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Shelf life prediction with parameters of photosynthesis

Rapport B610

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Confidential

Patricia Alves

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Patricia Alves

ATO B.V.
Agrotechnological Research Institute
Bornsesteeg 59
P.O. Box 17
6700 AA Wageningen
The Netherlands
Tel: +31.0317.475024
Fax: +31.317.475347

225217

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I. INTRODUCTION

I. 1. General

Nowadays, new food products appear in food market, because of the enormous progress in food science and technology. This abrupt appearance of new products makes modern societies become more exigent relating to the quality of food products. Because of this interest, consumers try to find safe products in chemistry, physics and microbiology. These products must be nutritious, aesthetically appealing, easy to use and reasonable priced (Daun 1993). In this way, producers' and food makers' concern and responsibility increase, being the great objective of them to satisfy the consumer's exigencies, after being offered the demanded products. These products should have all the quality attributes demanded by the consumers. But their responsibilities and objectives are not only to offer the products looked for by the consumers with such desired characteristics, but also to draw out these characteristics and, in this way, to draw out the lifetime of the product.

Every kind of food product has its own quality attributes. Those quality attributes characterise the product and make of it a quality product or a non-quality product.

Nutritional and Organoleptic (sensory) characteristics include almost all the attributes that a product can have.

Nutritional characteristics of food are its composition in terms of in proteins, lipids, sugars, vitamins, minerals, and water. For each product this composition is different. Some groups of food have a high amount of: proteins (e.g. meat, fish eggs); lipids (e.g. oils); sugars (e.g. cereals like bread). Other groups, such as fruits and vegetables, have a high amount of vitamins (e.g. A, C, B_6, thiamin, niacin), minerals and water.

Organoleptic characteristics are the smell, touch, sight, and all the other characteristics that are related to human senses. According to Floros (1993), organoleptically fruits and vegetables are valued for their supreme flavour and aroma, crisp texture, attractive colours, and their overall appeal to human senses of smell, taste, touch and sight.

When we look at a piece of meat, bread, or at a lettuce leaf, it is difficult to

realise how strong its protein composition is. However, we can see if it has attractive colours or not, a good or a bad smell, a smooth or a coarse texture when we touch them, a pleasant or an unpleasant aroma.

Since the moment that a product is ready to be commercialised until the moment it is bought, the product loses some of its characteristics. Products, in general, are surely different (they have different characteristics), so the way and fastness they lose their characteristics is different as well. The time a product can be bought depends on the product and its organoleptic characteristics, because we can realise these ones and not the nutritional ones. That is why organoleptic characteristics are a factor with great importance. During their processing, storage and transport, several changes occur in foods, chemical, physical and microbiological changes are the leading causes of food deterioration (Singh 1994).

Foods are exposed to a wide range of environmental conditions such as temperature, humidity, oxygen and light. As a consequence of these conditions, many mechanisms can occur and influence quality attributes of food. After these mechanisms happen, some of those attributes can reach an undesirable state and products may become unsuitable for consumption and be rejected by consumers. When a food product reaches this condition, we say that it is at the end of its shelf life (Singh 1994).

Shelf life of a product is the period of time between the production and packaging of the product and the point at which it becomes unacceptable (Ellis 1994) for the consumer. During this period of time, the product will be safe and have the desired sensory, chemical, physical and microbiological characteristics (AAVV 1992).

Fruits` and vegetables` shelf life can be extended, by retarding or inactivating certain physiological and deterioration processes. Processes such as heat sterilisation, dehydration or freezing reduce and inactive physiological and microbiological degradation (so prolonging shelf life). Packaging is also important in shelf life extension of fruits and vegetables (Floros 1993).

In this research work, the aim is to predict shelf life of fresh products. Shelf life prediction is a very important subject (Daun 1993) and not new. Its importance needs to be considered with respect to each of the groups involved in the food chain: growers, manufacturers; distributors; retailers and consumers (AAVV 1992). Until the product is available to the consumer, it has a long journey.

First of all, growers and primary producers try, more than ever, to produce

products with certain specifications that make the products able to be used in special processes and make of them products with more quality attributes which may have a direct influence on the shelf life of manufactured food products (AAVV 1992).

After the growers', there is the manufactures' contribution, which must try to predict product shelf life and extend it. The problem of shelf life lays on the difficulty of finding out how long a product's shelf life will be, because manufactures cannot wait until the product changes its own organoleptic characteristics. When we know a product's shelf life, we can decide what destiny shall be given to the product: if it must be sold tomorrow or if it must be exported (never forgetting the transportation days). As mentioned before, consumers accept products based on those organoleptic characteristics. But sometimes, only using the human senses, it is not possible to detect if there are differences among products, because there are often some chemical and physical changes that are slowly occurring and are not detectable externally. These are called "cryptic changes" (Daun 1993). Also, although products have a same appearance, they can have been developed in a slightly different way, so their shelf life can probably be different.

For these reasons, if we could develop a method which could give information about products quality and detect differences between products, it would be an enormous aid for food shelf life prediction. The main goal of this work is to try to predict shelf life of fresh products, based on two Photosynthetic Parameters, being aware that they can give us information about a product quality, in the case of products that, though different, seem equal.

I. 2. Product

I. 2.1. Lettuce

Lettuce is a member of *Compositae* family, and its scientific name is *Lactuca Sativa* (Maroto 2002).

Europe, Asia and Northern Africa are considered the places from where lettuce has its origins (Halfacre & Barden 1979).

According to some authors, there are 4 types of lettuce: butterhead; cos (or romaine); crisphead (or iceburg); and loose leaf (Halfacre & Barden 1979). Other authors say that there is one more type: stem lettuce, known as celtuce (Yamaguchi 1983).

The most nutritious part of the lettuce is the leaf. Regardless of the types of lettuce, all of them have a high quantity of water, vitamins and minerals. In butterhead lettuce, the most frequent vitamin is ascorbic acid, and the mineral is calcium. In the case of cos and crisphead lettuce, the vitamin is ascorbis acid and the mineral is potassium (Yamaguchi 1983).

This vegetable is usually eaten in all kind of salads, i.e. fresh.

I.2.2. Endive

Endive is a biennial plant, which belongs to *Compositae* family or sunflower family, its scientific name is *Cichorium endivia* (Maroto 2002).

This plant is originated in the region of East India and has been eaten since the days of the Egyptians (Halfacre & Barden 1979).

There are two types (Yamaguchi 1983). One type is endive with very curled and serrated narrow leaves. The other type is escarole, with broad leaves. Like others vegetables, endive has a strong composition in water, vitamins and minerals. The other nutrients (in small portions) are: proteins, carbon hydrates and fats. The most found vitamin in endive is ascorbic acid. Calcium and potassium are the most found minerals (Yamaguchi 1983).

This vegetable is usually eaten fresh in salads, but in some parts of the world it is cooked.

I. 2.3. Leek

Leek, whose scientific name is *Allium Porrum*, is (like onions) a member of *Liliaceas* family (Maroto 2002), but it does not form bulbs (Spliltstoesser 1979). Leeks' origins are Europe and Occidental Asia (Maroto 2002) and they have been cultivated since pre-historical ages (Spliltstoesser 1979).

It is a biennial plant, with white and numerous roots (Maroto 2002). The leaves

are long and green and, on the basis, they are white. Leek is particularly adapted to cold weather and is more resistant than the onion (Yamaguchi 1993).

Leek has high quantities of water, vitamins and minerals, but also of carbon hydrates and proteins. Ascorbic acid is the most abundant vitamin in leek's composition and potassium is the most frequent mineral (Maroto 2002).

It is usually used in soups and vegetarian meals.

I. 3. Photosynthesis

Much of the structure, function, and evolution of cells and organisms can be related to their need for energy (Alberts et al. 1994). It means that there are many ways of obtaining the energy that the organisms and cells need to live. For obtaining energy, all animals and most microorganisms rely on the continual uptake of large amounts of organic compounds from their environment (Alberts et al. 1994). Other organisms and microorganisms have the capacity to do one of the most important biological processes: Photosynthesis. Only with CO_2, water or H_2S and light energy do these organisms satisfy their needs in energy and do they release O_2 (used by animals and other microorganisms that do not have that capacity) to the environment.

Photosynthetic organisms can be Prokaryotes or Eukaryotes. In the Prokaryotes group, there are anaerobic forms and aerobic forms. The anaerobic forms are the photosynthetic bacteria which use H_2S as a source of electrons (never water). The aerobic forms are blue-green algae, also called Cyanobacteria, which are the most advanced photosynthetic bacteria which have minimal nutrient requirements (Alberts et al. 1994). These bacteria use water as a source of electrons. The Eukaryotes photosynthetic organisms are all aerobic forms: it means that they use water as a source of electrons (e.g. some Algae like Green-Chlorophyceae, Red-Rhodophyceae and the higher plants like Bryophyta, Angiospermae, and Gymnospermae) (Lawlor 1993).

The photosynthetic organisms that will be studied in this research work are plants. In plants, photosynthesis occurs in a specialised intracellular organelle–the chloroplast (Alberts et al. 1994).

All green parts of a plant, including green stems and unripened fruit, have chloroplasts, but the leaves are the major sites of photosynthesis in most plants (Campbell & Mitchel).The leaf has the upper and lower epidermis, mesophyll cells,

vein, and bundle sheath cells (Fig. 1).The epidermis has a specialised structure known as stomata (sing.: stoma), which allow gas to enter and leave the leaf. These structure are flanked by two guard cells.

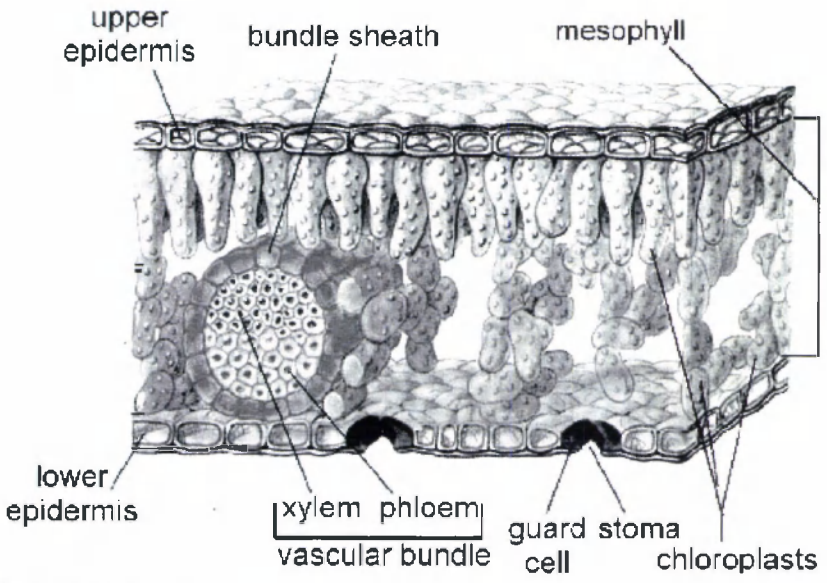

Fig. 1: Leaf's structure.

In the interior of the leaf there are the mesophyll cells. The chloroplasts are found mainly in these cells (Campbell & Mitchel).The chloroplasts (Fig.2) contain: the outer membrane, the inner membrane, intermembrane space, the stroma, and the thylakoids. The thylakoids are concentrated in stacks called grana and have a membrane, which separates the stroma from the thylakoid space (Campbell & Mitchel).

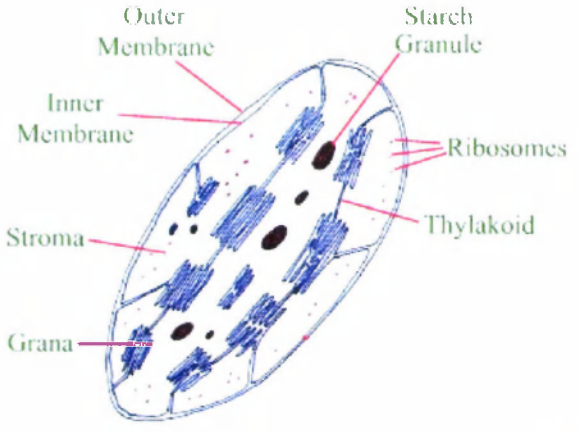

Fig.2: Chloroplast.

The CO_2 enters and O_2 exits by the stoma. The water is absorbed by the roots and is delivered to the leaves in vein (Campbell & Mitchel), and light energy is captured by pigments, which are found in the thylakoids membranes.

The thylakoid membranes contains all of the energy-generating systems of the chloroplasts (Alberts et al. 1994): the light-harvesting proteins, reaction centers and electron-transport chains.

Plants use sunlight as a source of light energy. Light is a form of energy known as electromagnetic energy, also called radiation (Campbell & Mitchel). Electromagnetic radiation can be explained in two ways: waves and particles theory. When light interacts with light, the best description is the wave theory. The wave theory describes light as coupled sinusoidal oscillations of electric field and a magnetic fields (Hipkins & Baker 2002). But when light interacts with matter and transfer energy to it, it is necessary to use the quantum description (Hipkins & Baker 2002). Light must be thought as a stream of energy-carrying particles. These particles are called photon. A photon does not have mass, but carries energy (Hipkins & Baker 2002).

Both theories are related.

$$E = h . \nu = h . c / \lambda$$

E = Energy of a quantum;

h = Plank's constant ($6{,}626 \times 10^{-3}$ sec);

ν = Frequency (sec^{-1});

c = Velocity of light ($2{,}988 \times 10^{8}$ m sec^{-1});

λ = Wavelength - distance between the crests of electromagnetic waves (nm for Gamma rays – microwaves and m for radio waves).

Electromagnetic spectrum (Fig.3) is the entire range of radiation. Only the visible region of the spectrum is detected by the human eye (380 – 720 nm).

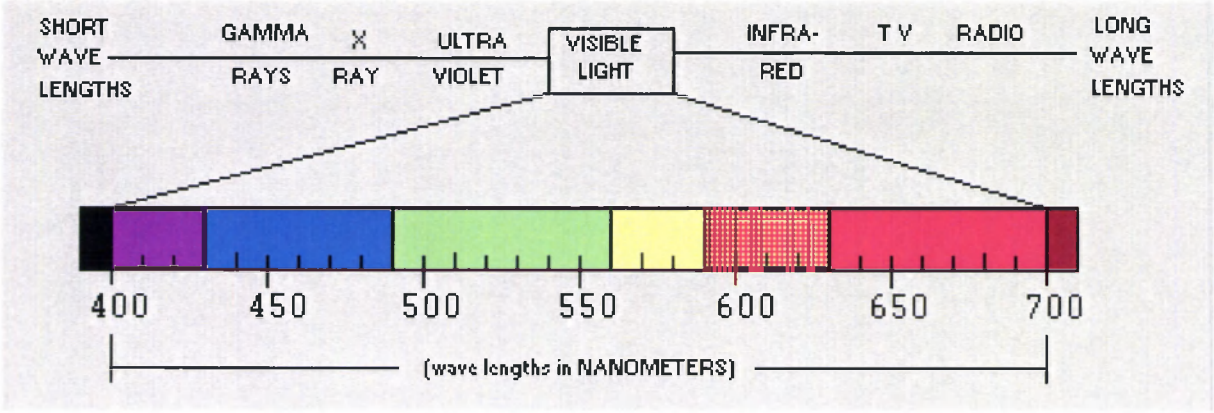

Fig.3: Electromagnetic spectrum

Photosynthesis process can be grouped into two stages. Light reactions or photosynthetic electron-transfer reactions and dark reactions also called carbon-fixation reaction. Light reactions are one of the two steps of photosynthesis process and consist in the conversion of solar energy in chemical energy – ATP and NADPH. This step occurs in thylakoid membrane. Dark reactions begin in the chloroplast stroma and continue in the cytosol (Alberts et al. 1994). In this step ATP and NADPH which were produced in light reactions step are used as a source of energy and CO_2 is converted into carbon hydrate.

- Light reactions

In light reactions (Fig.4), ATP and NADPH are produced by a two-stepped process called noncyclic photophosphorylation. It is called two-stepped process, because there are two photosystems namely I and II (Alberts et al. 1994). A photosystem is composed of two components: antenna complex and photochemical reaction centre.

Antenna complex consists of a cluster of a few hundred of pigment molecules (chlorophyll a, b, and carotenoids) (Campbell & Mitchel). A pigment is a substance that absorbs visible light. These pigment molecules are linked together by proteins that hold them on the thylakoid membrane (Alberts et al. 1994).

The photochemical reaction centre is a protein-pigment complex that enables light energy to be converted into chemical energy. The reaction centre has chlorophyll

molecules which act as a trap, because the excited electron is not able to come back and is immediately put in a electron transport chain (Alberts et al. 1994).

The reaction-center chlorophyll of photosystem I is known as P700 because this pigment is best absorbing light having a wavelength of 700nm (the far-red part of the spectrum) (Campbell & Mitchel). Photosystem II chlorophyll reaction centre is called P680, for the same reason.

The light reactions can be divided into the following steps:

1 – Light energy (photon) is absorbed by PSII through its antenna complex and transferred to P680. An electron from P680 is excited and moved from one molecular orbital to another of higher energy (Alberts et al. 1994). This electron must return to its original unexcited state and there are three ways: Fluorescence, converting the extra energy into heat or a combination of heat and light with a longer wavelength; resonance energy transfer, transferring the energy to another chlorophyll molecule that is neighbour; or transferring the electron to another closer electron acceptor. The last two ways are used in the photosynthesis process (Alberts et al. 1994).

2 – The excited electron from P680 is captured by a primary electron acceptor.

3 – The P680 has now a hole that will be filled with electrons that come from water molecules. When two water molecules are split, they release $4H^+$ and one molecule of oxygen.

4 – The photoexcited electrons pass from the primary electron acceptor of PSII to PSI through an electron transport chain. The electrons are transferred from a molecule to another molecule. The molecule that receives the electron becomes reduced and the molecule that transfers the electron becomes oxidised.

5 – In this electron transport chain, ATP is formed by a process called noncyclic photophosphorylation.

6 – The electron reaches the PSI. P700 has now a hole, because light energy drives an electron from P700 to the primary acceptor of PSI. Now $P700^+$ is going to

receive the electron that comes from PSII's electron transport chain and becomes P700 that is again able to be excited.

7 – The primary acceptor transmits the photoexcited electron to another electron transport chain that belongs to the PSI. Sometimes, the electron that comes from PSI does not go to its electron transport chain, but it goes to the PSII electron transport chain to produce ATP by a process called cyclic photophosphorylation. In this process, NADPH is not produced, and oxygen is not released. The function of this process is to produce more ATP (Campbell & Mitchel).

8 – In this electron transport chain, NADPH is produced.

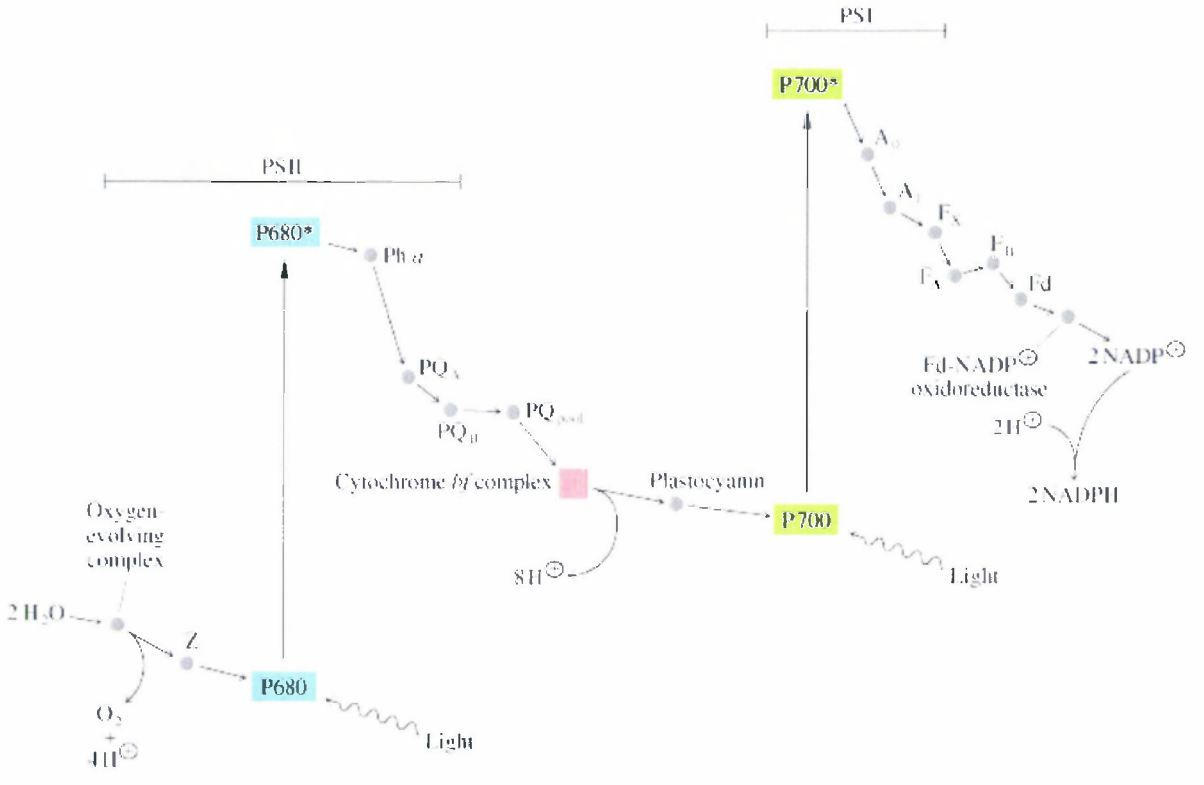

Fig. 4: Light reactions. Photosystems I and II.

- Dark reactions

CO_2 is fixed by 3 molecules of ribulose 1,5–bisphosphate (RuBP carboxylase or rubisco). In this first step, one molecule of 3–phosphoglycerate (3 carbons) is produced.

To produce RuBP, a series of reactions are needed. These reactions need a large amount of NADPH and ATP (Alberts et al. 1994). For 3 molecules of CO_2, 9 ATP and 6 NADPH are needed (Campbell & Mitchel). These reactions belong to a cyclic, carbon fixation cycle or Calvin cyclic.

In this cyclic (Fig.5), glyceraldehyde 3–phosphate is produced. Most part of this molecule is exported to the cytosol and then it can be converted in fructose 6-phosphate and glucose 1-phosphate by the inverse glycolysis reactions and the sucrose used by the plant is produced. The other part of glyceraldehyde 3-phosphate stays in the chloroplasts and is converted in starch in the stroma. The starch is stored as large grains in the chloroplast stroma. During the night, the plants need to support their necessities, thus the starch is broken and used (Alberts et al. 1994). This process is made by C3 plants. But there is at least one more way: the C4 plants way. C4, because the first compound produced after CO_2 being fixed is a compound with 4 carbons, and Calvin cyclic occurs in the chloroplasts of Bundle-sheath cells.

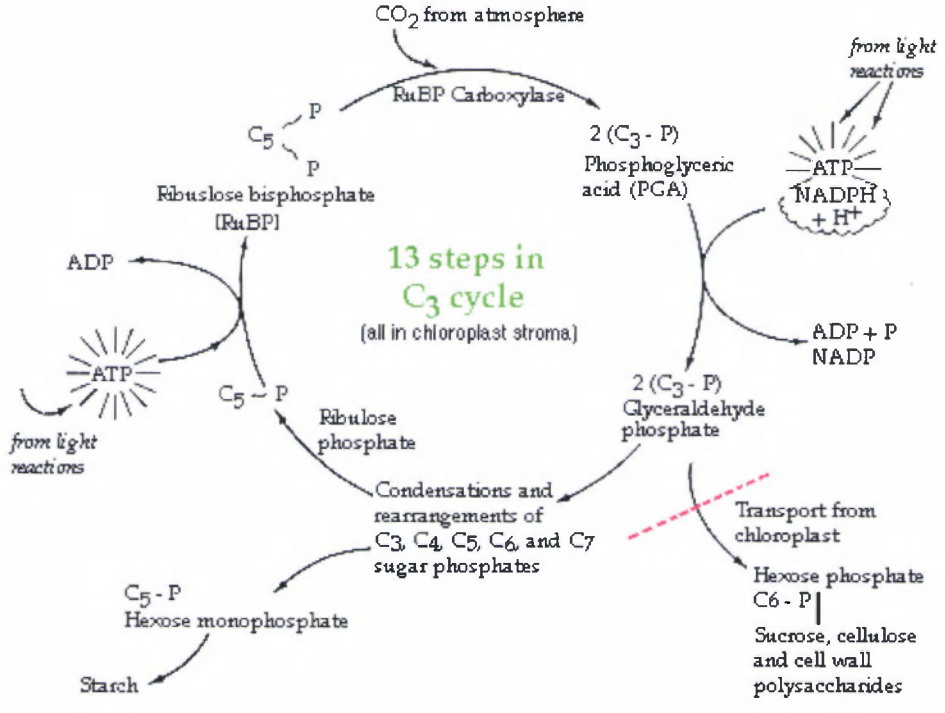

Fig. 5: Calvin cycle.

I. 4. PSI Efficiency / On-Off Parameter

When a chlorophyll molecule is excited by a quantum of light (a photon) and an electron is moved from one molecular orbital to another of higher energy the excited molecule is unstable and will tend to return to its original, unexcited state (Alberts et al. 1994).

Fluorescence is one of the ways in which an excited molecule can regain the ground state (Baker & Hipkins 2002). It involves the emission of radiation of a longer wavelength.

The chlorophyll fluorescence measurement is the most widely used technique for probing the photochemical and electrochemical processes occurring in and around thylacoids *in vivo* and *in vitro* (Harbinson & Woodward 1986). Chlorophyll fluorescence has several advantages (e.g. it is a non-destructive method and can be performed relatively fast and with a great precision by minimally trained personnel) (DeEll et al. 1999).

Although Chlorophyll fluorescence is an extremely powerful tool, interpretation of simple measurements of chlorophyll fluorescence are rarely unambiguous because of the multiplicity of factors that control the fluorescence yield from PSII (Harbinson & Woodward 1986).

The yield of chlorophyll fluorescence is influenced by numerous factors in a very complex manner" (Krause & Weis 1988): e.g. light intensity, temperature, pre-illumination, light-adaptation state, gas composition, humidity, tissue age and the entire "pre-history" of the plant, including possible exposure to environmental stresses (Renger & Schreiber 1986).

For all these reasons, new techniques shall be developed. Two of these techniques are related to PSI: PSI Efficiency and On-Off Parameter.

Another reason for the high importance of this work is the fact that chlorophyll fluorescence technique has been already used to predict shelf life in some products. Good results were obtained for cucumbers, but not that good for leek and lettuce.

O. van Kooten et al. (unpublished data) were unable to use chlorophyll fluorescence techniques to predict the shelf-life of leeks obtained from an auction in The Netherlands due to large variations in leek quality and thus in chlorophyll fluorescence measurements. Leeks are not as homogeneous as cucumbers, with

considerable variation in diameters and lengths. Difference in shelf-life among cultivars are not known, nor are differences between the storability of thick and thin or long and short leeks. Such unknown information and the fact that leeks are not very morphologically homogeneous makes it virtually impossible to use chlorophyll fluorescence to predict the shelf-life of leeks. Similar large variations in product quality and chlorophyll fluorescence measurements make it difficult to predict shelf life of 'iceberg' lettuce (DeEll et al. 1999).

These methods (PSI Efficiency and On-Off Parameter) have a big potential to overcome the disadvantages of chlorophyll fluorescence method.

It will be investigated if the quality of a crop can be determined by using these two methods. This information is also important, because ATO has a Patent for this equipment used to determine one of these quality indicators, namely the On-Off Parameter (Boogaard et al. 2001).

II. Materials and Methods

II. 1. Equipment

II. 1.1. PSI/On-Off measurements

Products' shelf life is influenced by a number of factors. Most of them are so ambiguous that they become difficult to find a method that can give us precise and reliable information about shelf life prediction.

The tests used to predict shelf life are:

- sensory evaluation (this kind of test must be done under controlled environmental conditions–e.g. standard lighting and quietness–and the staff used must be a trained sensory panel);
- microbiological examination;
- chemical analysis;
- physical examination.

(AAVV 1992)

Most of these tests have a lot of disadvantages (e.g. trained people are needed, the product is destroyed and some time is needed to know the results).

It would be a great aid, especially to the manufactures, to have a quality indicator that could offer valuable information about quality and that could be useful in shelf life prediction and, at the same time, could not offer so many disadvantages as the previous tests.

With this equipment, it is possible to study two important photosynthetic parameters: On-Off Parameter and PS1 Efficiency–both belonging to Photosystem I (PSI).

On-Off Parameter gives us information about the speed at which $P700^+$ molecules are reduced after the light has been switched off.

PS1 Efficiency is an efficiency (so the values are between 0 and 1) and tells us about the percentage of P700 that is oxidised when light is caught by P700.

$$PS1\ Efficiency = \frac{Max.P700^{+} - realP700^{+}}{Max.P700^{+}}$$

To obtain these two parameters, three important measurements must be done: light On / light Off; Far-red; and Flash.

For the On-Off Parameter, the light On / light Off measurement must be done; for the PS1 efficiency, the amount of real $P700^{+}$ obtained with light On / light Off measurement as well as the amount of maximum $P700^{+}$ obtained with Far-red and Flash measurements must be measured.

To do these measurements, the most important parts of the equipment are: light source; CO_2 recorder; PS1 recorder; Nicolet and the cuvet.

The cuvet is the place where the plant (leaf) is put (Fig. 6). Inside the cuvet, the plant (leaf) should be flat, because of the gas flow, which should be 250 ml/min.

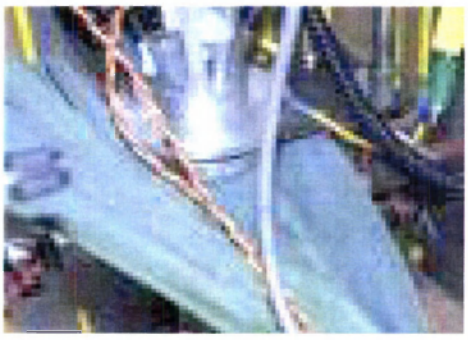

Fig.6: Cuvet, place in the equipment where the vegetable is put to do the measurements.

Above the cuvet and the plant, there is a light source. For these parameters, the light used was visible light, because for the photosynthesis process plants use light from the visible part of the spectrum. The bulb used was a halogene bulb. The light intensity varies between dark and 6000 mV (1886 $\mu mol/m^2.s$).

When the plant is either with light or without light, CO_2 recorder records information about the CO_2 that is absorbed by the plant. The recorder draws an informative line about CO_2 uptake.

As we can see in Fig.7: when the light is switched off, the CO_2 uptake line changes, so the difference (mm) between light-on line and light-off line gives us information about how much CO_2 is absorbed by the plant.

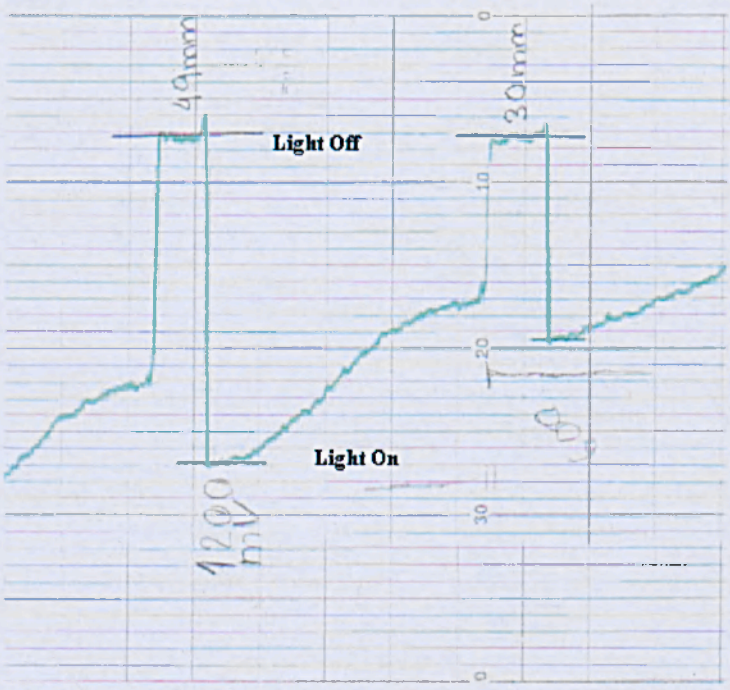

Fig. 7: The line drawn by CO2 recorder when the plant is under light-on conditions and light-off conditions. It is perceptible how the lines changes when light is switched off.

The PS1 recorder, as the name says, has a straight relation with Photosystem I of the photosynthesis process.

This recorder records information about light on / light Off and Far-red measurements. It makes two kinds of graphs: Fig. 8 is related to light On / light Off measurements, which give us the real amount of $P700^{+}$; Fig.9 is related to the Far-red measurement, and give information about maximum $P700^{+}$. The results of this two measurements are in mm.

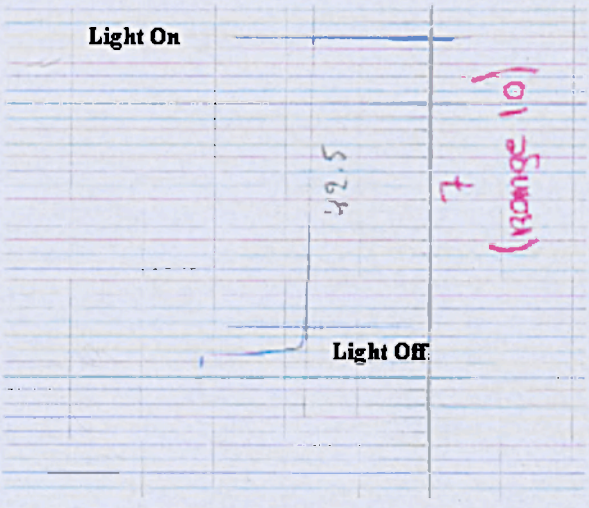

Fig. 8: The difference (mm) between the top line and the bottom line gives the real amount of $P700^{+}$.

Fig. 9: Far-red measurements give us the maximum of $P700^{+}$.

Both recorders have a range. For CO_2 recorder, the usually used range is 20-60, but we can increase or decrease it, which respectively implies putting the graphs smaller or bigger. The same happens with PSI recorder: if we increase the range, the graphs made by this recorder will be smaller; if we decrease the range, the graphs will be bigger.

At last, the Nicolet gives us the On-Off Parameter and the results about Flash measurements, which were missing to have the Maximum of $P700^{+}$. All this information about light On / light Off measurements and flash measurement is saved on a floppy and then worked in two different computer programmes. For light On / light Off measurements is used Matlab, for Flash measuremesnt is used Qbasic.

- On-Off Parameter

To do this measurement, the protocol exposed in the appendix was followed.

For this measurement, the plants must be under light conditions during equal time periods. This time period can vary. Sometimes, it is until CO_2 is stable (CO_2 recorder). In this research work, the chosen time period was only 5 minutes based on a preliminary test (see section III. 2.1.).

When the plant is in light, the photosynthesis process begins and P700 molecules become excited (P700 $\rightarrow$ P700*). When a P700 molecule is excited, it is able to lose

an electron. When the excited molecule loses the electron, it becomes oxidised ($P700^+$) and the electron goes to an electron transfer chain to form NADPH.

When the light is switched off, the $P700^+$ molecules are reduced and the On-Off Parameter gives us information about the speed at which $P700^+$ molecules are reduced.

The Nicolet is the part of the equipment that gives us the On-Off Parameter.

So, to obtain this Parameter, we need to keep the plant under light conditions during some time and then switch off the light. It is what is called light On / light Off measurement.

- PSI Efficiency

When the plant is in light, the photosynthesis process begins and P700 molecules are excited and then oxidised. PSI Efficiency gives the percentage of the P700 molecules that are oxidised.

In order to be able to calculate the PSI Efficiency, three measurements must be done. The first is light On / light Off, which is also made for the On-Off Parameter. However, for the PSI Efficiency the information that is needed is the graph made with the PSI recorder (that gives us the real amount of $P700^+$). This amount is obtained by measuring the distance (mm) between light On line and light Off line. The other two measurements needed are Far-red and Flash. The sum of these two measurements gives us the maximum amount of $P700^+$.

After the light On / light Off measurements, the light remains switched off to measure the maximum amount of $P700^+$, the plant must be under far-red light conditions. This kind of light has a long wavelength of 720nm and under this light only PSI is functioning. It means that all P700 molecules will be excited and oxidised and none of the oxidised molecules will be reduced, because the electron transfer chain that comes from PSII to PSI does not work. The Flash measurement is just a way of being sure that all P700 molecules are oxidised. Flash is visible light and the plant is under this light for a few seconds. The Flash results are measured with the Nicolet and subsequently analysed with a computer programme (Qbasic).

II. 1.2. Colour measurements

The human eye can distinguish colours, but cannot see small differences between objects that have similar colours.

For these reasons, colour measurements can give us more precise information about colours.

The equipment used was the Mercury Portable Spectrophotometer, which uses the L, a, b system (Fig.10). The L, a, b measuring mode closely represents human sensitivity to colour and thus is one of the most popular colour notation systems (Advertisement for Minolta's Chroma Meter Cr-200).

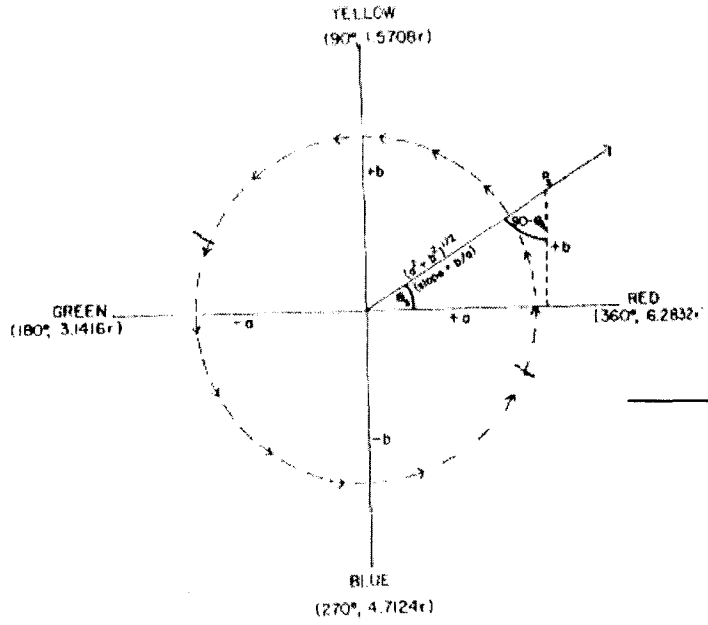

Fig. 10: The way how hue angle varies along the axis.

L = Distance of the Batch along the lightness-darkness axis;
a = Distance of the Batch along the red-green axis;
b = Distance of the Batch along the yellow-blue axis;
H= Hue Angle = arctan b/a;
$C= (a^2+b^2)^{½}$.

(Little 1975)

II. 1.3. Weight Measurements

The balance used was Mettler Toledo, type SR16001.

II. 1.4. Statistical Analyses

Measured values were analyzed for significant differences by analysis of variance (ANOVA) with the statistical package Genstat. Differences were considered statistically significant if $p < 0.05$. Columns in the figures (results' chapters) marked with a same letter are not statistically different.

II. 2. Products

All the products used are vegetables.

All of them were used in their natural form. They were harvested and did not suffer any type of processing like cutting, blanching or freezing, etc.

As the objective is to predict their shelf life, it is interesting to use new and old products to compare if there are any differences between them. It is also important to follow the physical, chemical and sensorial alterations during their shelf life. In this way, a more exactly shelf life prevision can be made. For this reason, the only process to which the products were submitted was storage.

Depending on the aim of the short tests and the main experiments, different products were used and the state of the products were also different. Some products some were new, other were old or ripe.

The products studied were lettuce (*Lactuca Sativa*), leek (*Allium Porrum*) and endive (*Cichorium Endivia*) (Table 1).

Table 1. Amount of products used for each experiment.

	Test 1	Test 2	Test 3	Main Experiment 1	Main Experiment 2
Lettuce	2	1		-	22
Leek	-	-	1	25	26
Endive	-	-		-	20

For test 1, 2 , 3 and main experiment 1, fresh products were used. All of them were bought in a supermarket, but neither the growers are known nor their exactly age. The lettuces used were the butterhead type.

The products used for the main experiment 2 were lettuces (crisphead type), endives (endive type) and leeks. Each type of product was achieved from the same grower and their age was more or less known. The lettuces were produced at the same time, but 11 of them were harvested at an optimal harvest time and the other 11 were harvested several days later. The same happened with the endives: 10 were harvested in time and the other 10 several days later.

The leeks used for main experiment 2 were different. While 13 leeks had been in a temperature controlled room until one day before measurements during a year (below 6°C), the other 13 leeks had been harvested one week before measurements.

Until the day before measurements, they had been in a temperature controlled room where the temperature was also 6ªC.

II. 3. Experiments

II. 3.1. Test 1

II. 3.1.1. Objective

The objective was to find out whether there were differences in PSI Efficiency between 2 lettuces. One of them was fresh and the other was stored more than 5 days.

II. 3.1.2. Material

- 2 lettuces (butterhead type), which were bought in a supermarket on the same day (*on 29th March 2002*); the age was unknown;
- PSI, On-Off Equipment;
- Temperature controlled room.

II. 3.1.3. Procedure

On March 29th, the lettuces were bought. One of them was put in the temperature controlled room. The temperature inside the room was 10°C and it was dark. The lettuce remained there for 5 days and only *on April 3rd 2002* it was removed. The other lettuce was submitted to the PSI and On-Off measurements *on April 3rd 2002*.

These measurements were done under 4 different intensity lights: 150 mV (49,1μmol/m^2.s); 300 mV (96,2 μmol/m^2.s); 600 mV (190,4μmol/m^2.s); and 3600 mV (1132,4 μmol/m^2.s). Temperature during measurements was 18°C, the relative humidity was 100%, and the gas flow was 250ml/min. The leaf used to do the measurements was leaf number 1 of the lettuce (most outer leave) and the measuring spot was on the top of the leaf.

The equipment was switched on.

Approximately 1 hour was waited until the measurements started, because CO_2 and temperature should be stable.

The first light intensity tested was 150mv (49,1 μmol/m^2.s). As usual, the first measurement done was light on / light off. With this measurement, the PS1 recorder gave the real amount of $P700^+$. Subsequently the Far-red was given by the PSI recorder and Flash given by the Nicolet. These two together gave the maximum of $P700^+$.

When the light intensity was changed, we had to wait until CO_2 uptake was stable: sometimes only 5 minutes but other times more, because, with higher intensity, it is more difficult for the CO_2 uptake to stabilise. *On April 3rd 2002*, the second lettuce was used. The light On / light Off, Far-red and Flash measurements were done. The procedure for this lettuce's measurements was the same as for the first lettuce and the measurement conditions were the same. The only difference was that more light intensities were tested: 150 mV (49,1μmol/m^2.s); 300 mV (96,2μmol/m^2.s); 600 mV (190,4μmol/m^2.s); 1200 mV (378,8μmol/m^2.s); 2400 mV (755,6μmol/m^2.s); 3600 mV (1132,4 μmol/m^2.s).

After the measurements were done, all the graphs from the CO_2 and PSI recorder were analysed as described in the Material and Methods section II. 1.1.

II. 3.2. Test 2

II. 3.2.1. Objective

When the light intensity changes, the plant needs time to get used to the new light intensity. This is shown by the CO2 uptake which does not get stable immediately. The higher the light intensity, the more time the plant needs to adapt to this new situation.

The goal is to get an answer to this question: will PSI Efficiency be influenced by the time waited before the start of the light On / light Off measurements?

II. 3.2.2. Material

- A crop – Lettuce (butterhead type) bought at a supermarket on *April 8th 2002*; the age of the product was unknown;
- PSI, On-Off Equipment.

II. 3.2.3.Procedure

The goal was to find out whether adaptation time was so important for the PSI Efficiency measurements, so 3 open assembly times had to be tested: 5; 15; 30 minutes.

The equipment was switched on. As always, when the equipment is switched on, 45 minutes or one hour must be waited.

The measurements were done at 18°C, and the relative humidity was 100%. The gas flow should have been 250ml/min, but sometimes varied a little bit.

For each open assembly time 4 different light intensities were tested: 150 mV (49,1μmol/m^2.s); 300 mV (96,2μmol/m^2.s); 600 mV (190,4μmol/m^2.s); 3600 mV (1132,4 μmol/m^2.s).

The first open assembly time to be tested was 5 minutes.

After the light On / light Off measurement was done, the Far-red and Flash were also done. This same procedure happened for all light intensities as well as for the 3 open assembly time.

All the measurements were done on the same lettuce and the leaf chosen was leaf number 1 (on its top).

All the graphs were made on the CO_2 and PSI recorders and on the Nicolet.

II. 3.3.Test 3

II. 3.3.1.Objective

The objective of this test was: How is the evolution of PS1 Efficiency under different light intensities of a crop?

The chosen crop was leek, because of its morphology. It appeared to be an easy crop to put in the cuvet, without provoking any damages on it.

II. 3.3.2. Material

- Crop: Leek, bought in a supermarket on *April 16th 2002*; the age was unknown;
- PSI, On-Off equipment.

II. 3.3.3. Procedure

At the same day, light On / light Off, Far-red and Flash measurements were done to obtain both Photosynthetic Parameters.

The light intensities tested were: 160 mV (52,2 μmol/m^2.s); 320 mV (102,5 μmol/m^2.s); 640 mV (202,9μmol/m^2.s); 1280 mV (403,9μmol/m^2.s); 2500 mV (787μmol/m^2.s); 3700 mV (1163,8μmol/m^2.s); 4200 mV (1320,8 μmol/m^2.s).

During these measurements, the range of PS1 recorder had to be changed, because some of the graphs were very long, so the paper from the PS1 recorder was not wide enough. The normal range used by this equipment is 5. On this day, the range had to be changed for both measurements: for the Far-red measurement, the range used was always 20; for the light On / light Off until 1280 mV (403,9 μmol/m^2.s) (intensity light), the range used was 5; above 2500 mV (787 μmol/m^2.s) (inclusive) the range used was 10.

The conditions during the measurements were: a temperature of 18°C; relative humidity level of 100%; gas flow of 250ml/min, with some deviations because of the crop's morphology. The time waited for the light on / light Off measurement was 5

minutes.

After the measurements were done, all the calculations were done to obtain the On-Off parameter and PS1 Efficiency.

II. 3.4.Main Experiment 1

II. 3.4.1.Objective

The objective was to follow the alterations in On-Off parameter and PS1 Efficiency values of a crop (Leek).

II. 3.4.2.Material

- Crop: 25 Leeks, bought in a supermarket *on April 18th 2002*; the age was unknown;
- Shelf life room, temperature 18ª C, relative humidity 65 % (a room with the same conditions as a shop);
- On-Off, PSI equipment;
- Balance - Mettler Toledo, type SR16001;
- Mercury Portable Spectrophotometer.

II. 3.4.3.Procedure

25 leeks were bought in a supermarket *on April 18th 2002*. All of them were numbered.

Since *18 p.m. of April 18th* until *9 a.m. of April 19th 2002*, the leeks stayed inside the shelf life room. The leeks stayed there all night covered to be in the dark until the measurements.

On April 19th 2002, the light On / light Off (for On–Off Parameter and PS1 Efficiency calculations), Far-red, Flash (for PS1 Efficiency calculations), colour and weight measurements were done for each leek.

The first measurements done were the light On / light Off, Far-red and Flash. The equipment for these measurements was switched on, and for this experiment only

one light intensity was chosen: 1280mV (403,9 μmol/m^2.s). This one was chosen, because it was the light intensity where the PSI Efficiency started to decrease in test 3. The ranges used for the PSI recorder were; for light On / light Off, 10 and, for the Far-red, 20. For the CO_2 recorder, the used range was 20-60, and the gas flow was 250ml/min., but sometimes it was difficult to reach this value. The temperature was 18° C and relative humidity was 100%. The time waited until the light On / light Off measurement was only 5 minutes.

The leaf where the measurements were done depended on the leek. Mostly, the outer leave (leave 1) was measured but, in other cases, the leave chosen was the second or the third one. The choice of the leaf was not that easy, because the product should not have any damages and sometimes, when it was tried to put the first or the second leaves in the cuvet, some damages could happen. So it was decided to choose the leaf that was the easiest to put in the cuvet without provoking any damages.

The PSI, CO_2 recorder, and Nicolet made all the graphs, so the On–Off Parameter and PSI Efficiency could be calculated.

After it, each leek's colour measurement was done at the same spot where the light On / light Off, Far-red and Flash measurements were made.

At last, the weight was also measured.

All these measurements were done on the same day: *April 19th 2002.*

After the measurements, the leeks were put again in the shelf life room, but at this time they were not covered. They stayed under light conditions but *on April 22nd 2002* the leeks were covered again, because it was the day before the next measurements.

On April 23rd 2002 morning, the measurements started again. The measurements were repeated to investigate how the evolution of all these attributes and parameters was. On this day, all the measurements were repeated: light On / light Off, Far-red, Flash, colour, and weight.

The sequence was the same: light On / light Off, Far-red and Flash were the first. The ranges used varied from leek to leek: for light On / light Off measurements, the range used was always 10; for Far-red, the most used was 20; for leeks number 3 and 22, the range used was 10; range 5 was used for leeks number 1,7,11 and 25. The leaf used was the same leaf as used for the first day measurements. The measurements

conditions were the same.

The colour and weight were the next measurements.

All the measurements were always done on the same leaf and on the same spot of the leaf.

After it, the calculations were done. The On-Off Parameter was obtained in the computer programme. PS1 Efficiency was also calculated as well as the Hue angle for the colour measurements.

II. 3.5.Main Experiment 2

II. 3.5.1. Objective

The objective was to investigate whether there are differences in On-Off Parameter and PS1 Efficiency between a normal harvest crop and a late harvest crop, and between a stored crop and an fresh (young) crop, and to investigate whether these parameters are related to shelf life.

II. 3.5.2. Material

- 26 Leeks (Fig.11): 13 were stored during a year (old);
  13 harvested *on July 24th 2002* (young).

Fig. 11: At the top, the old leeks that were stored during a year are shown; on the bottom, the leeks that were harvested a week before the experiment (fresh leeks).

- 22 Lettuce (crisphead type) (Fig.12): 11 the harvest was done late;
  11 the harvest was in time.

Fig. 12: At the top, the lettuces that were harvested later and on bottom the lettuces harvested in time.

- 20 Endive (endive type) (Fig.13): 10 the harvest was done late;
  10 the harvest was done in time.

Fig. 13: The endives that were harvested later are at the top of the figure; on bottom, the endives are those that were harvested in time.

Since the harvest all the products were stored in a temperature controlled room. The temperature was 6°C and the room was dark.

- Shelf life room;
- On – Off, PSI equipment;
- Balance - Mettler Toledo, type SR16001;
- Mercury Portable Spectrophotometer.

II. 3.5.3 Procedure

Leek

On July 1st 2002 evening, the leeks were put in shelf life room. Before this day and since the harvest day, they were always in a temperature controlled room. On this same day, colour measurements were done. For each leek 4 measurements were done on different leaves, but all measurements at the same level of the leaves.

The leeks remained there until *July 2nd 2002 morning* (measurements day). The leeks stayed uncovered in order to get used to the light.

On July 2nd 2002, the light On / light Off, Far-red, Flash and weight measurements were done.

The equipment to do the light On / light Off, Far-red and Flash measurements was switched on.

The light intensity used was 1200mV (378,8 μmol/m^2.s). The range for the CO_2 recorder was 20-60 for all leeks and for the PS1 recorder it was 10 for both measurements.

Almost all the measurements were made on leaf number 1 (outer leave), except some of them that were made on leaf number 2 or 3. Measurements were always done at the same level where the colour measurements were made.

The gas flow should have been 250ml/min, but this could not always be realised because this product was very irregular: leaves were not flat, so it was difficult to remain the flow stable. The temperature was 18° C and the relative humidity was 100%. The time waited to do the light On / light Off measurement was only 5 minutes.

The graphs were made by the Nicolet and by CO_2 and PS1 recorders. With the

graphs, all the calculations could be made and On-Off Parameter and PS1 Efficiency obtained.

On this same day, the weight was also measured.

On July 5th 2002, the first measurements made were light On / light Off, Far-red and Flash. The light intensity used was the same: 1200mV (378,8 μmol/m^2.s). The range for CO_2 was 20-60 for all leeks, and the range for the PS1 recorder was 5 for both measurements. The flow should have been 250ml/min, but, as it was on the other day, it was very difficult to achieve this flow, because the surface of the leaves was not flat. The leaf where the measurements were made was the same leaf as measured *on July 2nd 2002*. The measurement conditions (temperature, humidity and time) were the same.

After it, 4 colour measurements were done on the same level of the leaves and also the weight was measured.

Lettuce

On July 2nd 2002 evening, the lettuces were numbered and put uncovered in the shelf life room.

The measurements for the On-Off Parameter (light On / light Off) and PSI Efficiency (light On / light Off, Far-red and Flash) were done *on July 3rd 2002*. Also the colour and weight measurements were done on this same day.

The On-Off Parameter and PSI Efficiency measurements conditions were: a temperature of 18° C; a relative humidity level of 100%; a light intensity of 600 mV (190,4 μmol/m^2.s). This light intensity was chosen, because, as found for test 1, this light intensity was the one where PSI started to have the deepest decrease. The gas flow should have been 250ml/min, but, because of the leaf's morphology, it was difficult to reach it, so sometimes it was higher or lower. The range used for the CO_2 recorder was 20-60 for all measurements, and for the PSI recorder it was always 5. The lettuces' leaves chosen to do these measurements were leaves number 1 (outer leave), with the exception of those from lettuces number 8 and 14. In these cases, leaves number 3 were chosen. These measurements were all made at the top of the leaves. The time waited to do the light On / light Off was 5 minutes.

4 colour measurements were also made at the top of the leaves of each lettuce.

The Nicolet, CO_2 recorder, and PSI recorder made all the graphs, and the calculations could be done. The On-Off Parameter and PSI efficiency were obtained.

The Hue angle was also calculated for the colour measurements. On the same day, the weight was also measured.

On July 5th 2002, only colour and weight measurements were done. Colour measurements were done according to the same procedure as *on July 3rd 2002.*

Endive

The measurements done and the used procedure were the same as used for lettuce. The only difference was the light intensity: for endive it was 1200mV (378,8 μmol/m^2.s). The temperature, humidity, ranges, flow and time waited were the same as those used for lettuces.

On-Off Parameter and PSI Efficiency: the measurements needed were done *on July 4th 2002.*

The colour and weight were measured either *on July 4th 2002* and *on July 5th 2002.*

III. RESULTS AND DISCUSSION

III. 1. Test 1

III. 1.1. Results

According to the PSI Efficiency values, there was not a great difference between values from a fresh lettuce and a lettuce after 5 days storage.

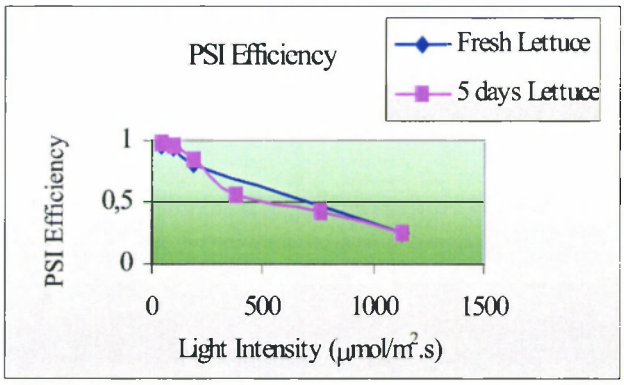

Fig. 14: PSI evolution. Comparison of a fresh lettuce to a lettuce with 5 days more.

While the light intensity increased, the PSI efficiency decreased (Fig. 14). It appeared that the plant is not so efficient at higher light intensity.

III. 1.2. Discussion

Although the plant uses less light energy at a lower light intensity (less available), the plant will use the available light energy more efficient, resulting in a higher PSI efficiency.

It was not expected that no differences could be found between the fresh and the stored lettuce. Perhaps 5 days were not enough to find alterations in PSI Efficiency value or the fresh lettuce was initially older than the stored one. An important reason may be the plant's morphology. Sometimes, because of the plant's morphology, putting the leaf in the cuvet is a difficult task. In this case, it can be difficult, because mechanical damages are not desired and when the lettuce is put in the cuvet, sometimes

some mechanical alterations may happen. Because of the leaf's morphology (e.g. veins) it is sometimes difficult to have a stable flow; sometimes the flow is higher than 250 ml/min, other times is lower than 250 ml/min. However in this experiment the flow did not vary that much.

At last, when the light intensity is changed to another one, the time needed until CO_2 uptake is stable varies a lot. With increasing light intensity , more time is needed. So, it can be a problem, when we want to have quick measurements.

Will the time be so important to calculate PSI Efficiency? This question will be answered in the next test (test 2).

III. 2. Test 2

III. 2.1. Results

The difference between the 3 different open assembly times for each light intensity was small (Fig. 15).

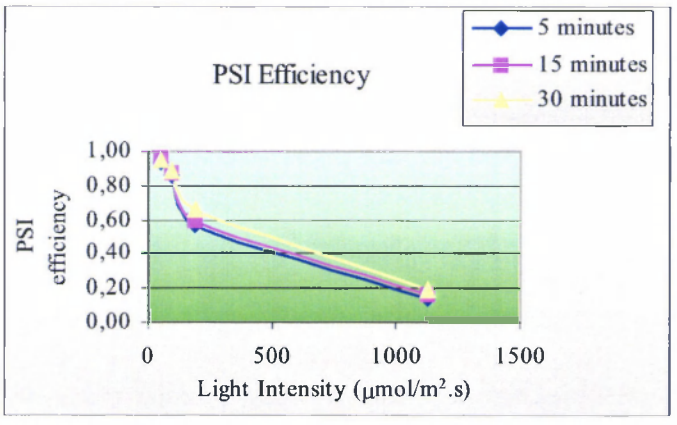

Fig. 15: The difference in PSI Efficiency values between 3 different open assembly times: 5, 15, 30 minutes.

It means that PSI Efficiency is not influenced by the time that is waited before light On / light Off measurements.

III. 2.2. Discussion

These results are a great aid for this work. If the open assembly time does not influence the PSI Efficiency value, it means that it is not necessary to wait so much time to do the measurements. In this way, it is possible to do quick measurements.

In the next test and in the main experiments, the waited time before light On / light Off will be only 5 minutes.

III. 3. Test 3

III. 3.1. Results

PSI Efficiency was decreasing while light intensity was increasing (Fig. 16).

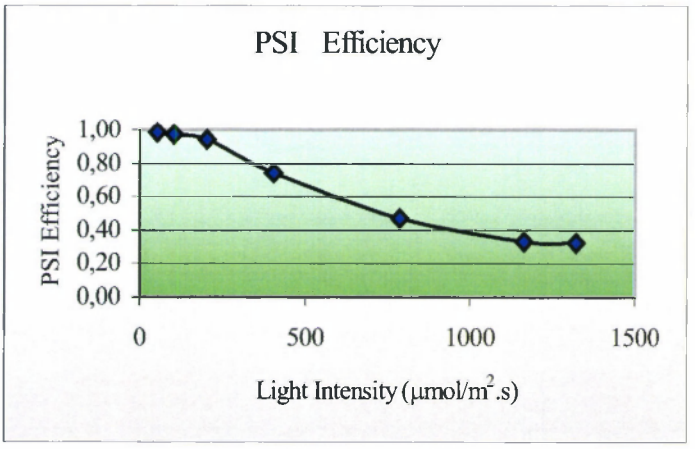

Fig. 16: Leek's PSI Efficiency evolution. PSI Efficiency decreases while light intensity is increasing.

On Fig. 17 it is observed that while light intensity is increasing also On-Off Parameter is increasing.

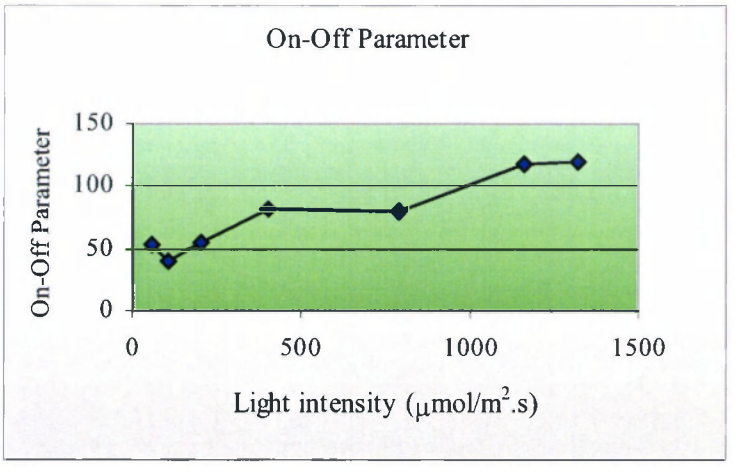

Fig. 17: While light intensity is increasing, the fastness at which $P700^+$ are reduced is also increasing.

III. 3.2. Discussion

Indeed, this crop was a good choice, because until now it was the easiest to be put in the cuvet. However, it was difficult to have the gas flow stable.

Another problem was the light intensity. When it is 1000mV (316 μmol/m^2.s) or higher, it is difficult to keep it stable.

Though, this crop was chosen to be studied in the following experiment and the chosen light intensity was 1280mV (378,8μmol/m^2.s) for all measurements, because it was the one at which PSI Efficiency started having a faster decrease.

III. 4. Main Experiment 1

III. 4.1. Results

After On-Off Parameter, PSI Efficiency, Hue angle and weight were obtained, some statistic calculations were done. The mean and standard deviation was calculated for Photosynthetic Parameter (On-Off Parameter and PSI Efficiency), for Hue angle and for weight in both days.

Another statistical test was done: ANOVA.

With this statistical test, it was possible to compare the data for Photosynthetic Parameter, for Hue angle, and for weight between both days.

Both Photosynthetic Parameters decreased (Fig. 18 and 19). The same happened

with Hue angle (Fig. 20), but not with the weight, which did not have a significant difference (Fig. 21).

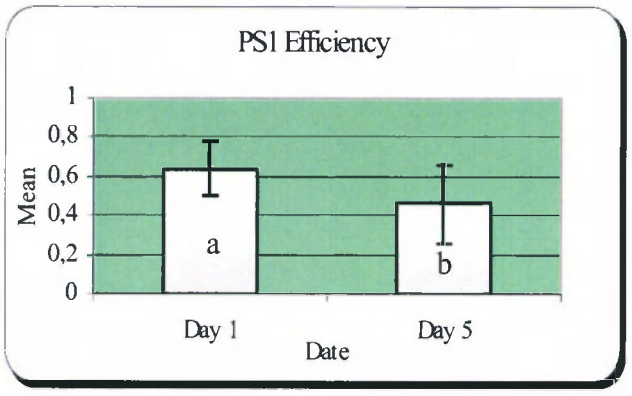

Fig. 18: PSI Efficiency decreased in time: it was higher on day 1 and lower on day 5.

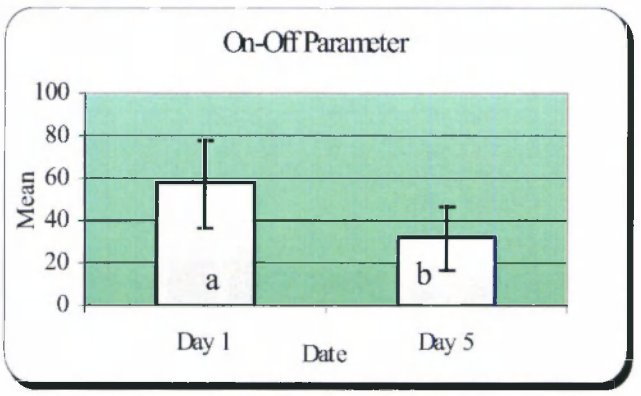

Fig. 19: On-Off Parameter decreased in time: it was higher on day 1 and lower on day 5.

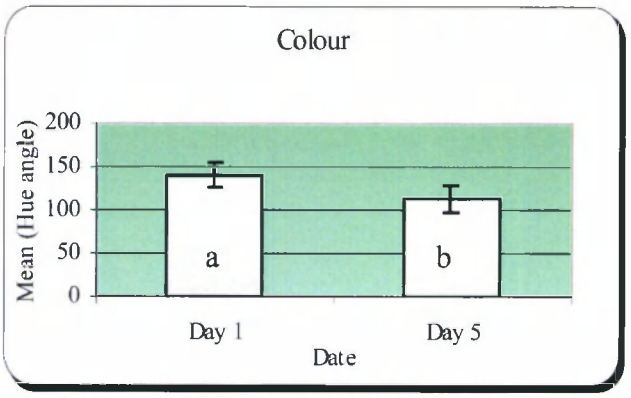

Fig. 20: The leek was greener on day 1 and lost its colour in 5 days.

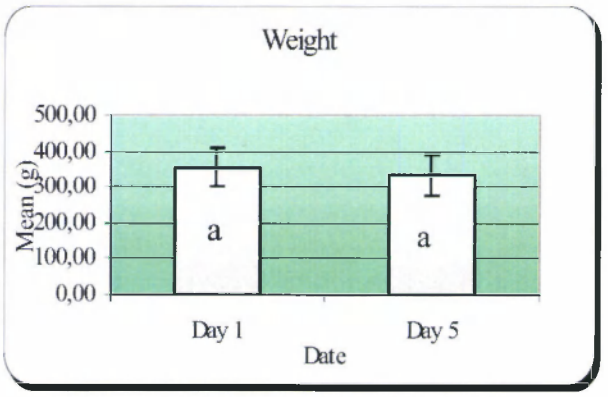

Fig. 21: During the 5 days, leeks did not lose weight.

III. 4.2. Discussion

One point that is clear is the fact that PSI Efficiency and On-Off Parameter decreased in time. Hue angle also decreased in time, which is not a surprise. Leeks lost their green colour with time.

Perhaps this decrease in On-Off Parameter and in PSI Efficiency values in time could be related with the decrease in crop quality. So, if it is possible to relate both

Parameters with crop quality, maybe it will be possible to predict shelf life, because for both days both Parameters had different values and while crop quality was decreasing both Parameters were also decreasing.

The aim of the next main experiment was to investigate if there are any differences in On-Off Parameter and PSI efficiency in 2 crops with different levels of quality.

If PSI Efficiency and On-Off Parameter will be different for crops with different levels of quality, it could indeed mean that these Photosynthetic Parameters could be related with crop quality, which could be a great aid for shelf life prediction.

III. 5. Main Experiment 2

III. 5.1. Leek

III. 5.1.1. Results

PSI Efficiency was obtained for each leek in both days, while the On-Off Parameter was only obtained on day 1.

In this main experiment, statistical tests were also made. The mean and standard deviation were calculated for the new leek and for the old leek in both days, for both Photosynthetic Parameters and for the Hue angle and weight.

ANOVA tests were also made to compare new leeks and old leeks data for both days. It was investigated whether there was any difference between new leek and old leek data on each day and also whether there were differences between day 1 and day 4.

As we can see in Fig. 22, which is referring to PSI Efficiency, there is no difference between new leek mean and old leek mean on day 1 and day 4. But there are differences between days. For both kinds of leeks, PSI Efficiency decreased in time: on day 4, it was lower.

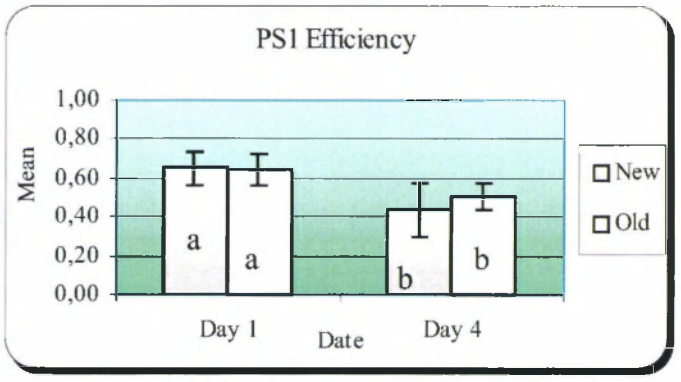

Fig.22: PSI Efficiency. For both leeks this parameter decreased in time, but there is not any difference among a old and new leek.

Mean, standard deviation and ANOVA were also made for the On-Off Parameter.

ANOVA test was made between new and old leeks on day 1 only, because the measurement for this Parameter on day 4 was unable to be made because of a technical problem.

In Fig. 23, it is showed that the On-Off Parameter had different values for new and old leeks. It was higher for old leeks than for new leeks.

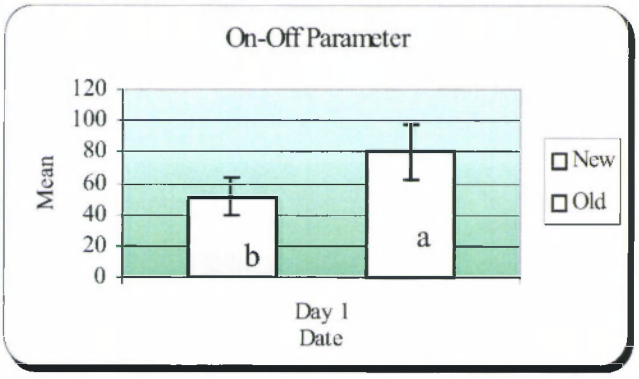

Fig.23: On-Off Parameter was higher for the old leeks than for new leeks.

Fig. 24 shows colour results. There was no difference between new and old leek, but there were differences between days. As it was expected, the hue angle from both kind of leeks on day 4 was lower: it means that both new and old leeks were less green after storage.

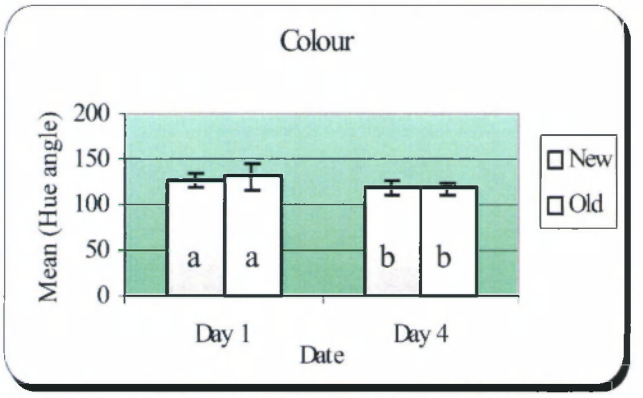

Fig.24: There are no differences between the leeks on both days. Hue angle decreased from day 1 to day 4.

Also the weight was measured. The ANOVA test between new and old leek on both days was not important, but between days it was important. From day 1 to day 4, leeks did not lose weight significantly (Fig.25).

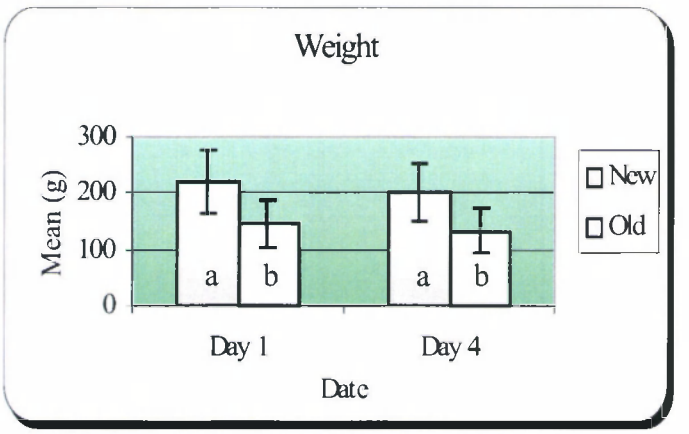

Fig.25: Weight. There are no differences between day 1 and day 4 for both leeks. The weight is different for different kinds of leeks.

III. 5.1.2. Discussion

When PSI Efficiency and On-Off Parameter results were analysed (having as hypothesis that both could give information about crop quality), PSI Efficiency value was the same for both kinds of leeks on both days. If PS1 efficiency is indeed a parameter for quality, it would mean that both kinds of leeks had the same quality level. If we tried to predict

shelf life, we would say that both kind of leeks would have the same shelf life.

But when the On-Off Parameter was analysed, it was observed that there was difference between the fresh and stored leek, which could mean that they had different quality levels. But it was unexpected that the old leeks (with probably a lower quality level) had a higher On-Off Parameter value.

To clear up every doubt, the leeks were observed and cut after 3 days shelf-life

Differences between new and old leek were clearly visible (Fig. 26 and 27).

Fig. 26: Fresh leek, the leaves were green.

Fig. 27: Stored leek, inside the leaves were already yellow.

So, the new and the old did not have the same quality level.

Our prediction based on PSI Efficiency did not work well. It means that PSI efficiency for leeks does not give any information about crop quality and that it is not possible to predict shelf life based on this photosynthetic parameter.

Regarding the On-Off Parameter, results were really strange. In main experiment 1 the On-Off parameter was decreasing while crop quality was decreasing. However now the inverse happened: the oldest leeks had a higher value for On-Off Parameter. The only explanation that can be given is that something wrong happened during the On-Off measurements.

III. 5.2. Lettuce

III. 5.2.1. Results

For this crop, the mean and standard deviation of PSI Efficiency, On-Off Parameter, Hue angle and weight were also calculated.

As we can see in Fig. 28, PSI Efficiency was the same for both kinds of lettuce: that which was harvested in time and that which was harvest later. So, our prediction was: they will have the same shelf life, based on this Photosynthetic Parameter.

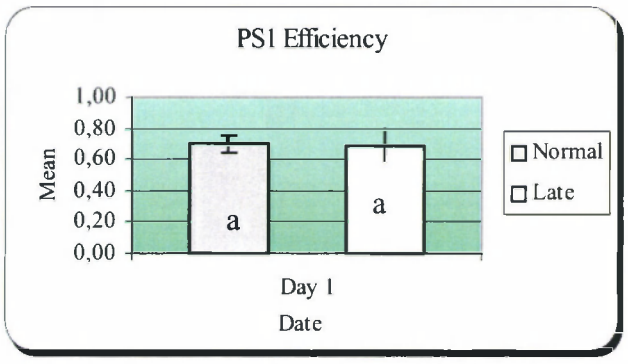

Fig. 28: There were any differences in PSI Efficiency value for both different types of lettuce.

The same prediction could be made when we based our prediction on the On-Off Parameter, (Fig. 29) because this Parameter had the same value for different kinds of lettuce.

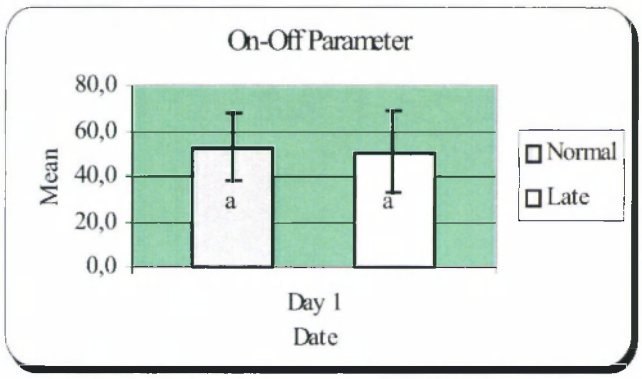

Fig. 29: Any differences in On-Off Parameter. Different lettuces had the same On-Off Parameter value.

About Hue Angle (Fig.30), the colour between different lettuces was different on day 1 and on day 2. However, it was also different between days: it means that the hue angle of lettuces that were harvested in time decreased (more yellow). The same happened with lettuces that were harvested later: the hue angle decreased (more yellow).

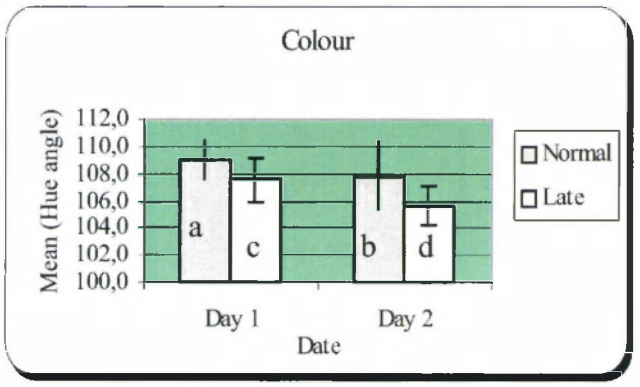

Fig. 30: The Hue angle was different among the different lettuces on both days, and was different for the same lettuce on both days.

The different kinds of lettuces had different weights (Fig.31), but it was not important. Nevertheless, between days both kinds of lettuce did not lose a significantly amount of weight.

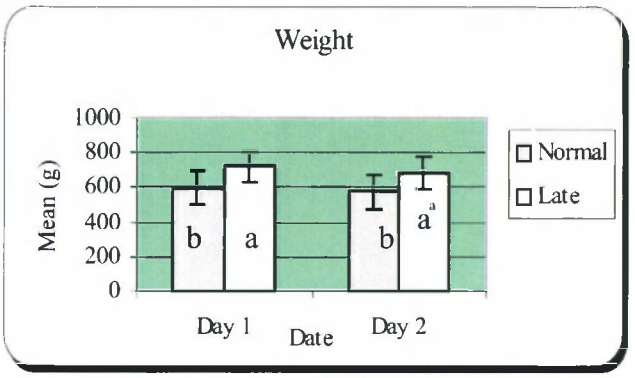

Fig. 31:Both lettuces did not decrease their weight, but they (among them) had different weight on both days.

III. 5.2.2. Discussion

Shelf life prediction based on these photosynthetic parameters was: the lettuces that were harvested in time would have the same shelf life as those lettuces that had been harvested later.

To know if this prediction was correct, the lettuces were observed and cut after 2 days shelf-life (Fig 32, 33 and 34).

Fig. 32: Lettuce that was harvested in time. It was yet green and eatable.

Fig. 33: Lettuce that was harvested later. It was eatable, but less green, with a lower quality.

Fig. 34: A lettuce that was harvested later. It was uneatable.

Again the prediction was wrong. Although they had the same values for both photosynthetic parameters, they did not have the same shelf life. The lettuces that were harvested later had a shorter shelf life.

III. 5.3. Endive

III. 5.3.1.Results

As it was done for the other products, for endives the mean and standard deviation for both Photosynthetic Parameters were also calculated, and also for Hue angle and weight on both days.

A statistic test was also made: ANOVA.

PSI Efficiency was compared between the endive harvested in time and the endive harvested later. There was no difference (Fig.35) between both kinds of endives. So the shelf life prediction is: they will have the same shelf life.

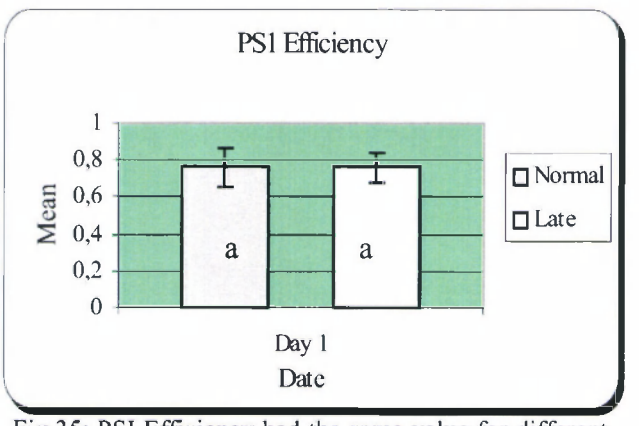

Fig.35: PSI Efficiency had the same value for different endives

For the On-Off Parameter the same happened: there was no difference between the values of the endive harvested in time and the endive harvested later (Fig. 36). So, it could be predicted that they have the same shelf life.

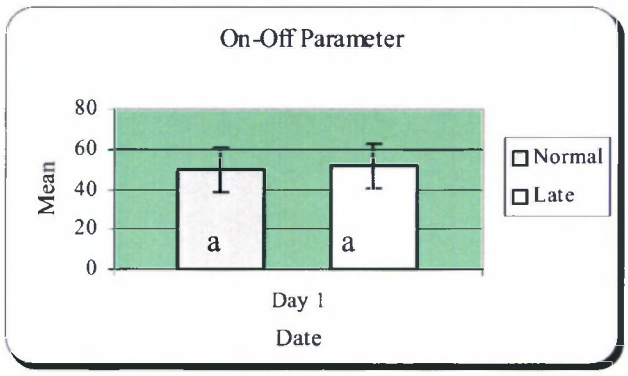

Fig.36: On-Off Parameter was the same value, for endives harvested in time and for endives harvested later.

For each day, there were no statistically significant differences between different endives in colour and weight (Fig. 37 and 38). During 1 day shelf-life, the colour and the weight did not change significantly.

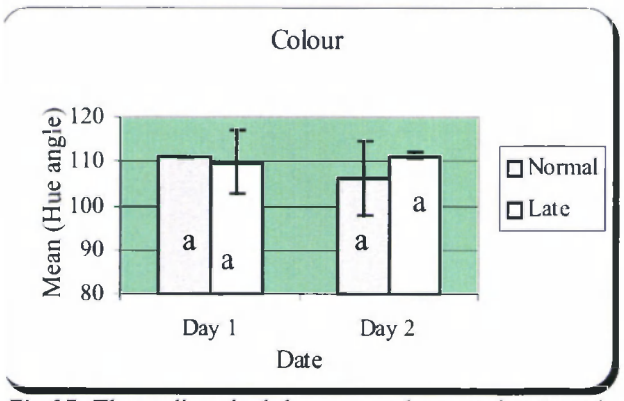

Fig.37: The endives had the same colour on day 1, and they did not lose that colour.

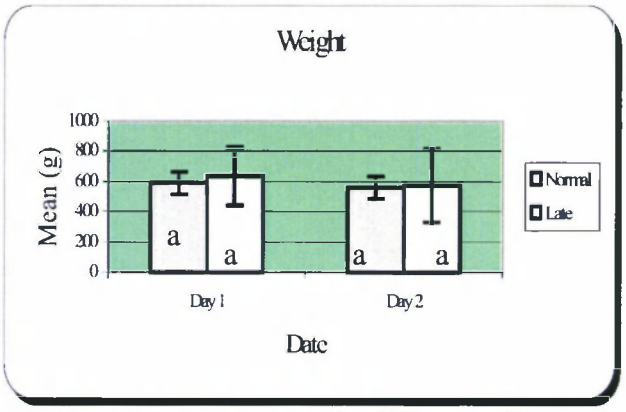

Fig.38: Weight was the same for different endives, and both kinds of endives did not lose the weight.

III. 5.3.2. Discussion

Shelf life prediction was: the endives that were harvested in time would have the same shelf life as those endives that were harvested later.

But when endives were observed and cut, they were not at the same level of quality.

Fig.39: On the top there the endives that were harvested in time, on bottom the endives that were harvested later, these were less green inside.

The endives that were harvested later had bigger leaves and were less green (inside). Similar to the leeks and the lettuces, both photosynthetic parameters were not useful to predict shelf life and it was not possible to relate them with crop quality

IV. CONCLUSION

Unfortunately, it was not possible to relate the two studied Photosynthetic Parameters (On-Off parameter and PSI efficiency) with crop quality. This was shown by the fact that crops with different levels of quality had the same PSI Efficiency and On-Off parameter.

All shelf life predictions based on these two parameters did not work well. Different crops (normal versus late harvest; old versus young product) had equal values for photosynthetic parameters while differences in shelf-life were observed.

So, it is advised that ATO should quit the patent (On-Off Parameter). Besides, the equipment used to obtain these photosynthetic parameters has some disadvantages:

- it causes some damages on the products when they are put in the cuvet;
- when light intensity is higher than 1000 mV (316 μmol / m^2.s) is difficult to get the light intensity stable;
- If the leaf is not flat, it is difficult to apply the desired gas flow;
- The computer programme used to get the flash value, does not give an exact value. The value obtained depends of the person that is doing the interpretation.

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APPENDIX

On-Off Parameter and PSI Efficiency Equipment

How to use and calculate both Photosynthetic Parameters

On-Off Parameter and PSI Efficiency Equipment

On-Off Parameter gives us information about the speed at which $P700^+$ molecules are reduced after the light has been switched off.

- Light On / light Off measurement.

PS1 Efficiency is an efficiency (so the values are between 0 and 1) and tells us about the percentage of P700 that is oxidised when light is caught by P700.

$$PS1\ Efficiency = \frac{Max.P700^+ - realP700^+}{Max.P700^+}$$

- Real $P700^+$ - Light On / light Off measurements;
- Maximum $P700^+$- Far-red and Flash measurements.

To do these measurements, the most important parts of the equipment are:

- Light source - halogene bulb. The light intensity varies between dark and 6000 mV;
- Cuvet - the place where the plant (leaf) is put;
- CO2 recorder - records information about the CO2 that is absorbed by the plant;
- PSI recorder - records information about light-On / light-Off, far-red and flash measurements;

- Nicolet - Gives us On-Off Parameter and the results about flash measurements.

Start of day

The equipment must be switched an hour before the measurements start.

- Gas O2 on (large valve, on the right);
- Gas CO2 on (large valve, on the left);
- Gas N2 on (small valve, on the table);
- Gasmix on (small valve, on the table);
- 2 English plugs on (on the floor);
- Channel 4 = 25 (250 ml/min);
- ADC pump on (hold pump);
- CO2 recorder on : 1 – Power ;
  2 – Chart start;
  3 – Pen Lift (green = CO2, red = Temperature);
  4 – Range 20 – 60 (can vary, depends on the product)
- Close cuvet (without plant);
- Check ADC (250);
- Electricity block on;
- Halogene light on;
- Temperature (* and arrows);
- Fill humidifier;
- Floppy in Nicolet;

Wait until temperature and CO2 constant (± 1 hour)

Light On / light Off, Far-red and Flash measurements preparations

Preparation for measurements:

- Shutter off (NC)- Apply new light intensity: 1 – filters;
  2 – shutter on;
  3 – moving, to choose the
- Shutter off (NC) + White sticker (up) = Light on ;
- Green light / Red light off
- Cable to In;
- PS1 fiber;
- Intensity PS1 flash on low;
- Pens in PS1 recorder;
- Put the plant in the cuvet;

Wait until CO2 constant (± 20 minutes)

Preparation for the light on / light off measurement:

- Nicolet on: 1 – 1 V or 2 V when light intensity is higher then 1000 mV;
  2 – The line should be put in middle or higher, depends of the product;
- PSI recorder : 1 – on the right;
  2 – Speed 5;
  3 – Range 5;
  4 – pens down;
  5 – On.

PSI recorder is on. It is drawing a line.

Light on / light off measurement:

The light is on, PSI recorder is drawing a line (which represents light on). To know the amount of $P700^+$ and the fastness how they are reduced the light must be switched off.

- Light off (white sticker down);
- PS1 recorder off;
- Store in the nicolet.

The PSI recorder has the graph made, Nicolet also has the graph made.

Far-red and Flash measurements preparation.

- Intensity PS1 flash on 1;
- Cable flash;

Wait until CO2 constant (± 20 minutes)

- PS1 recorder on: 1 – on the left;
  2 – Speed 1;
  3 – Range 5;
  4 – Pens down;
  5 – On.
- Nicolet on: 1 – 2 V;
  2 – Line in the middle.

Far-red and Flash measurements

- Far-red on;
- Hendle;
- Flash (after 3 seconds);
- Far-red off;
- PS1 Recorder off;
- Store in the nicolet.

End of day

- Flow at 250 ml/min;
- 8% Filter;
- Nicolet on 1 V;
- PS1 flash on low;
- Bulb off;
- Take both paper;
- Electricity block off;
- CO2 recorder : 1 – Lift pens;
  2 – Power off.
- ADC (pumps) off;
- English plugs out;
- Gasses closed;
- Pens from PSI recorder out;
- Cuvet open;
- Take off floppy;

YOKOGAWA - CO2 recorder

Normal: range 20-60, flow 250 ml/min.

- **If peak is to high**: 1 - FLOW 350 ml/min;
  2 - values recorder paper*350/250(1.4)

- **If peak still to high**: 1 - Range 0-60;
  2 - values recorder paper*60/40*350/250(2.1)

- **If peak still to high**: 1 - Flow 450 ml/min;
  2 - values recorder paper* 60/40*450/250(2.7)

Point 2 is the way to convert the values obtain in CO2 recorder, when the range is changed in those ways.

Change range:

- Shift + Range;
- With ∇ to 'span L ...MV';
- F3 (to delete);
- Give new value for instance: 20.00MV;
- With ∇ to 'span R...MV';
- F3 (to delete);
- Give new value for instance: 60.00MV;
- Enter;
- Enter;

PSI recorder

Normal range: 5.

If graph is too long: 1 - To change range to 10;
2 – PSI recorder values * 2.

If graph still too long: 1 – To change range to 20;
2 – PSI recorder values *4.

Point 2 is the way to convert the values obtain in CO_2 recorder, when the range is changed in those ways.

Calculations

- **On-Off Parameter**

In a computer programme – **Matlab** to use the information stored in the floppy in a computer programme. The values are immediately obtained.

- **PSI Efficiency**

$$PS1\ Efficiency = \frac{Max.P700^{+} - realP700^{+}}{Max.P700^{+}}$$

Max.P700^{+} is obtained: Far-red + Flash.

- Far-red = The difference measured between the top and bottom of the Far-red measurement graph in the PSI recorder;
- Flash = In Qbasic (computer programme) is obtained a value let call it flash. The flash must be multiplied for (200/5).

RealP700^{+} is obtained measuring the different between the top and the bottom of the light on / light Off measurement graph in PSI recorder.

Tables

- Main experiment 1

	PSI Efficiency		*On-Off Parameter*		*Hue angle*		*Weight (g)*	
Leeks	*19-4-02*	*23-4-02*	*19-4-02*	*23-4-02*	*19-4-02*	*23-4-02*	*19-4-02*	*23-4-02*
1	0.80	0.80	76.14	13.96	138.86	91.90	414.5	391.2
2	0.85	0.59	89.72	46.10	138.05	129.23	357	328.6
3	0.58	0.38	36.33	13.83	137.34	88.90	352.6	324.1
4	0.74	0.22	107.76	31.03	137.13	109.01	263.2	234.8
5	0.71	0.36	43.82	22.71	145.05	107.11	331.3	296.5
6	0.59	0.14	62.48	15.63	150.80	103.06	317.5	292.8
7	0.69	0.28	52.22	15.04	124.29	92.11	438.6	412.7
8	0.56	0.48	38.95	49.80	155.67	131.51	405.8	391.7
9	0.67	0.41	78.17	47.22	157.00	132.48	374.3	350.1
10	0.70	0.57	59.28	46.54	169.43	146.40	306.5	284.3
11	0.47	0.44	32.08	0.11	122.76	89.72	270.9	252.9
12	0.81	0.33	76.71	40.15	143.87	126.58	301.5	286.6
13	0.77	0.35	81.76	45.83	131.00	133.07	374.2	354.1
14	0.74	0.92	77.27	34.66	129.52	122.88	314.4	295.6
15	0.54	0.40	61.80	51.79	126.92	115.50	385.7	358.9
16	0.46	0.38	39.73	31.37	133.24	117.32	418.6	400.2
17	0.64	0.46	57.74	15.63	135.01	134.48	410.4	385.5
18	0.62	0.48	42.64	45.05	144.57	101.36	395.6	378.5
19	0.66	0.43	71.08	43.09	124.13	124.96	274	260.4
20	0.75	0.31	56.91	20.01	133.34	108.89	290.9	264.7
21	0.40	0.28	29.52	22.16	151.47	111.08	463.7	444.4
22	0.72	0.69	50.72	19.58	185.62	101.25	373.3	353.5
23	0.67	0.53	42.10	31.29	135.46	114.61	399.8	379.5
24	0.49	0.40	47.22	49.22	127.93	82.41	345.8	320.4
25	0.25	0.92	27.13	11.66	129.93	112.58	308.6	287.3
Average	0.64 (a) *	0.46 (b)	57.57 (a)	30.54 (b)	140.33 (a)	113.13 (b)	355,58 (a)	333,17 (a)
Standard Deviation	0.14	0.20	20.62	15.20	14.93	16.83	55,88	56,32

*Means for each parameter within each row followed by different letters are significantly different ($p < 0,05$).

- Main experiment 2

Leek

PS1 Efficiency					
New leeks	2-7-2002	5-7-2002	Old leeks	2-7-2002	5-7-2002
1	0,70	0,39	2	0,65	0,60
3	0,70	0,51	4	0,73	0,51
5	0,69	0,48	6	0,60	0,48
7	0,57	0,39	8	0,80	0,61
9	0,45	0,30	10	0,73	0,57
11	0,64	0,41	12	0,69	0,60
13	0,71	0,44	14	0,57	0,46
15	0,56	0,26	16	0,63	0,44
17	0,60	0,30	18	0,58	0,52
19	0,72	0,44	20	0,52	0,52
21	0,76	0,72	22	0,66	0,42
23	0,74	0,41	24	0,54	0,42
25	0,63	0,71	26	0,69	0,43
Average	0,65 (a)*	0,44 (b)	Average	0,64 (a)	0,51 (b)
Standard Deviation	0,09	0,14	Standard Deviation	0,08	0,07

On-Off Parameter					
New leeks	2-7-2002	5-7-2002	Old leeks	2-7-2002	5-7-2002
1	65,50	-	2	74,00	-
3	39,30	-	4	106,40	-
5	44,30	-	6	74,50	-
7	50,60	-	8	117,10	-
9	29,00	-	10	96,90	-
11	44,40	-	12	73,90	-
13	48,70	-	14	52,40	-
15	42,60	-	16	86,10	-
17	51,30	-	18	69,00	-
19	61,40	-	20	73,80	-
21	61,20	-	22	73,00	-
23	70,00	-	24	66,90	-
25	60,00	-	26	78,60	-
Average	51,41 (b)*	-	Average	80,20 (a)	-
Standard Deviation	11,72	-	Standard Deviation	17,42	-

*Means within each row followed by different letters are significantly different ($p < 0,05$).

Hue angle					
New leek	1-7-2002	5-7-2002	old leek	1-7-2002	5-7-2002
1	125,93	125,50	2	112,04	115,99
3	122,76	117,00	4	126,49	125,40
5	123,43	121,95	6	119,89	118,41
7	121,69	110,36	8	141,82	127,04
9	118,18	103,85	10	123,11	114,70
11	130,56	128,02	12	134,94	123,61
13	131,64	129,16	14	140,86	119,79
15	129,88	111,39	16	128,28	120,56
17	118,98	115,65	18	145,40	112,65
19	129,63	116,32	20	121,19	112,32
21	123,85	119,47	22	136,49	105,95
23	136,73	121,05	24	128,48	110,59
25	130,09	127,06	26	136,75	116,62
Average	125,92 (a)*	118,98 (b)	Average	130,44 (a)	117,48(b)
Standard Deviation	8,38	7,58	Standard Deviation	15,08	6,11

Weight (g)					
New leeks	2-7-2002	5-7-2002	Old leeks	2-7-2002	5-7-2002
1	312,60	291,40	1	161,80	142,70
3	176,90	162,20	3	128,10	117,00
5	312,80	288,20	5	165,90	149,20
7	138,10	126,70	7	131,60	123,30
9	202,60	183,80	9	183,30	170,10
11	268,00	241,50	11	222,00	203,50
13	203,60	186,40	13	123,40	108,40
15	203,10	188,60	15	138,10	116,70
17	179,60	166,20	17	86,80	73,90
19	278,90	256,50	19	152,60	137,10
21	158,60	144,80	21	62,60	53,60
23	208,80	188,20	23	158,60	143,60
25	218,10	201,40	25	184,30	170,50
Average	220,13 (a)*	201,99 (a)	Average	148,08 (b)	131,51 (b)
Standard Deviation	56,21	52,21	Standard Deviation	41,88	40,03

*Means within each row followed by different letters are significantly different ($p < 0,05$).

Lettuce

PS1 Efficiency			
Normal Harvest Lettuce	3-7-2002	Later Harvest Lettuce	3-7-2002
1	0,74	2	0,58
3	0,64	4	0,75
5	0,70	6	0,74
7	0,77	8	0,75
9	0,82	10	0,90
11	0,68	12	0,66
13	0,69	14	0,67
15	0,68	16	0,57
17	0,59	18	0,70
19	0,71	20	0,59
21	-	22	-
Average	0,70 (a)*	Average	0,69 (a)
Standard Deviation	0,06	Standard Deviation	0,10

On-Off			
Normal Harvest Lettuce	3-7-2002	Later Harvest Lettuce	3-7-2002
1	40,80	2	39,60
3	57,90	4	-
5	48,20	6	52,50
7	55,20	8	-
9	91,60	10	87,40
11	54,70	12	53,20
13	50,20	14	26,80
15	37,20	16	45,10
17	41,50	18	39,20
19	49,60	20	60,90
21	-	22	-
Average	52,69 (a)*	Average	50,59 (a)
Standard Deviation	15,25	Standard Deviation	18,20

*Means within each row followed by different letters are significantly different ($p < 0,05$).

Hue angle					
Normal			Later		
Harvest Lettuce	3-7-2002	5-5-2002	Harvest Lettuce	3-7-2002	5-5-2002
1	109,68	107,48	2	108,76	106,30
3	110,48	102,20	4	106,60	102,29
5	107,92	107,05	6	109,15	107,09
7	106,52	106,67	8	104,59	105,15
9	110,21	110,48	10	109,93	105,77
11	110,65	110,07	12	106,70	105,85
13	107,51	107,77	14	106,67	105,61
15	107,99	107,65	16	106,91	105,07
17	110,21	109,18	18	107,52	105,58
19	109,24	109,85	20	108,96	107,70
21	-	-	22	-	-
Average	109,04 (a)*	107,84 (b)	Average	107,58 ©	105,64 (d)
Standard Deviation	1,45	2,40	Standard Deviation	1,61	1,44

Weight (g)					
Normal			Later		
Harvest Lettuce	3-7-2002	5-7-2002	Harvest Lettuce	3-7-2002	5-7-2002
1	718,00	696,00	2	748,40	721,20
3	378,70	364,60	4	680,00	651,20
5	641,20	612,00	6	833,50	809,00
7	602,30	560,60	8	708,10	562,30
9	620,10	591,40	10	868,90	840,60
11	606,70	581,30	12	623,00	601,00
13	587,30	567,60	14	647,50	624,80
15	491,20	474,80	16	769,90	745,90
17	592,30	574,80	18	698,00	674,90
19	744,50	720,00	20	627,50	610,30
21	-	-	22	-	-
Average	598,23*	574,31	Average	720,48	684,12
Standard Deviation	104,33	101,06	Standard Deviation	84,19	92,59

*Means within each row followed by different letters are significantly different ($p < 0,05$).

Endive

PS1Efficiency			
Normal Harvest Endive	4-7-2002	Later Harvest Endive	4-7-2002
1	0,71	2	0,72
3	0,85	4	0,74
5	0,79	6	0,60
7	0,76	8	0,68
9	0,83	10	0,81
11	0,76	12	0,81
13	0,55	14	0,79
15	0,66	16	0,88
17	0,87	18	0,78
19	0,84	20	0,81
Average*	0,76 (a)*	Average	0,76 (a)
Standard deviation	0,10	Standard Deviation	0,08

On-Off			
Normal Harvest Endive	4-7-2002	Later Harvest Endive	4-7-2002
1	35,90	2	46,40
3	50,70	4	46,50
5	46,00	6	47,20
7	55,90	8	39,00
9	60,10	10	44,30
11	52,20	12	48,70
13	34,00	14	46,30
15	37,90	16	61,30
17	65,90	18	58,40
19	56,70	20	80,10
Average	49,53 8 (a)*	Average	51,82 (a)
Standard deviation	10,83	Standard Deviation	11,89

*Means within each row followed by different letters are significantly different ($p < 0,05$)

Hue angle					
Normal Harvest Endive	4-7-2002	5-7-2002	Later Harvest Endive	4-7-2002	5-7-2002
1	110,40	110,53	2	114,06	111,60
3	110,96	111,60	4	111,52	112,19
5	110,59	87,08	6	110,54	109,82
7	110,67	94,32	8	113,05	111,49
9	111,08	110,34	10	114,22	110,98
11	110,93	110,79	12	111,64	111,75
13	110,70	110,14	14	112,37	111,35
15	110,85	107,84	16	111,06	110,93
17	111,90	111,05	18	89,47	110,88
19	110,63	109,30	20	109,93	111,57
Average	110,87 (a)*	106,30 (a)	Average	109,79 (a)	111,26 (a)
Standard deviation	0,41	8,46	Standard Deviation	7,28	0,65

Weight (g)					
Normal Harvest Endive	4-7-02	5-7-02	Later Harvest Endive	4-7-02	5-7-02
1	586.8	545.2	2	572.6	552.2
3	507	487.8	4	769.3	744.7
5	703.6	683.5	6	808.1	775.8
7	639	611.1	8	425.5	432.3
9	627.4	602.4	10	514.2	432.3
11	641.7	599.4	12	469.1	470.4
13	461.3	436	14	995.8	956.8
15	527.6	495.6	16	803.1	769.8
17	616.4	586.3	18	484	456.5
19	568.7	540.8	20	511.1	111.6
Average	587.95 (a)*	558.81 (a)	Average	635.28 (a)	570.24 (a)
Standard deviation	72.91	72.43	Standard Deviation	192.78	243.49

*Means within each row followed by different letters are significantly different ($p < 0,05$).