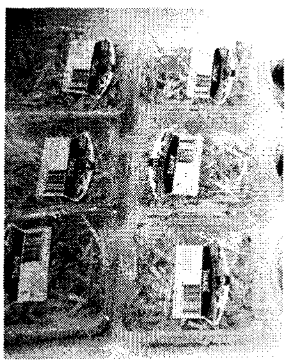
**"MAP technology for fresh cut products is still in its infancy and there are many gaps in our understanding"**

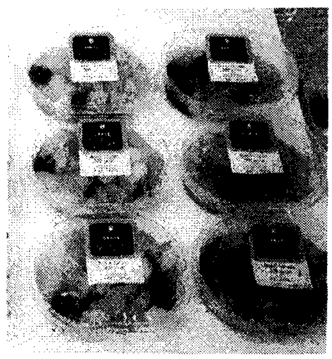
(Gorny, 1997)

# Acknowledgement

I gratefully acknowledge everybody in ATO that helped me in one way or another, especially: Aart Zegveld, Anneke Polderdijk, Eugene Schijvens, Fatima Kreft, Gerard Boogaard, Harmannus Harkema, Herman Peppelenbos, Janneke ten Donkelaar, Raimund Jaeger and Rudolf Bakker.

# Commercial packaged salads





**Bean sprouts**
**Mixed salads**
**Fruit salad**

# 1. Introduction

## 1.1. Markets and Expectations

Changes in the technology and practice of packaging fresh horticultural commodities has been driven by a rapidly evolving confluence of changes in consumer expectations, globalization of produce markets, and technological advances that are resulting in the rapid development of ever more sophisticated ways to maintain produce in a "fresh" condition for extended periods of time. As the market for fresh fruits and vegetables has become global, consumers have come to expect and to demand safe, clean, high quality fresh produce every day of the year. Surveys of consumers have repeatedly shown that "freshness" of produce is one of the most important factors in selection of where people shop and of the produce that they purchase (Zind, 1989).

Minimally processed, precut produce and other value added presentation of fresh fruits and vegetables are in increasing demand by individual and, especially, by restaurants and other foodservice operations. Packages that can increase shelf life, maintain quality, protect from injury, ensure cleanliness, reduce disease, carry a recognized brand label, and attractively present fresh fruits and vegetables can help satisfy these newly evolving demands of the marketplace. For these reasons, many produce packers and processor have renewed their interest in modified atmosphere packaging (MAP) to help them anticipate the demands of the market.

Modified atmosphere packaging is a technology appropriate to the needs of the produce industry, particularly the precut or minimally processed industry which is the fastest growing sector (Farber *et al*, 1995).

The quality of fruits and vegetables is comprised of several different elements. Flavor, nutrition, texture, aroma, appearance, and safety are all important elements of produce quality and when any one of these parameters falls bellow a certain desirable level, the quality of the product is compromised. When the quality falls significantly, the end of product shelf life results. This will be different for different kinds of produce and will be different for produce bound for different markets.

Consumers expect fresh cut product to be without defects, of optimum maturity and in fresh condition. The fresh like condition covers general appearance, sensory quality (texture/firmness and taste) and nutrient quality.

There has been a surge in the production of "ready to eat" or minimally processed vegetables and fruit products, and packaged salads currently comprise about half the total volume. The volume of packaged salads has dramatically increased in recent years (Lopez-Galvez *et al*, 1997).

## 1.2. Shelf life extension by MAP

MAP is able to slow quality loss and extend shelf life of fresh fruits and vegetables through suppression of a number of processes that would otherwise deplete the tissue of their components or hasten ripening and subsequent senescence. MAP is able to have such a diversity of effects on plant tissue due to the differential effects of low $O_2$ and elevated $CO_2$, suppression of the effects of the ripening hormone ethylene ($C_2H_4$), and reduction of moisture loss due to the moisture barrier properties of the plastic film.

## 1.3. The importance of $O_2$

Because $O_2$ acts as the terminal electron acceptor in many metabolic reactions, the rates of some essential metabolic processes are sensitive to $O_2$ concentration. Reducing $O_2$ concentrations below about 10% around many fresh fruits and vegetables slows their respiration rate and indirectly slows the rates at which they ripen, age and decay. Reducing the $O_2$ concentration can, in some cases, reduce oxidative browning reactions which can be of particular concern in precut leafy vegetables. Reduced $O_2$ can delay compositional changes such as fruit softening, pigment development, toughening of some vegetables, and development of flavor (Kader, 1986).

However, $O_2$ is required for normal metabolism to proceed. $O_2$ concentrations below about 1 - 2% can lead to anaerobic metabolism and associated production of ethanol and acetaldehyde resulting in off flavors, off odors and loss of quality. Of even greater concern is the potential growth of anaerobic bacteria, some of which are pathogenic to humans, under low $O_2$ conditions. The proper $O_2$ concentration will depend upon the fruit or vegetable and its tolerance to low $O_2$, the temperature and the time that the product will be exposed to low $O_2$ (Farber *et al*, 1995).

## 1.4. The importance of $CO_2$

$CO_2$ occurs in a small amounts in air ($\approx$ 0.03%) but, at elevated levels, has important metabolic effects on both produce and microorganisms.

At concentrations above 1 - 2%, $CO_2$ reduces the sensitivity of plant tissues to the ripening hormone ethylene. Ethylene can cause premature ripening, fruit softening, yellowing of leafy vegetables, increased respiration rate and senescence of many fruits and vegetables.

Elevated $CO_2$ can, like reduced $O_2$, slow respiratory processes thereby extending shelf life. Although the effects of elevated $CO_2$ on respiration are not as dramatic as those of low $O_2$, high $CO_2$ and low $O_2$ together can, in some cases, reduce respiration more than either gas alone (Kader *et al*, 1989).

$CO_2$ at relatively high concentration (>10%) has been shown to suppress the growth of number of decay-causing fungi and bacteria. However these levels of $CO_2$ do not suppress some human pathogenic bacteria of potential concern on fresh produce.

For example, *Clostridium botulinum* and *Listeria monocytogenes* are relatively resistant to the effects of $CO_2$ (Farber, 1991).

## 1.5. Fresh cuts and MAP

Fresh cut fruits and vegetables are products that are partially prepared so that no additional preparation is necessary for their use.

The operations involved in preparing fruits and vegetables as fresh-cut products generally increase the rates of deterioration of the products. The physical damage or wounding caused by preparation increases respiration and phenolic metabolism within minutes (Bolin *et al*, 1977; Ohta and Sugawara, 1987). These increases in the rates of other biochemical reactions are responsible for changes in colour (including browning) (Ballantyne *et al*, 1988; Heimdal *et al*, 1995), flavour (McDonald *et al*, 1990), texture, and nutritional quality (Brecht, 1995).

Browning the most common colour defect found in fresh cuts is caused by the oxidation of phenolic compounds in the presence of the enzyme polyphenol oxidase (Murata *et al*, 1995; Sharples *et al*, 1963).

The rapid wide spread use of what is often referred to as the "fresh cut revolution" has been made possible, in part, by improvements in MAP.

MAP facilitated the extension of product shelf life to meet the demands of long distance distribution; the maintenance of product quality through reduction of respiration rate, browning reactions, fruit softening and decay; added product convenience through portion control and resealable bags; and enhanced marketing through identification of brands and improved visibility of the product (Zagory, 1997).

Modified atmosphere within fresh cut containers or bags can be beneficial in maintaining quality of fresh cut product (Gorny, 1997). Fresh cut products probably can tolerate more extreme levels of $O_2$ and $CO_2$, because they do not have as much cuticle or skin to restrict gas diffusion, and the distance of gas diffusion from the centre to outside of the product is much less than for the whole product. However, threshold levels that might cause injury should be avoided because gas mixture of modified atmosphere packages cannot be regulated closely (Watada *et al*, 1999).

Fresh cut products are highly susceptible to weight loss because internal tissues are exposed and lack skin or cuticle. However, relative humidity generally is very high in film bags or containers overwrapped with film, so dehydration typically is not a problem (Baldwin *et al*, 1995). Although films provide high relative humidity, two potential problems exist. Moisture condenses on the inner surface of the packaging films which detracts in viewing the material and the droplets can become a site for possible breakdown and microbial growth. The second problem is that, if the film does not allow sufficient transmission of $O_2$ or $CO_2$, to high $CO_2$ or too low $O_2$ levels can cause injury to the product. Thus, films need to be selected not only for maintaining high relative humidity, but also for allowing proper transmission of gases (Watada *et al*, 1999).

Although fresh cut products are highly perishable, high quality products can be assured by selecting the proper maturity for processing, controlling defects and disorders, and maintaining temperature, atmosphere and relative humidity.

## 2. Experimental Procedures

Four commercial types of packaged salad products were evaluated:
salad A ( a mix of iceberg lettuce and red lettuce); salad B ( a mix iceberg lettuce, carrot, white cabbage and red lettuce) (Fig.1); fruit salad (grapes, apple, melon, pineapple and peach); bean sprouts (Fig. 2).

**Fig. 1** Commercial packages of mixed salads.

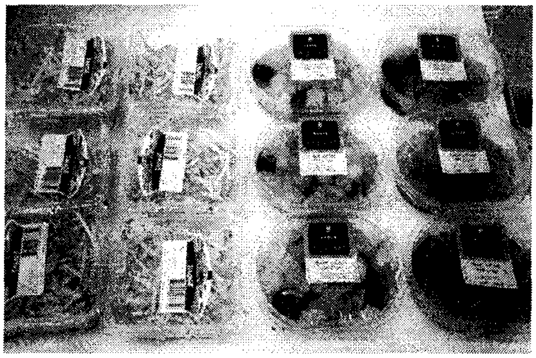


**Fig. 2** Commercial packages of bean sprouts and fruit salad.

There were six samples per each different group of salad which were stored at 7° C. Most of the products reached the "best before" date three days from the day of package. Three packages were opened on the last day of the "best before" and three the day after. Packages have a typical shelf life period of 5 days (Johma, private communication).

The package atmosphere was measured every day. Gas samples were taken and the $CO_2$ and $O_2$ concentrations were determined on a gas chomatograph (Chrompack micro GC 2002 automatic sampling and calibration).

Product quality was evaluated by an untrained panel. In order to have a general impression of the product, the visual characteristics, textural properties, evaluation of odours and flavour defects were determined.

The most important visual quality parameter was the browning and dehydration. The texture was evaluated by breaking some pieces. The evaluation of odours was initially determined in the headspace when the bags were opened .

For the fruit salad the evaluation was made for each individual component.

# 3. Results

## 3.1. Package atmosphere

The $CO_2$ concentrations increased more than the $O_2$ concentrations decreased (Figs. 3 and 4).

The $CO_2$ concentrations are higher in the salad B than the A. For the fruit salad the level of $CO_2$ during the last two days increased strongly. Since the $CO_2$ was not produced by the product because there wasn't much $O_2$ it was presumably produced by microorganisms.

The gas concentrations in the bean sprouts packages remained constant during the storage period. This was a result of perforation applied to the film: in this case a 'equilibrium' or passive MA condition was applied.

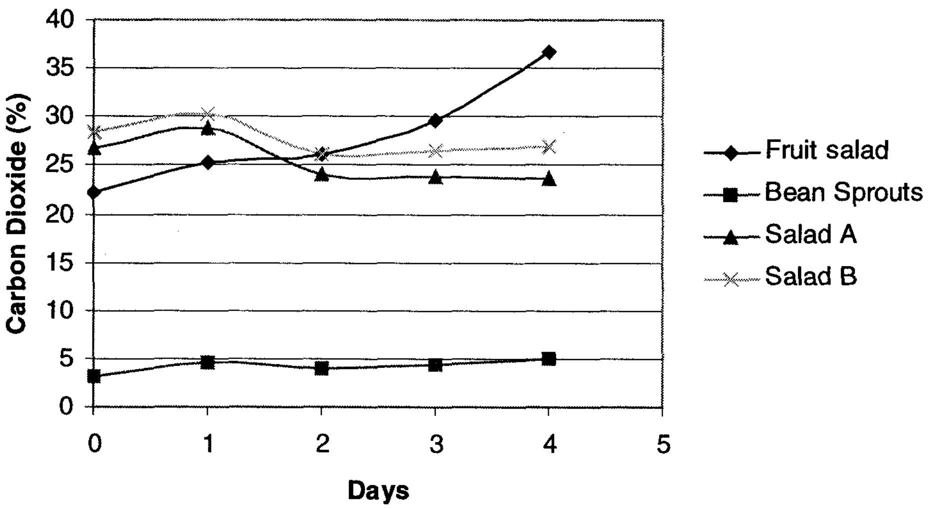



**Fig. 3** Carbon Dioxide concentrations in the packs during storage at 7° C for 4 days.

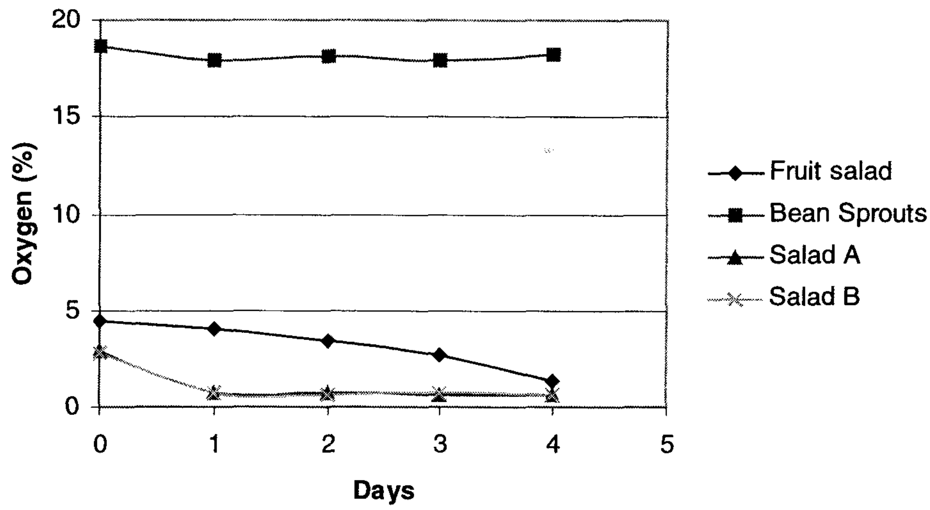



**Fig.4** Oxygen concentrations in the packs during storage at 7° C for 4 days.

## 3.2. Product quality evaluation

- Fruit salad

The general impression of the product was not fresh, especially for apples, melon and pineapple. There were significant differences in general overall visual and textural quality among all fruit due to browning and crispiness in the apples, colourless and limp melons and coloration and softness in the pineapple. The visual characteristics and the textural properties were not so bad for the grapes and the peach.
A fermentative odour and flavour was detected.
The fruit salad was not acceptable at the "best before" date.

- Bean sprouts

The main visual defects were dark tips due to dehydration and dark beans.
Texture was not so much affected in the commercial bean sprouts, apart from the tips. Bean sprouts developed a musty off-odour.

- Mixed Salad

The most important visual characteristics that decreased in quality was the browning in the lettuce and the surface whiteness in the carrots in the salad B.
General good textural properties were observed. The development of off-odours was one of the main defects in both kind of salads.

# 4. Conclusion

Based on the gas measurements in the initial period of the experiment, we can conclude that elevated $CO_2$ and decreased $O_2$ concentrations were applied during packaging. In the commercial packaged salads the $O_2$ and $CO_2$ concentrations in the bags were very similar, except for the bean sprouts which were packaged in a perforated package.

The quality of the commercial salads decreased in time but was acceptable at the "best before" date. The fruit salad was an exception, it was not acceptable at the "best before" date.

Browning and off-odours were the most noticeable defects.

# Minimally processed cut mangoes

II

# 1. Introduction

Mango fruit are very popular for their succulence, exotic flavour and delicious taste. Mango is a fairly good source of carbohydrates, vitamin C and is a rich source of provitamin A (Jain, 1961; Bhatnagar and Subramanyam, 1973; Lakshminarayana, 1980; Doreyapa Gowda and Ramanjaneya, 1994).

The mango (*Mangifera indica* L.) has traditionally been grown in an area that extends southward and eastward from India through Myanmar and Vietnam to Indonesia. It probably is not indigenous to the Philippines, where it has long been cultivated (Valmayor, 1962), but related *Mangifera* species are endemic there (Bondad, 1982).

There are criteria for cultivar description (Mulkherjee, 1985) and there are also a list of different mango cultivars.

In our research we used a well-known cultivar named "Kent". The fruit is greenish-yellow with red or crimson blush, numerous small yellow dots, oval, with rounded base; the skin is thick, tough and adherent; the flesh is firm, tender, melting and juicy with little fibre, deep yellow to orange yellow, sweet with a rich flavour and pleasant aroma, of excellent quality; the seed is momoembryonic in thick , woody stone.

"Kent" is successful commercial cultivar in drier parts of Mexico, Central America, West and South Africa (Campbell, 1992), from where we obtained the mangoes used in the experiments.

The import of (sub)tropical and off-season fruits and vegetables into the European Union (EU) is growing substantially, with the Netherlands as an important import and transit market. The volumes of EU and Dutch imports of mangoes of 1990 and 1994 are shown in table (Anon, 1995) (Table 1).

**Table 1** Growth of European Union (EU) and Dutch imports of mangoes

| | **Volume EU (tonnes)** | | **Volume The Netherlands (tonnes)** | |
|---|---|---|---|---|
| | **1990** | **1994** | **1990** | **1994** |
| **Mangoes** | 31.602 | 48.977 | 7.537 | 21.914 |

In most mango growing countries, the fruit is generally consumed fresh it is there for desirable to explore possibilities to maintain the fresh like characteristics of minimally processed mangoes by MAP.

Mango may be preserved or processed in a way to produce a product which retains at least some degree of cellular integrity and structure. Being generally more delicate than most other tissue foods, mangoes bear a correspondingly greater risk of structural and textural damage.

Minimally processed fruits, developed through the application of "hurdle technology", are claimed to better retain the organoleptic properties of the fresh materials since the factors or "hurdles" responsible for the short to medium term preservation of such products are applied at relatively low intensities or concentrations (Leistner 1985, 1992; Alzamora *et al* 1989, 1993; Huxsoll *et al* 1989).

The mangoes required some preparation such as peeling, removal of flesh from the seed and slicing before they can be consumed. Thus, the fruit probably would have a greater appeal if they were peeled and sliced for immediate consumption. Very little is known about the potential shelf life and quality changes of fresh cut mangoes.

In general, lowering the oxygen in storage atmosphere can be beneficial in maintaining quality and extend shelf life of fresh produce (Lipton, 1975; Ulrich, 1975; Robinson *et al*, 1975). For fresh cut mangoes, the lower limit of acceptable $O_2$ level is unknown. A very low $O_2$ level in packaged whole mango fruit have been found to be undesirable (Yahia *et al*, 1997). Limbanyen *et al* (1998) also reported that mangoes should be ripe but not over-ripe for the use as fresh cut product.

Maturity is an important quality attribute of fresh cut fruit because immature fruit lack good sensory quality and over-mature fruit has a limited shelf life. Thus, with the limited shelf life of the fresh cut fruits, it is essential to use mature fruit with acceptable eating quality for minimally processing, but not overmature fruit, which will deteriorate rapidly (Watada *et al*, 1999)

When a modified atmosphere package (MAP) is flushed with the ideal gas levels for preserving a product, it is preferable if the gas levels remain close to their initial values (i e., maintain steady state conditions). Technically, this would result in controlled atmosphere (CA) or passive (equilibrium) MA rather than MAP (Brecht, 1980).

To maintain the desired gas levels within the package, two factors must be considered. First, the produce in the package is continually respiring and consuming $O_2$ while producing $CO_2$. Second, $O_2$ will diffuse into the bag and $CO_2$ will diffuse out. This occurs because the package contains low levels of $O_2$ and high levels of $CO_2$, and the atmosphere outside the package contains 21% $O_2$ and trace levels of $CO_2$. To control the atmosphere within the package successfully, the permeability of the packaging film must be selected such that the rate of $O_2$ diffusion into the package equals the rate of $O_2$ consumption by the product (Segall *et al*, 1996).

The aim of this study was to examine the mangoes reaction to different gas concentrations, several films and the reaction of distinct parts of the fruit.

# 2. Experimental Procedures

Ready to eat Kent mangoes were washed and hygienically cut and gas packed, using a gas packer (Ilpra Food Packer 500 UG) and gas mixer (Witt KM 300/3 M), in different types of packages with different initial concentrations of $O_2$ and $CO_2$ (Table 2).

**Table 2** Types of packages and initial concentrations of $O_2$ and $CO_2$.

| | $O_2$ (%) | $CO_2$ (%) |
|---|---|---|
| **PET** | 0 | 10 |
| **PET perforated** | 21 | 0 |
| **PET perforated** | 5 | 15 |
| **PET perforated** | 5 | 0 |
| **Hyplast ST3** | 21 | 0 |
| **PA90 (outer flesh)** | 21 | 0 |
| **PA90 (inner flesh)** | 21 | 0 |

Four replicates per each type of package were prepared. Each one contained 300g of product. A representative sample of different mangoes and different parts of the fruit were prepared. The packs with the film type PA 90 were packed differently. In these one slices cut near the seed (inner flesh) were packed and in the other slices cut just under the mangoes peel (outer flesh). All packs were stored at 10° C.

The $O_2$ and $CO_2$ concentrations inside the packs were measured daily with a gas chomatograph (model Chrompack micro GC 2002 automatic sampling and calibration).
The quality evaluation of the mangoes was tested after 2 days. The following aspects were evaluated: Browning were scored on 0 to 5 scale, where 0 = none and 5 = severe browning. In addition odour and flavour were evaluated. For these two parameter was only evaluated whether the product had or had no off odour/flavour.

# 3. Results

## 3.1. Package atmosphere

There was more variance in the $CO_2$ than in the $O_2$ concentrations, the increase of $CO_2$ concentrations was higher compared to the decrease of $O_2$ concentration. (Figs. 5 and 6) The explanation could be microbial activity.

The development of $CO_2$ and $O_2$ concentrations in the packages with the Hyplast ST3 film was different due its selectivity (ratio permeabilities $CO_2/O_2 \approx 10$).

There was no difference between inner part and outer part of the fruits with respect to the $O_2$ concentrations, but the built up of $CO_2$ was little bit higher for the inner fleshes.

For the perforated packs the $CO_2$ and $O_2$ levels will reach some equilibrium value (equilibrium MA), depending on the initial concentrations and variations in the size of the perforation thus the $CO_2$ levels for these packs were circa 10% - 20% and $O_2$ were 10% - 15%.

However, the $CO_2$ concentrations increased in the PET with perforation stronger than in the PA 90 (ratio permeabilities $CO_2/O_2 \approx 1.18$), possibly due to microbial activity.
The packs with higher % $CO_2$ (15%) had a slower built up % $O_2$ compared with the packs with 0% $CO_2$.

The PET packs with 0% $O_2$ and 10% $CO_2$ was the ones that had the highest increase for $CO_2$, it was the tightest film. It almost reached 30% $CO_2$ in the end of the storage period.

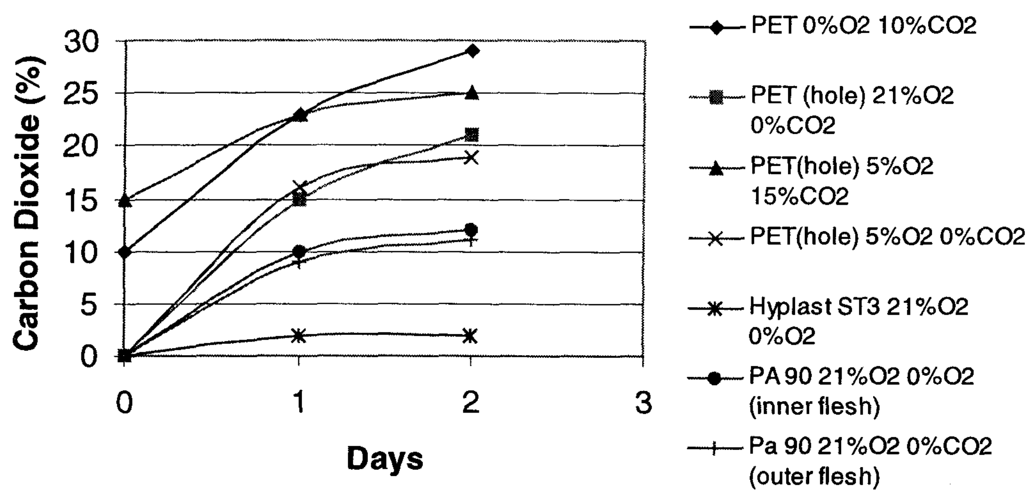



**Fig. 5** Carbon Dioxide concentrations in the packs during storage at 10° C for 2 days.

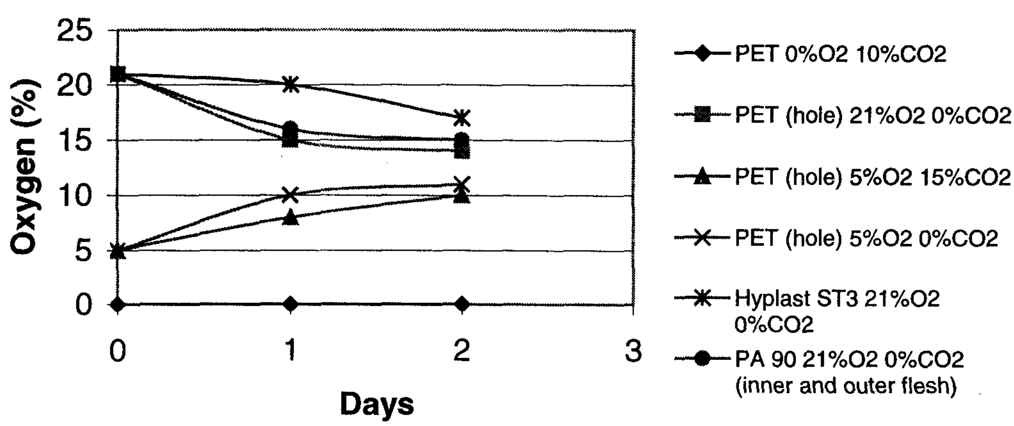



**Fig.6** Oxygen concentrations in the packs during storage at 10° C for 2 days

## 3.2. Product quality evaluation

The mangoes packed in PET packs with perforation, independently of the initial gas concentrations, got brown after 2 days of storage (Table 3). These packs got a 3 for browning which means according to the browning scale that they were not acceptable anymore (Figs. 7 and 9). The odour evaluation of all packs resulted in off odour, off flavour was only detected for the discoloured pieces, the yellow ones still tasted good.

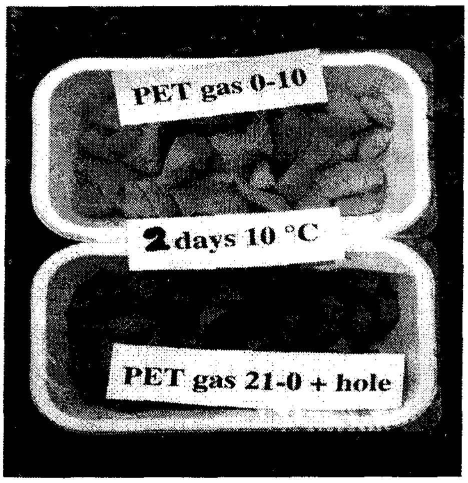



**Fig. 7** Effect of gas composition on browning.

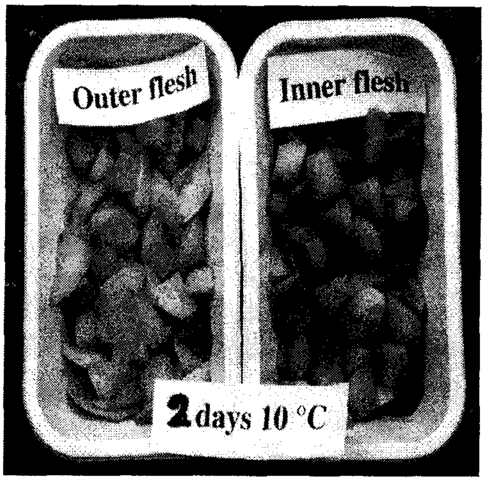



**Fig. 8** Differences in browning for inner and outer part of the mangoes.

The best score in browning was for the PET packs without oxygen (Figs. 7 and 9). These packs didn't get any off odour or off taste after 2 days storage, but the taste wasn't like fresh mangoes. It was similar to canned fruits.

The Hyplast ST3 and PA 90 with the outer flesh got the same score for browning (Fig. 9 and Table 3). These packs was acceptable in terms of coloration, but the smell and flavour for the latter ones was less good. Only the yellow pieces were good, like in the PET packs with hole. The Hyplast ST3 packs were the best ones considering the odour and the flavour. These ones had much more aroma.

The PA 90 packs with only the inner flesh had the worst score for browning (Figs. 8 and 9), but the flavour and odour was similar to the others packs with the outer fleshes.

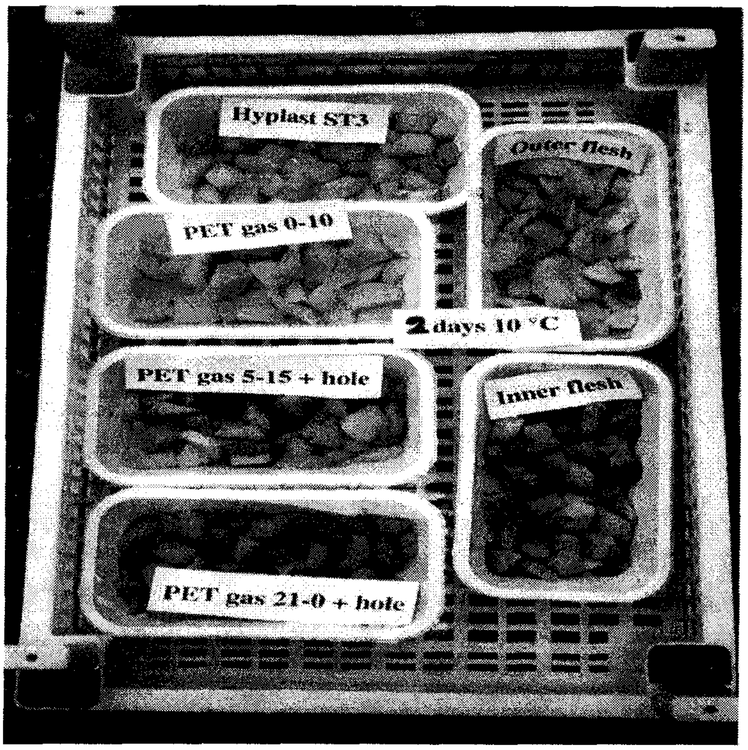



**Fig. 9** General effect of different gas concentrations on browning.

**Table 3** Quality evaluation for browning, flavour and odour in the packs after 2 days storage at 10° C.

| Type of package | % $O_2$ - % $CO_2$ (initial) | % $O_2$ - % $CO_2$ (final) | Browning (0 – 5) | Flavour/Odour |
|---|---|---|---|---|
| PET | 0 – 10 | 0 – 29 | 0 | No off |
| PET perforated | 21 – 0 | 14 –21 | 3 | Off |
| PET perforated | 5 – 15 | 10 – 25 | 3 | Off |
| PET perforated | 5 - 0 | 11 – 19 | 3 | Off |
| Hyplast ST3 | 21 – 0 | 17 – 2 | 2 | No off |
| PA 90 (inner flesh) | 21 – 0 | 15 – 12 | 5 | Off |
| PA 90 (outer flesh) | 21 – 0 | 15 – 11 | 2 | Off |

# 4. Conclusion

Quality of cut mangoes decreased during the storage period due to the presence of $O_2$ which caused rapid browning, especially at the presence of elevated $CO_2$ levels. High $CO_2$ caused off-flavour in the brown discoloured pieces.

The absence of $O_2$ prevents browning but the fruit lost its fresh flavour: the product tasted like canned fruit and the tissue became slightly translucent.

Inner fruit darkened more than the outer fruit due to differences in ripening near the seed and near the peel, which was also noted by Limbanyen *et al.* (1998).

The use of selective films like Hyplast ST3 seemed to be a good way to avoid negative scores for browning and also to have closest as possible fresh mangoes taste. It is a promising solution in terms of preventing browning and preserving flavour.

# 5. Further Research

In order to get accurate results and go deeper into the subject, it will be interesting focus in low $O_2$ and also low $CO_2$, using selective films or $CO_2$ scrubber, or to combine both.

For the next experiment the store temperature should be lower than this time. Using temperature around 5° C must bring some benefits as was showed by Rattanapanone and Watada. In addition, the sensitivity of different cultivars of mangoes for browning should be evaluated.

# Minimally processed cut lettuce

# III

# 1. Introduction

Minimally processed iceberg lettuce is a product that should offer convenience and quality. However, the physiological stresses that the lettuce is subjected to during processing and storage limit its shelf life. These stresses include deteriorative reactions of wounded or senescing leaf tissue, decay caused by the growth of microorganisms, and water loss from the tissue (King and Bolin, 1989).

Methods currently used for preservation include low temperature storage and packaging in a plastic film. Although these methods work reasonably well, it is believed that an increase in shelf life would result from storing the lettuce under a modified atmosphere (MA), as for other produce (Kader *et al*, 1989)

Controlled or modified atmosphere benefit fresh cut products principally by reducing respiration rates, retarding microbial growth, and retarding discoloration. Discoloration of the cut surfaces is a common problem with mildly processed products. Cutting stimulates enzymes involved in phenolic metabolism which in turn leads to the formation of undesirable brown pigments. The shelf life of many lettuce salads, for example, is limited by discoloration (Cantwell, 1997)

Controlled or modified atmospheres used for fresh cut products may differ from the atmosphere recommended for the intact product. Lettuce is a good example: intact lettuce heads are damage by high $CO_2$ concentrations. However a very effective way to retard discoloration at the cut surfaces of lettuce is to increase the $CO_2$ concentrations, below the critical levels (Cantwell, 1997).

## 1.1. Respiration rate

The tissues in fresh cut products are still living. To maintain life, their metabolic processes must derive energy primarily through the process of respiration that is regulated by many enzymes whose activities are controlled by temperature.

Respiration involves the consumption, using atmospheric oxygen, of carbohydrates and organic acids and consequent production of metabolic energy, heat, carbon dioxide, ethylene and moisture vapour. Different fruit and vegetables, and even different varieties of a given fruit or vegetable, will vary in their respiration rates. Those that have high respiration rates tend to be the most perishable while those with low respiration rates tend to be the least perishable. Furthermore, when fruits or vegetables are cut, sliced, shredded or otherwise processed, their respiration rates increase (Table 4). This is probably due to the increased surface area exposed to the atmosphere after cutting that allows oxygen to diffuse into the interior cells more rapidly and to the increased metabolic activity of injured cells (Zagory, 1997).

**Table 4** Respiration rates (mL.$Kg^{-1}$.$h^{-1}$) of whole and fresh cut iceberg lettuce.

| | Respiration rate (mL. $Kg^{-1}$. $h^{-1}$) | | |
|---|---|---|---|
| | **Temperature (°C)** | **Whole** | **Cut** |
| **Cantwell (1997)** | 7.5 | 5.0 | 12.5 |
| **Watada *et al* (1996)** | 7.5 | 5.0 | 12.6 |

Higher respiration rate mean a faster overall metabolism and a faster deterioration rates. Also higher respiration rates cause a faster loss of sugar and other components that determine flavour quality.

Fresh cut products generally have higher respiration rates than the intact products (Hyvonen *et al*, 1995).

The respiration rate is determined by measuring the consumption of $0_2$ or the generation of $CO_2$ over time (Kays, 1991). This may be done in a closed system in which the product is sealed in an air tight container and samples of the atmosphere are taken regularly. As there is no exchange of gases, the $0_2$ levels are continuously dropping while the $CO_2$ levels are continuously increasing.

The form of the mathematical model that best predicts respiration behaviour is a matter of debate. Some researchers have argued that the presence of $CO_2$ does not significantly affect the rate of $0_2$ consumption and that, for the sake of simplicity, the model can neglect the presence of $CO_2$ (Andrich *et al*, 1991; Cameron et al, 1989, 1994; Gong and Corey, 1994; Joles *et al*, 1994; Jurin and Karel, 1963; Lopez-Briones *et al*, 1993) However, other researchers have shown that the rate of $0_2$ consumption by the commodity depends on the concentration of $CO_2$ in the atmosphere (Herne, 1987).

Several models have been developed that incorporate the effect of $CO_2$. These models can be classified based on the mathematical functions used: enzymatic (Haggar et al, 1992; Lee *et al*, 1991, 1994;Peppelenbos, 1996; Yam *et al*, 1993), quadratic (Lee, 1986), or linear (Hayakawa *et al*, 1975).

The relationship between $0_2$ concentration and $0_2$ consumption rates can be described using Michaelis-Menten Kinetics (Chevillote, 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 influence of $CO_2$ can be modelled using the kinetics of a single enzyme, as is done with $0_2$ influence on $0_2$ consumption. In general three types of inhibition on the reaction rate of an enzyme are distinguished: competitive, non-competitive and uncompetitive (Chang, 1981).

In this study we used the non-competitive type of inhibition for reasons of simplicity. It gives very similar and good results.

The non-competitive type of inhibition occurs where the inhibitor reacts both with the enzyme and with the enzyme-substrate complex. The model with non-competitive inhibition can be described as (Chevilotte, 1973; Peppelenbos and van't Leven, 1996):

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

Where $Kmn_{CO2}$ is the Michaelis constant for the non-competitive $CO_2$ inhibition of $O_2$ consumption ($\%CO_2$), $Km_{O2}$ is the Michaelis constant for $O_2$ consumption ($\%O_2$) and $Vm_{O2}$ is the maximum $O_2$ consumption rate ($mL.Kg^{-1}.h^{-1}$). 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.

The model to describe total $CO_2$ production (Peppelenbos, 1993) is calculated as:

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

Where $Vmf_{CO2}$ is the maximum fermentative $CO_2$ production rate ($mL.Kg^{-1}.h^{-1}$), $Kmf_{O2}$ is the Michaelis constant for the inhibition of fermentative $CO_2$ production by $O_2$ and $RQ_{Ox}$ is the ratio between oxidative $CO_2$ production and $O_2$ uptake.

The first goal of this study was select the best range for $O_2$ and $CO_2$ concentrations required to preserve lettuce quality and consequently to extend shelf life. The second goal was to determine oxygen consumption rates

# 2. Experimental Procedures

## 2.1. Plant Material

Fresh cut iceberg lettuce was obtained from a local commercial trader in Renkum. The lettuce had been harvested in Spain four days earlier.

## 2.2. MA Package Material

Samples of 250g were weighed in CPET trays. The packages were flushed with a gas packer (ILPRA Food Packer 500 UG) and a gas mixer (Witt KM 300/3 M) (Fig. 10) with different concentrations of $O_2$ and $CO_2$ as showed in table 5.

In order to get the desired concentrations, the gas packer created a 98% vacuum before flushed. After flushing the gas packer sealed the trays with a CPET film.

**Fig. 10** Gas packer (ILPRA Food Packer 500 UG) and gas mixer (Witt KM 300/3M)

**Table 5** $O_2$ and $CO_2$ concentrations applied to the packages and number of replicates.

| $CO_2$ / $O_2$ | 0 | 5 | 10 | 15 | 20 | 40 |
|---|---|---|---|---|---|---|
| 0 | 6 | 6 | 6 | 6 | 6 | 6 |
| 5 | 6 | 6 | 6 | 6 | 6 | 6 |
| 10 | 6 | 6 | 6 | 6 | 6 | 6 |
| 15 | 6 | 6 | 6 | 6 | 6 | 6 |
| 21 | 6 | 6 | 6 | 6 | 6 | 6 |
| 40 | 4 | 4 | 4 | 6 | 6 | 6 |
| 60 | 4 | 4 | 4 | 4 | 4 | 4 |

All packs were stored in a room at constant 7° C.

## 2.3. Package Atmosphere Measurements

One replicate of each concentrations was connected to a gas chromatograph (Chrompack micro GC 2002 automatic sampling and calibration) (Fig. 11). Samples were taken with a needle inserted into the packs. In order to avoid leakage, the insertion point was sealed with silicone rubber. The $O_2$ and $CO_2$ concentrations were measured by the gas chromatograph that took 4 mL for sample volume and 10 samples per day for the first 3 days. After the third day till the end of the experiment samples were taken twice daily.

The gas composition in the remaining packs was monitored daily with another gas chromatograph. In order to know the concentration at the day when packs were opened and evaluated, one replicate per each concentrations was measured.

**Fig. 11** Gas chromatograph

## 2.4. Product Quality Evaluation

To study the quality changes in the packages during storage, packages were open after 5, 7 and 11 days of storage.

Product quality was evaluated by an untrained panel (Fig 12). The panel was composed of three group of two persons. Evaluation were conducted as a blind test so the judge did not know the $O_2$ and $CO_2$ concentrations in each pack.

Several visual quality parameters were evaluated. Browning and decay (macroscopic breakdown of the product) were evaluated using a score of 0 to 5, where 0 = none and 5 = severe. Translucency (glassy or transparent surface appearance) and cutting damage (level of injury caused by the cutter) were evaluated only by 0 or 1, where 0 = absence and 1 = present.

Texture was evaluated by manually breaking pieces of lettuce and was expressed in the crispness score based on a 0 to 5 scale, where 0 = crispy and 5 = limp.

The evaluation of odours in the headspace were determined immediately after opening the bag and after some minutes to check if it persisted (Fig. 13). Off-odours were scored on 0 = none; 1 = acid smell; 2 = sweet smell; 3 = rotten smell.

The general quality of the lettuce were evaluated using a scale of 0 to 5, where 0 = excellent and 5 = very bad.

**Fig. 12** Panel doing the quality evaluation.

**Fig 13** Evaluation of odours.

## 2.5. pH Measurements

In order to measure the pH, selected packs were taken after each evaluation. To carry out all measurements at the same time, the lettuce was frozen in liquid nitrogen and stored at -80° C until the end of all experiments. The pH was measured with Schott-pH-Meter CG 840 B (Fig. 14).

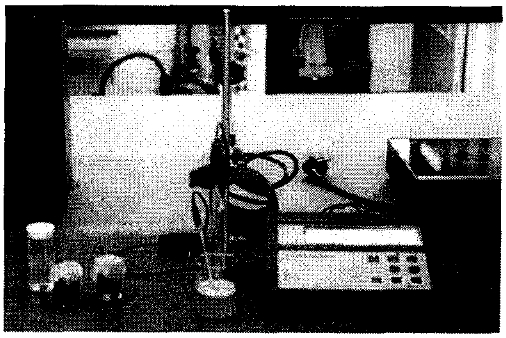


**Fig. 14** pH equipment

## 2.6. Volume determination

The headspace volume was estimated by water displacement (Baumann and Henze, 1983)

## 2.7. Permeability study

To study the permeability of the entire package an experiment was carried out during the same period of time with empty packs filled with 20% $CO_2$ and 0% $O_2$ in order to have equal gradient for $O_2$ and $CO_2$.
The gas concentrations were followed in time in order to determine the tightness of the packs.

## 2.8. Statistical Analysis

The data was subjected to a analysis of variance (Genstat 5). LSD values were calculated at the $P \leq 0.05$ level. Correlation analyses were performed to establish the association between the subjective parameters evaluated and the objectives gas concentrations.
In order to obtain the respiration rate of the cut lettuce, the data were compared with the models using non-linear regression in the statistical package Genstat (release 5).

## 2.9. Respiration Rate

The best fit line for $O_2$ uptake was based in the non-competitive model. For the $CO_2$ production we distinguished oxidative and the fermentative $CO_2$ production. The $CO_2$ production was fitted using the $O_2$ measurements.
Both determinations, $O_2$ uptake and $CO_2$ production, were determined by using the gas measurements after one day.
In order to avoid errors all the data with a R.Q. < 0.6 were not used. Also the data that had intensive respiration in the second day compared with the first day were not used.

# 3. Results

## 3.1. Package atmosphere

The initial $O_2$ and $CO_2$ were different from the desired and required ones. The real initial concentrations are shown in table 6.
In the remainder of this report we refer to the intended concentrations.

**Table 6** Difference between real initial $O_2$ and $CO_2$ range (in red) flushed in to the packs and the $O_2$ and $CO_2$ intended concentrations (in black).

| $CO_2$ / $O_2$ | 0 - 2 | 5 - 5 | 10 - 10 | 15 - 15 | 20 - 18 | 40 - 35 |
|---|---|---|---|---|---|---|
| 0 – 0 | 6 | 6 | 6 | 6 | 6 | 6 |
| 5 - 3 | 6 | 6 | 6 | 6 | 6 | 6 |
| 10 - 8 | 6 | 6 | 6 | 6 | 6 | 6 |
| 15 – 12 | 6 | 6 | 6 | 6 | 6 | 6 |
| 21 - 17 | 6 | 6 | 6 | 6 | 6 | 6 |
| 40 - 27 | 4 | 4 | 4 | 6 | 6 | 6 |
| 60 - 38 | 4 | 4 | 4 | 4 | 4 | 4 |

The period that packs took to get anaerobic was proportional to the initial $O_2$ concentrations but it was independent the $CO_2$ concentrations (Fig.15), which indicates no $CO_2$ inhibition. All packs got anaerobic during the period of the experience, except the ones that began with very high $O_2$ (60%).

The $O_2$ concentrations decreased rapidly, the longest period that packs could stay without anaerobic atmosphere were 6 days for the packs which had in the beginning 40% $O_2$.

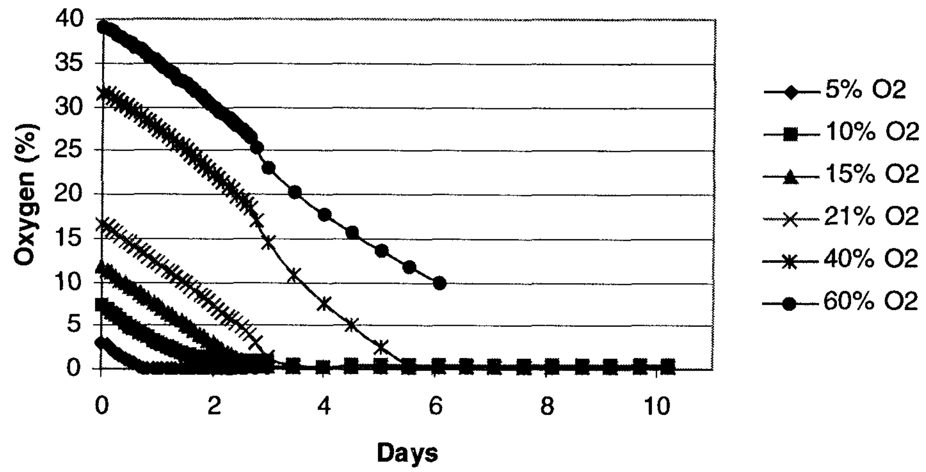



**Fig. 15** Time development of different Oxygen concentrations combined with 0% $CO_2$ during storage at 7° C.

The increase of $CO_2$ concentrations was essentially independent of its initial concentrations and also from the $O_2$ concentrations (Fig. 16).
The increase of $CO_2$ levels was similar to the rate of the decreasing $O_2$ concentrations. It did not take too long to get high concentrations of $CO_2$ even the packs with low initial $O_2$ levels.

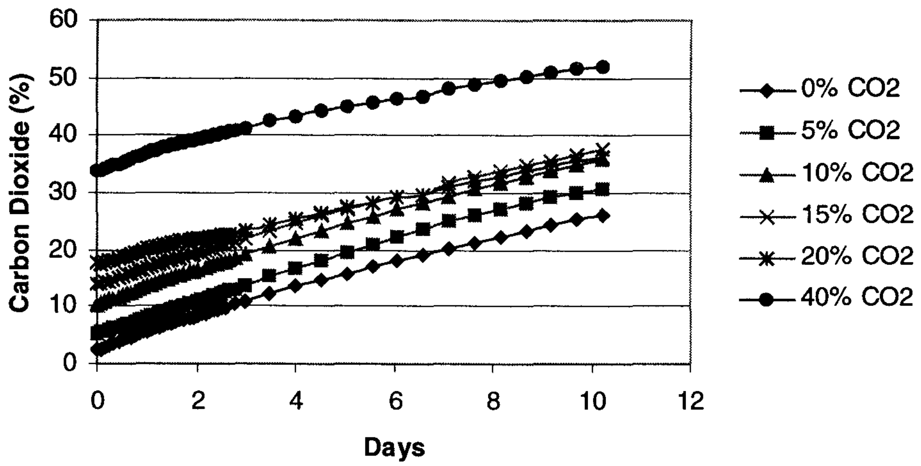



**Fig. 16** Time development of Carbon dioxide concentrations combined with 5% $O_2$ during storage at 7° C.

## 3.2. Product Quality Evaluation

The quality evaluation results are displayed in Appendix.

### 3.2.1. Browning

For $CO_2$ concentrations between 15% and 40% and all applied $O_2$ concentrations (0% up to 21%) there were no differences in the scores for browning at the three evaluation times, after 5, 7 and 11 days. The only exceptions were 15% $CO_2$ - 0% $O_2$ in the 7[th] day and 40% $CO_2$ – 0% $O_2$ in the 11[th] day. The first combination was the worst in terms of increasing browning but the last one was still acceptable (Fig. 18).

For 5% and 10% $CO_2$ the browning defect was only detected in combination with 21% $O_2$. It was acceptable in the first 7 days but on the 11[th] day it exceeded the threshold.

In the packs with 5% $CO_2$-10% $O_2$ it was possible to see a slight increase in browning at the end of the two last opening moments, 7 and 11 days, but in both cases the quality was still acceptable.

The packs that started with no $CO_2$ showed browning in combination with 21% $O_2$.

Even after the first five days we already observed browning for lettuce packed with relatively high $O_2$ concentrations and low $CO_2$ concentrations and after the 7[th] day it was more noticeable (Fig. 17).

There was a better correlation between $CO_2$ and browning than between $O_2$ and browning.

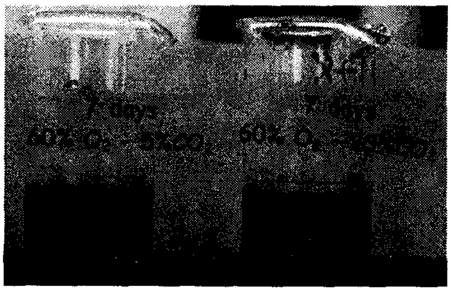


**Fig. 17** Different coloration of the lettuce juice from product that was packed with 60% $O_2$ in combination with 5% and 20% $CO_2$.

The packs that were not within acceptable limits was the ones with 15% $CO_2$ - 0% $O_2$ (Fig. 18) and the packs with high $O_2$ (40% - 60%) in combination with low $CO_2$ (0% - 10%), this already after 5 days storage (Fig.19).


**Fig. 18** Differences in browning after 7 days storage for the packs with 15 % $CO_2$ combined with different $O_2$ concentrations (0% - 60%).

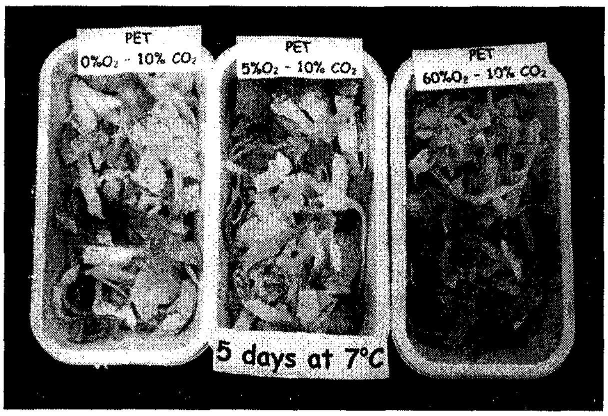



**Fig. 19** Differences in browning between packs with different initial $O_2$ concentrations (0%, 5% and 60%) and identical initial $CO_2$ concentrations (10%).

The best scores after 7 days storage were for high $CO_2$ (40%) combined with all $O_2$ concentrations (0% - 60%) (Fig. 20) and for 10% and 15% $CO_2$ combined with low $O_2$ (0% - 15%) (Fig. 21).


**Fig. 20** Browning defect in the packs with high $CO_2$ (40%) in combination with 5%, 15% and 40% $O_2$.

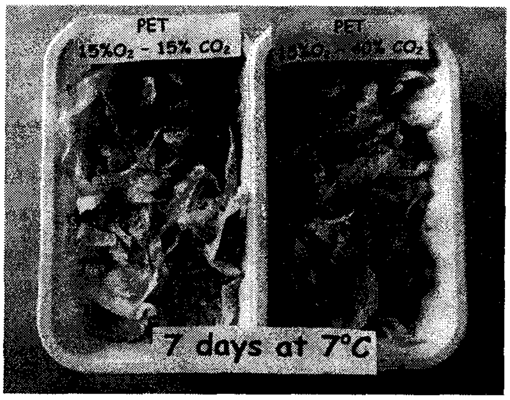



**Fig. 21** Browning defect in the packs with 15% $O_2$ in combination with 15% and 40% $CO_2$.

### 3.2.2. Crispness

This parameter decreased during the storage time. Crispness in the 11 day was worst mostly for the high $O_2$ concentrations.

In the first five storage days, all different concentrations resulted in a crispy lettuce, but in the end of 7$^{th}$ day all the packs with high $O_2$ (40% - 60%) combined with high $CO_2$ (15% - 40%) lost its crispness. The product was so limp that was not anymore acceptable.

All the packs with 20% and 40% $CO_2$ were limp where the latter ones were all unacceptable according to the crispness scores.

The best combination in the end of 11 days storage at 70° C was 15% -21% $O_2$ in combination with 0% $CO_2$.

Correlation coefficients were higher for $CO_2$ than $O_2$ levels.

### 3.2.3. Decay

Decay was not a dominant defect in the first five day storage, but after seven days most of the packs showed first indication of decay, although they were still acceptable. Only one combination, 60% $O_2$ – 40% $CO_2$, was not acceptable after seven days.

At the 11$^{th}$ day the scores were below acceptable limits for the packs with 10%, 20% and 40% $CO_2$.

The best concentration in the end of 11 days was 21% $O_2$ – 0% $CO_2$.

The correlation between decay evaluation and $CO_2$ levels was very high.

### 3.2.4. Translucency

The lettuce pieces got translucent during the storage period. In the 5th days only packs with very high $CO_2$ (20% – 40%) showed translucency but in the 11th day all products in almost all packs got translucency.

High $O_2$ combined with low $CO_2$ seemed to show potential avoiding this defect.

The correlation analyses showed a higher correlation between $CO_2$ and translucency than $O_2$ and translucency.

### 3.2.5. Odour

High $O_2$ (15% - 60%) seemed to be the most efficient to avoid off-odour, but also low $O_2$ combined with high $CO_2$ seemed to be also a good combination to prevent off-odours. All the rest packages with low $O_2$ developed off-odours after 5 days.

The off-odours that developed after 5th day for low $O_2$ was in the majority of the packs a rotten smell. For the intermediate $O_2$ concentration (10%), it was difficult to distinguish between sweet and acid smell. In all the packs where sweet or acid smell was detected on the 5th day, a distinctly rotten smell was detected after 11 days.

At the 11th day all the packs that were evaluated in the 5th day with no off-odour were evaluated with some off-odour, sweet or acid smell.

Off-odour was more correlated with $CO_2$ levels.

### 3.2.6. General quality

The range of initial gas concentrations that had the best scores was for 5% - 21% $O_2$ in combination with 0% - 20% $CO_2$.

Most packs with 0% $O_2$ score significantly worst than the above mentioned packs .For 0% $O_2$ there was significant differences in evaluated parameters combined with all $CO_2$ concentration, except in combination with 10% $CO_2$.

There was significant differences in score for all concentrations of $O_2$ combined with 40% $CO_2$.

In the first five days all packs had good scores, just the packs with high $O_2$ (40% - 60%) got bad scores but were still above the limit of acceptance. In the 7th day these packs were not acceptable anymore.

In the 11th day all the packs with 20% and 40% $CO_2$ combined with all $O_2$ level (0% - 21%) were below tolerable marks.

There was a high correlation coefficient for $CO_2$ in relation of the general impression.

## 3.3. pH

In spite of being a weak acid the $CO_2$ dissolved in the lettuce influences the pH as showed in figure 22.

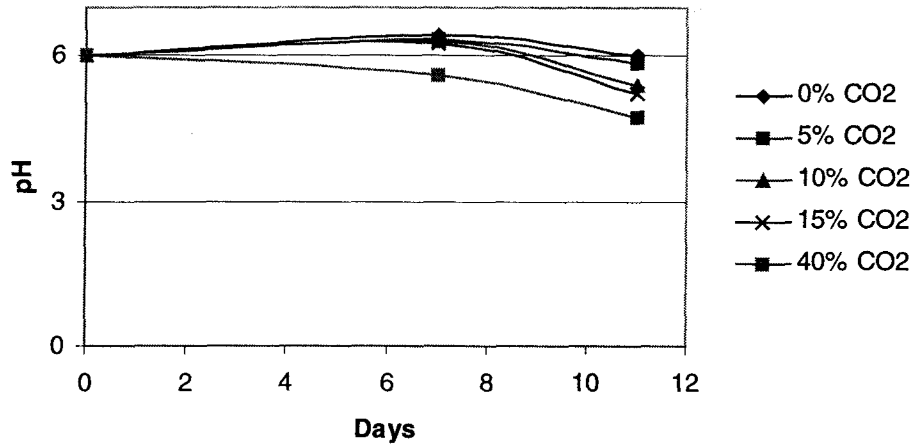



**Fig. 22** pH variations in different $CO_2$ concentrations combined with 5% $O_2$ measured after 5, 7 and 11 storage days at 7° C.

## 3.4. Volume determination

Volume calculations were done using the underwater weight and water density at 18° C.

The volumes becomes:

Total package volume = 0.00077 $m^3$
Lettuce volume = 0.0003 $m^3$
Headspace volume = 0.00047 $m^3$

## 3.5. Permeability study

Apart from the packs that were not well sealed and were leaking we did not observe any significant gas exchange in the packs during an experiment lasting 7 days. This is due to low $O_2$ and $CO_2$ permeabilities in CPET film and trays ($O_2$ = 21 mL/m$^2$.day.bar and $CO_2$ = 105 mL/m$^2$. day.bar, 100 μm thickness of the film).

## 3.6. Respiration Rate

At 7° C the estimated parameters were as shown in table 7 and 8 and the data were fitted in the model (Peppelenbos, 1996) as follows in the figure 23, 24 and 25.

**Table 7** Results of the non-linear regression analysis for $O_2$ consumption.

| | Estimated values | Standard error |
|---|---|---|
| **Vm** $_{O2}$ | 3.84 | 0.341 |
| **Km** $_{O2}$ | 0.883 | 0.318 |
| **Kmn** $_{CO2}$ | 121.1 | 68.5 |

**Table 8.** Results of the non-linear regression analysis for $CO_2$ production.

| | Estimated values | Standard error |
|---|---|---|
| **Vmf** $_{CO2}$ | 3.37 | 0.969 |
| **Kmf** $_{O2}$ | 0.174 | 0.134 |
| **RQ**$_{ox}$ | 0.8354 | 0.0340 |

Plotting respiration rate versus the corresponding gas concentration allows perdition of the respiration rate at the desired $O_2$ and $CO_2$ concentrations (Lee, 1986).

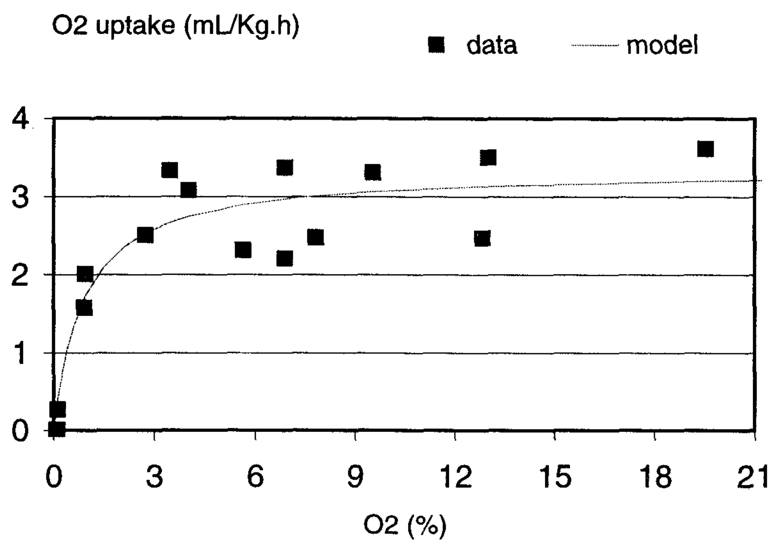



**Fig. 23** Average values for $O_2$ consumption data (mL.Kg$^{-1}$.h$^{-1}$) at several $O_2$ and $CO_2$ concentrations, fitted with the non-competitive type of inhibition.

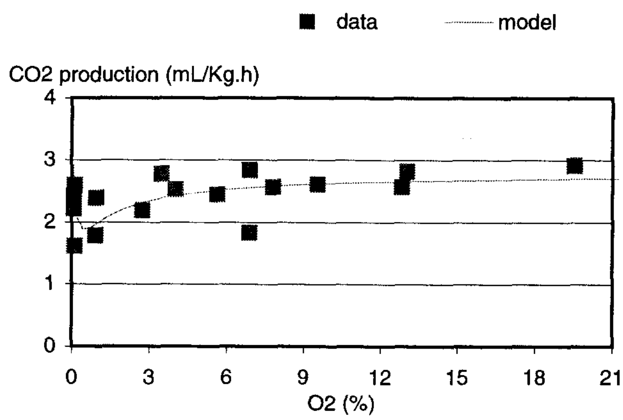



**Fig. 24** Average values for $CO_2$ production data ($mL.Kg^{-1}.h^{-1}$) fitted in the model.

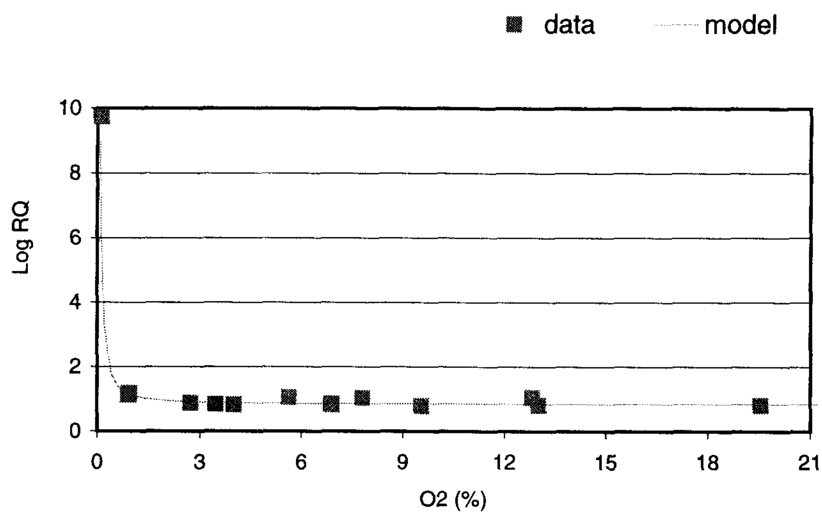



**Fig. 25** R. Q. values from the data and the model.

In summary we can say:

- We observed for all gas conditions a $O_2$ concentrations rate of approximately 4 $mL.Kg^{-1}.h^{-1}$;
- Within the experiment, we did not observe an inhibition of $O_2$ consumption by $CO_2$;
- The measured $O_2$ consumption rate do not agree with the literature values (Table 9);
- There were certain limitations in the set up of our experiment:
    - During the first 2 - 3 days, the product would still react as if it was stored under usual atmosphere conditions (21% $O_2$ – 0% $CO_2$), only after this period, the effects of actual MA will become visible;

♦ This limits our ability to make conclusive statements on $CO_2$ inhibition at lower $O_2$ concentrations, because the packs were anaerobic after 2 - 3 days.

♦ Nevertheless, we did not see any indication of $CO_2$ inhibition in packs which were initially packed under high $O_2$ levels, and which did not get anaerobic after 2 – 3 days. This gives us some confidence in our results.

♦ In addition, the delayed reaction on MA cannot explain the lower $O_2$ consumption rate, compared with the literature values, which we measured under all MA conditions.

**Table 9.** Respiration rates obtained from literature

| | Cantwell (1997) | Gorny (1997) | Watada *et al* (1996) |
|---|---|---|---|
| **Temperature (° C)** | 7.5 | 7.5 | 7.5 |
| **Respiration rates (mL.Kg$^{-1}$.h$^{-1}$)** | 12.5 | 12-17 | 12.6 |

# 4. Conclusion

According to the measurements and to the quality evaluation it was possible to describe a certain range of $O_2$ and $CO_2$ concentrations are all the defect were minimised or did not exist (Fig. 26), and where the quality of cut lettuce was maintained.

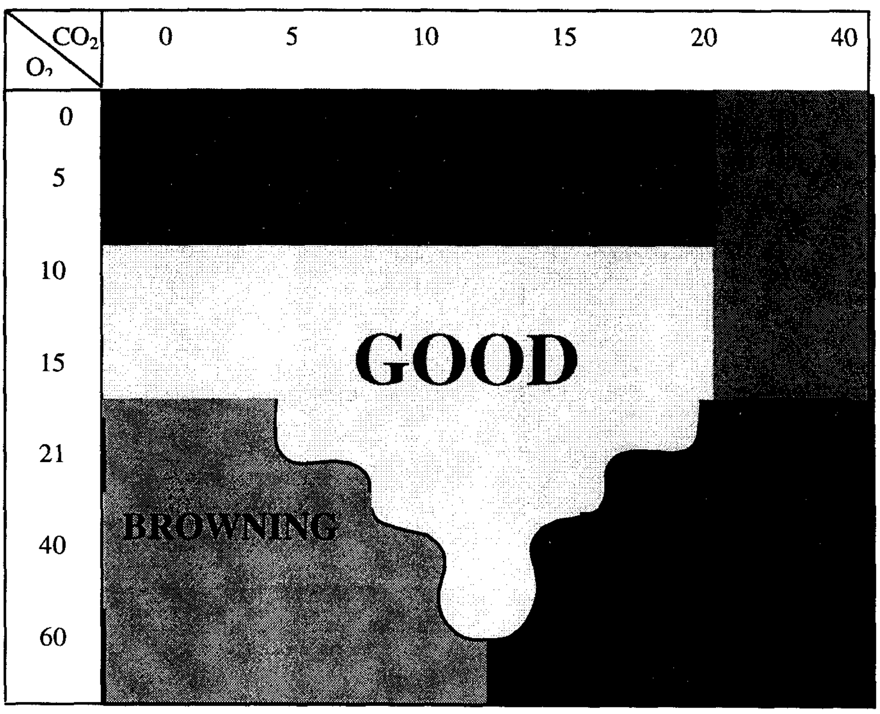



**Fig. 26** General effect of different $O_2$ and $CO_2$ concentration on product quality.

$O_2$ concentration between 10% and 15% in combination with almost all $CO_2$ levels resulted in a good product quality. These positive effects include inhibition of browning, crisper texture, no off-odour and no decay.

It was possible establish some limits:

♦ $CO_2$ is responsible for breakdown of plant tissue, which results in decay, so the limit for $CO_2$ in order to prevent damage is ≈ 15%;

♦ $O_2$ is responsible for the off-odour when its levels were too low. On the other hand it is responsible for browning when levels are too high, so the tolerance limits for $O_2$ have to be taken in to account in order to prevent both defects: lower $O_2$ limit is ≈ 5% ; higher $O_2$ limit is ≈ 15%

Over the course of the experiment it was noted that there were two main factors that limited the appearance of processed iceberg lettuce. These factors were the development of brown discoloration due to phenolic oxidation and the occurrence of off-odours probably because of anaerobic respiration.

The shelf life of the product under the experimental conditions is estimated in more than 7 days but less than 11.

The acid levels of cut iceberg lettuce was greater after MA package storage compared to air because $CO_2$ in solution is acid and the lower acid metabolism is associated with modified atmosphere.

The respiration rate estimated was ≈ 4 $mL.Kg^{-1}.h^{-1}$.This is lower in comparison with the literature and even lower compared with the respiration rates for whole lettuce found in the literature.

# 5. Further research

The trials carried out on minimally processed lettuce have demonstrated that MAP of iceberg lettuce is a very complex area which still requires further research.

The respiration rate should be estimated in comparison with the ones using steady-state conditions in order to judge about the validity of the data.

In order to reduce the subjectivity of the quality evaluation it would be interesting to use another methods in parallel such as electronic noise or study of the volatile compounds for the odour, browning using a colour chromatograph and for the texture evaluation a texturegraph.

The influence of MAP on the growth of microorganisms should be take into account.

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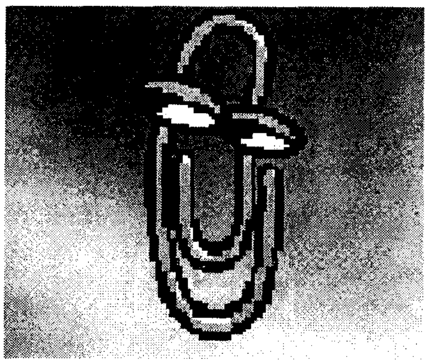
# Appendix



V

| O2 | CO2 | Day | Browning | Translucency | Decay | Cut. damage | Crispy | Smell | Mark |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 5 | 0 | 1 | 1 | 0 | 0 | 3 | 1 |
| 0 | 0 | 5 | 0 | 1 | 0 | 1 | 1 | 3 | 2 |
| 0 | 5 | 5 | 0 | 1 | 1 | 1 | 0 | 3 | 0 |
| 0 | 5 | 5 | 0 | 0 | 0 | 1 | 0 | 3 | 3 |
| 0 | 10 | 5 | 0 | 1 | 1 | 0 | 1 | 3 | 0 |
| 0 | 10 | 5 | 0 | 1 | 1 | 1 | 0 | 3 | 0 |
| 0 | 15 | 5 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 0 | 15 | 5 | 0 | 1 | 0 | 0 | 1 | 3 | 2 |
| 0 | 20 | 5 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| 0 | 20 | 5 | 0 | 1 | 1 | 0 | 1 | 2 | 1 |
| 0 | 40 | 5 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 0 | 40 | 5 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| 5 | 0 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 5 | 0 | 5 | 0 | 0 | 0 | 0 | 1 | 2 | 1 |
| 5 | 5 | 5 | 0 | 0 | 0 | 1 | 0 | 3 | 3 |
| 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| 5 | 10 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 5 | 10 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 5 | 15 | 5 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| 5 | 15 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 5 | 20 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 5 | 20 | 5 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 5 | 40 | 5 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| 5 | 40 | 5 | 0 | 0 | 1 | 1 | 2 | 1 | 2 |
| 10 | 0 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 10 | 0 | 5 | 1 | 1 | 0 | 1 | 0 | 2 | 1 |
| 10 | 5 | 5 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| 10 | 5 | 5 | 0 | 1 | 1 | 1 | 1 | 0 | 1 |
| 10 | 10 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 1 |
| 10 | 10 | 5 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 10 | 15 | 5 | 0 | 1 | 0 | 0 | 0 | 3 | 0 |
| 10 | 15 | 5 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 10 | 20 | 5 | 0 | 0 | 0 | 1 | 1 | 2 | 2 |
| 10 | 20 | 5 | 0 | 1 | 0 | 1 | 1 | 2 | 0 |
| 10 | 40 | 5 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| 10 | 40 | 5 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| 15 | 0 | 5 | 1 | 0 | 0 | 1 | 0 | 0 | 2 |
| 15 | 0 | 5 | 1 | 0 | 0 | 1 | 1 | 2 | 2 |
| 15 | 5 | 5 | 0 | 1 | 0 | 1 | 0 | 2 | 0 |
| 15 | 5 | 5 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| 15 | 10 | 5 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| 15 | 10 | 5 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| 15 | 15 | 5 | 0 | 1 | 0 | 1 | 1 | 0 | 0 |
| 15 | 15 | 5 | 0 | 1 | 0 | 1 | 0 | 2 | 0 |
| 15 | 20 | 5 | 0 | 1 | 0 | 0 | 0 | 3 | 0 |
| 15 | 20 | 5 | 0 | 1 | 0 | 1 | 1 | 2 | 0 |
| 15 | 40 | 5 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 15 | 40 | 5 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| 21 | 0 | 5 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| 21 | 0 | 5 | 3 | 1 | 0 | 1 | 1 | 0 | 2 |
| 21 | 5 | 5 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| 21 | 5 | 5 | 2 | 1 | 1 | 1 | 2 | 0 | 2 |
| 21 | 10 | 5 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| 21 | 10 | 5 | 1 | 0 | 0 | 1 | 1 | 2 | 1 |

| O2 | CO2 | Day | Browning | Translucency | Decay | Cut. damage | Crispy | Smell | Mark |
|---|---|---|---|---|---|---|---|---|---|
| 21 | 15 | 5 | 0 | 0 | 0 | 1 | 0 | 3 | 3 |
| 21 | 15 | 5 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| 21 | 20 | 5 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 21 | 20 | 5 | 0 | 1 | 0 | 0 | 1 | 2 | 0 |
| 21 | 40 | 5 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| 21 | 40 | 5 | 0 | 1 | 1 | 0 | 2 | 1 | 1 |
| 40 | 0 | 5 | 2 | 1 | 0 | 0 | 0 | 0 | 2 |
| 40 | 0 | 5 | 2 | 1 | 0 | 1 | 1 | 0 | 2 |
| 40 | 5 | 5 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| 40 | 5 | 5 | 2 | 0 | 0 | 1 | 1 | 0 | 2 |
| 40 | 10 | 5 | 2 | 0 | 0 | 1 | 1 | 2 | 2 |
| 40 | 10 | 5 | 1 | 1 | 2 | 1 | 1 | 2 | 1 |
| 40 | 15 | 5 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 40 | 15 | 5 | 1 | 1 | 1 | 1 | 2 | 0 | 1 |
| 40 | 20 | 5 | 1 | 1 | 1 | 1 | 2 | 0 | 2 |
| 40 | 20 | 5 | 0 | 1 | 1 | 1 | 1 | 0 | 1 |
| 40 | 40 | 5 | 0 | 1 | 1 | 1 | 1 | 2 | 1 |
| 40 | 40 | 5 | 0 | 1 | 1 | 1 | 1 | 2 | 1 |
| 60 | 0 | 5 | 2 | 1 | 0 | 0 | 0 | 0 | 2 |
| 60 | 0 | 5 | 3 | 0 | 0 | 1 | 1 | 0 | 3 |
| 60 | 5 | 5 | 3 | 1 | 1 | 1 | 1 | 0 | 2 |
| 60 | 5 | 5 | 2 | 1 | 1 | 1 | 1 | 0 | 2 |
| 60 | 10 | 5 | 2 | 0 | 1 | 0 | 1 | 0 | 2 |
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| 60 | 15 | 5 | 0 | 1 | 2 | 1 | 1 | 0 | 1 |
| 60 | 20 | 5 | 1 | 0 | 1 | 0 | 2 | 2 | 2 |
| 60 | 20 | 5 | 0 | 1 | 2 | 1 | 1 | 0 | 1 |
| 60 | 40 | 5 | 0 | 0 | 1 | 0 | 1 | 1 | 2 |
| 60 | 40 | 5 | 0 | 1 | 1 | 1 | 2 | 2 | 2 |
| 0 | 0 | 7 | 0 | 1 | 2 | 0 | 2 | 2 | 2 |
| 0 | 0 | 7 | 3 | 0 | 0 | 0 | 1 | 2 | 3 |
| 0 | 5 | 7 | 0 | 1 | 2 | 1 | 2 | 3 | 3 |
| 0 | 5 | 7 | 0 | 0 | 1 | 0 | 0 | 3 | 0 |
| 0 | 10 | 7 | 0 | 1 | 2 | 0 | 2 | 0 | 2 |
| 0 | 10 | 7 | 0 | 1 | 2 | 0 | 3 | 0 | 2 |
| 0 | 15 | 7 | 4 | 1 | 3 | 1 | 2 | 1 | 5 |
| 0 | 15 | 7 | 4 | 1 | 1 | 1 | 2 | 3 | 4 |
| 0 | 20 | 7 | 0 | 1 | 2 | 0 | 1 | 0 | 2 |
| 0 | 20 | 7 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| 0 | 40 | 7 | 0 | 1 | 2 | 0 | 1 | 1 | 2 |
| 0 | 40 | 7 | 0 | 1 | 2 | 1 | 2 | 3 | 3 |
| 5 | 0 | 7 | 0 | 1 | 1 | 0 | 1 | 2 | 0 |
| 5 | 0 | 7 | 0 | 1 | 1 | 0 | 0 | 3 | 2 |
| 5 | 5 | 7 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 5 | 5 | 7 | 0 | 1 | 1 | 0 | 0 | 2 | 1 |
| 5 | 10 | 7 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| 5 | 10 | 7 | 0 | 1 | 0 | 1 | 1 | 2 | 1 |
| 5 | 15 | 7 | 1 | 1 | 2 | 1 | 2 | 0 | 2 |
| 5 | 15 | 7 | 0 | 0 | 0 | 1 | 1 | 2 | 1 |
| 5 | 20 | 7 | 0 | 1 | 1 | 0 | 2 | 2 | 0 |
| 5 | 20 | 7 | 0 | 0 | 1 | 0 | 1 | 2 | 1 |

| O2 | CO2 | Day | Browning | Translucency | Decay | Cut. damage | Crispy | Smell | Mark |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 40 | 7 | 1 | 1 | 1 | 0 | 1 | 1 | 2 |
| 5 | 40 | 7 | 0 | 1 | 1 | 0 | 0 | 1 | 2 |
| 10 | 0 | 7 | 0 | 1 | 1 | 0 | 0 | 2 | 0 |
| 10 | 0 | 7 | 0 | 0 | 1 | 1 | 1 | 2 | 1 |
| 10 | 5 | 7 | 0 | 1 | 1 | 0 | 2 | 1 | 0 |
| 10 | 5 | 7 | 3 | 0 | 0 | 1 | 0 | 1 | 4 |
| 10 | 10 | 7 | 0 | 1 | 2 | 1 | 2 | 1 | 2 |
| 10 | 10 | 7 | 0 | 1 | 2 | 1 | 2 | 3 | 3 |
| 10 | 15 | 7 | 0 | 1 | 2 | 1 | 2 | 0 | 2 |
| 10 | 15 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 20 | 7 | 0 | 1 | 2 | 0 | 2 | 1 | 3 |
| 10 | 20 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 40 | 7 | 0 | 1 | 1 | 0 | 2 | 3 | 2 |
| 10 | 40 | 7 | 0 | 1 | 0 | 0 | 3 | 0 | 2 |
| 15 | 0 | 7 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| 15 | 0 | 7 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 15 | 5 | 7 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| 15 | 5 | 7 | 1 | 1 | 2 | 1 | 1 | 0 | 1 |
| 15 | 10 | 7 | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| 15 | 10 | 7 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| 15 | 15 | 7 | 0 | 1 | 2 | 0 | 1 | 0 | 1 |
| 15 | 15 | 7 | 0 | 0 | 1 | 1 | 1 | 0 | 2 |
| 15 | 20 | 7 | 1 | 1 | 2 | 1 | 2 | 0 | 1 |
| 15 | 20 | 7 | 0 | 1 | 1 | 0 | 2 | 2 | 2 |
| 15 | 40 | 7 | 0 | 1 | 1 | 1 | 2 | 0 | 1 |
| 15 | 40 | 7 | 0 | 1 | 2 | 0 | 2 | 1 | 2 |
| 21 | 0 | 7 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
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| 21 | 5 | 7 | 1 | 1 | 2 | 1 | 1 | 2 | 3 |
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| 21 | 10 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 21 | 15 | 7 | 0 | 0 | 1 | 0 | 2 | 2 | 2 |
| 21 | 20 | 7 | 1 | 1 | 1 | 1 | 2 | 2 | 2 |
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| 40 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
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| 40 | 5 | 7 | 4 | 0 | 2 | 1 | 0 | 0 | 4 |
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| 40 | 20 | 7 | 1 | 1 | 2 | 1 | 3 | 1 | 3 |
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| 60 | 0 | 7 | 2 | 1 | 0 | 1 | 2 | 2 | 3 |
| 60 | 5 | 7 | 3 | 1 | 2 | 1 | 1 | 2 | 4 |
| 60 | 5 | 7 | 2 | 0 | 0 | 1 | 2 | 1 | 3 |

| O2 | CO2 | Day | Browning | Translucency | Decay | Cut. damage | Crispy | Smell | Mark |
|---|---|---|---|---|---|---|---|---|---|
| 60 | 10 | 7 | 1 | 1 | 0 | 1 | 2 | 1 | 2 |
| 60 | 10 | 7 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 60 | 15 | 7 | 1 | 1 | 1 | 1 | 3 | 2 | 3 |
| 60 | 15 | 7 | 1 | 1 | 2 | 1 | 3 | 2 | 3 |
| 60 | 20 | 7 | 1 | 1 | 1 | 0 | 2 | 3 | 3 |
| 60 | 20 | 7 | 1 | 1 | 2 | 1 | 3 | 2 | 3 |
| 60 | 40 | 7 | 0 | 1 | 5 | 1 | 4 | 3 | 5 |
| 60 | 40 | 7 | 0 | 1 | 1 | 0 | 4 | 2 | 4 |
| | | | | | | | | | |
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| 0 | 0 | 11 | 0 | 1 | 3 | 0 | 3 | 3 | 3 |
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| 0 | 10 | 11 | 0 | 1 | 1 | 1 | 3 | 3 | 2 |
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| 15 | 15 | 11 | 0 | 1 | 1 | 0 | 1 | 2 | 1 |
| 15 | 15 | 11 | 0 | 1 | 1 | 1 | 1 | 2 | 1 |

| O2 | CO2 | Day | Browning | Translucency | Decay | Cut. damage | Crispy | Smell | Mark |
|---|---|---|---|---|---|---|---|---|---|
| 15 | 20 | 11 | 0 | 1 | 1 | 0 | 2 | 3 | 3 |
| 15 | 20 | 11 | 0 | 1 | 1 | 1 | 2 | 1 | 2 |
| 15 | 40 | 11 | 0 | 1 | 5 | 0 | 5 | 2 | 5 |
| 15 | 40 | 11 | 0 | 1 | 5 | 0 | 5 | 3 | 5 |
| 21 | 0 | 11 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |
| 21 | 0 | 11 | 1 | 1 | 0 | 1 | 0 | 1 | 1 |
| 21 | 5 | 11 | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| 21 | 5 | 11 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| 21 | 10 | 11 | 0 | 0 | 1 | 0 | 2 | 3 | 3 |
| 21 | 10 | 11 | 5 | 1 | 5 | 1 | 1 | 0 | 5 |
| 21 | 15 | 11 | 0 | 1 | 3 | 0 | 3 | 3 | 3 |
| 21 | 15 | 11 | 1 | 1 | 2 | 1 | 2 | 2 | 2 |
| 21 | 20 | 11 | 0 | 1 | 2 | 0 | 3 | 3 | 3 |
| 21 | 20 | 11 | 0 | 1 | 3 | 1 | 3 | 1 | 3 |
| 21 | 40 | 11 | 0 | 1 | 4 | 0 | 4 | 3 | 4 |
| 21 | 40 | 11 | 0 | 1 | 3 | 1 | 4 | 1 | 4 |
| 40 | 15 | 11 | 0 | 1 | 4 | 0 | 3 | 3 | 4 |
| 40 | 15 | 11 | 1 | 1 | 3 | 1 | 2 | 1 | 3 |
| 40 | 20 | 11 | 0 | 1 | 4 | 0 | 4 | 3 | 4 |
| 40 | 20 | 11 | 1 | 1 | 3 | 1 | 3 | 1 | 4 |
| 40 | 40 | 11 | 3 | 1 | 4 | 0 | 4 | 3 | 4 |
| 40 | 40 | 11 | 2 | 1 | 4 | 0 | 4 | 3 | 4 |