J. Elem. s. 269–278 DOI: 10.5601/jelem.2012.17.2.09

NON-INVASIVE QUALITY DETERMINATION OF SPINACH UNDER SIMULATED SALE CONDITIONS AND PREDICTION OF POSSIBLE CHANGES

Anna Jadwiga Keutgen, Jarosław Pobereżny, Elżbieta Wszelaczyńska

Department of Food Technology
University of Technology and Life Sciences in Bydgoszcz

Abstract

Chlorophyll fluorescence, especially the optimum quantum yield Fv/Fm, is used to quantify the physiological state of plants, because stress, infections or wilting may lower the optimum quantum yield. The characteristics of chlorophyll fluorescence have attracted our attention for its use to determine the quality of spinach, because the measurement of Fv/Fm during storage can be performed quickly and non-destructively, delivering information about spinach quality and its sale suitability.

Applicability of modern techniques for quick and non-invasive *in vivo* determination of such parameters as quality and freshness of spinach under simulated sale conditions was tested in our study. In this experiment, application of chlorophyll fluorescence for quantification of quality as well as physical and physiological changes which occur in fresh products was verified. The experiment was conducted on spinach, a product characterized by rapid appearance of postharvest losses. Spinach was harvested as leafy vegetable for direct consumption and stored for 5 days (16 h in a cooling room at 2-4°C, 96-98% relative humidity, followed by 8 h at room temperature: *ca* 22ºC, 65% relative humidity). Variants were represented by different cultivation conditions (fertilizer, leaf application of Previcur, $CaCl_2$ and Cerone). The investigations revealed that fresh mass losses in the postharvest phase could be described by changes of Fv/Fm spinach leaves adapted to darkness by a linear relationship of parameters (r=0.77). The disadvantage of chlorophyll fluorescence measurement was the less precise determination of the critical loss of fresh mass of 3%. Instead, losses of 4 to 5% resulted in a the decrease of optimum quantum yield below the critical value of 0.80, which indicates the occurrence of postharvest stress. Leaf application

dr hab. eng. Anna J. Keutgen, Department of Food Technology, University of Technology and Life Sciences in Bydgoszcz, Kordeckiego 20A, 85-225 Bydgoszcz, akeutgen@utp.edu.pl

of pesticides negatively influenced Fv/Fm. This relationship may be used to detect the application of plant protection products shortly before harvest, before the grace period is over. Hence, the determination of freshness using chlorophyll fluorescence offers, to a limited extent, protection of consumers.

Further investigations showed that optimum quantum yield was linearly and positively correlated with the calcium content in spinach leaves. This relationship may indicate worse cell membrane integrity and reduced cell wall stability postharvest. Additionally, the decrease of Fv/Fm during storage may indicate the occurrence of cold stress as well as altering processes (senescence) and loss of quality of spinach, independent of its cultivation method. In these investigations, the relationship between chlorophyll fluorescence and color changes of leaves, content of chlorophyll, total nitrogen and water soluble carbohydrates, as well as respiration intensity and ethylene emission could not be found.

Key words: chlorophyll fluorescence, quality, spinach, storage, fresh mass loss, calcium, starch.

NIEINWAZYJNE OZNACZANIE JAKOŚCI SZPINAKU PODCZAS SYMULOWANYCH WARUNKÓW SPRZEDAŻY ORAZ PRZEWIDYWANIE MOŻLIWYCH ZMIAN

Abstrakt

Fluorescencja chlorofilu, w szczególności optymalne wykorzystanie kwantów Fv/Fm, stosowana jest do oznaczania stanu fizjologicznego roślin, gdzie występowanie stresu, chorób czy procesów więdniecia może spowodować zmniejszenie optymalnego wykorzystania kwantów. W praktyce fluorescencja chlorofilu ma specjalne zastosowanie, ponieważ pomiary podczas przechowywania mogą zostać przeprowadzone szybko i bezdestrukcyjnie, natychmiast informując o stanie jakości szpinaku oraz jego przydatności do sprzedaży.

Możliwość zastosowania nowoczesnych technik w celu szybkiego i nieinwazyjnego oznaczenia *in vivo* takich parametrów, jak jakość i świeżość szpinaku podczas symulowanych warunków sprzedaży, stanowiła tematykę badań. W badaniach zweryfikowano zastosowanie fluorescencji chlorofilu do określenia zmian jakościowych, fizycznych i fizjologicznych, zachodzących w świeżym produkcie odznaczającym się szybkim występowaniem strat pozbiorczych – szpinaku będącym warzywem liściowym przeznaczonym do bezpośredniego spożycia, składowanym 5 dni (16 h w chłodni: 4ºC, 96% wilg. wzgl.; 8 h w temperaturze pokojowej: 22-23ºC, 70% wilg. wzgl.), z uwzględnieniem różnych warunków uprawy (nawożenie, stosowanie dolistne Previcuru, $CaCl_2$ oraz Cerone). Badania wykazały, że straty świeżej masy w fazie pozbiorczej mogły zostać opisane za pomocą zmian Fv/Fm zmierzonych w przystosowanych do ciemności liściach szpinaku ze względu na liniową zależność parametrów (r^2=0,77). Negatywny aspekt pomiaru fluorescencji chlorofilu stanowiła mała dokładność określenia krytycznej dla jakości handlowej szpinaku granicy strat świeżej masy wielkości 3%. Dopiero straty od 4 do 5% spowodowały zmniejszenie optymalnego wykorzystania kwantów poniżej krytycznej granicy 0,80, wskazującej na wystąpienie stresu pozbiorczego. Ponadto dolistne zastosowanie pestycydów negatywnie wpłynęło na pomiar Fv/Fm, co jednak może znaleźć zastosowanie w wykrywaniu stosowania środków ochrony roślin na krótko przed zbiorem, jeszcze przed upływem okresu karencji. Dlatego też oznaczenie stopnia świeżości za pomocą fluorescencji chlorofilu umożliwia również, w pewnym stopniu, ochronę konsumenta. Dalsze badania wykazały, że optymalne wykorzystanie kwantów Fv/Fm skorelowane było liniowo pozytywnie z zawartością wapnia w liściach szpinaku. Zależność ta wskazywałaby na zmniejszenie zarówno integracji błon komórkowych, jak i zmniejszenie stabilizacji ścian komórkowych podczas okresu pozbiorczego. Ponadto zmniejszenie wartości Fv/Fm podczas przechowywania może wskazywać na wystąpienie stresu

chłodu i procesów starzenia (senescencji) oraz utraty jakości badanego szpinaku, niezależnie od sposobu jego uprawy. W badaniach nie stwierdzono zależności między fluorescencją chlorofilu a zmianami barwy liści szpinaku, zawartością chlorofilu, azotu ogółem i węglowodanami rozpuszczalnymi w wodzie oraz intensywnością oddychania i emisją etylenu.

Słowa kluczowe: fluorescencja chlorofilu, jakość, szpinak, przechowywanie, straty świeżej masy, wapń, skrobia.

INTRODUCTION

Vegetables, especially their leaves, are highly perishable commodities. Harvested plants or plant organs must still be regarded as living tissue that is subjected to continuous changes with respect to its metabolism. They require energy for maintenance of cell organisation, membrane permeability, metabolism and metabolite transport within the tissue. Instead of photosynthetic assimilates, the required energy in harvested plants is supplied by oxidative breakdown of certain organic substances stored in the tissue (KEUTGEN 2000). From the consumer's point of view, most of the postharvest changes are undesirable because they result in plant and tissue senescence as well as tissue breakdown. They cause freshly harvested vegetables to deteriorate, shrivel, loose the green colour and to turn yellow due to chlorophyll degradation and lead to distinct shifting of sensory quality, especially visual deterioration and loss of palatability. In consequence, quality and shelf-life sustainability of leafy vegetables depends strongly on their vitality (FERRANTE, MAGGIOREA 2007). Recently, new kinds of *in vivo* analyses have provided detailed information on intrinsic value and storage as well as processing effects on fruits and vegetable (RUIZ-ALTISENTA et al. 2010, PAPAGEORGIOUA, GOVINDJEE 2011). These include the option of on-line control of quality characteristics of vegetable products during cultivation, storage and manufacture. Quality control as well as quality and shelf-life prediction are of prime importance to guarantee an optimal product. In practice, quality analyses should be fast, simple, robust, reproducible and reliable. Ideally, they should simultaneously describe chemical and physical parameters and, of course, should be non-destructive.

Recently, consumers focus not only on a good-looking product, but also ask for an excellent quality with regard to the nutritive value. Quality evaluation is a tool to ensure that new and existing products meet the consumers' requirements. New kinds of analyses should provide detailed information about ingredients and the effects of storage and processing. This includes the option of using on-line instruments to control the quality characteristics of vegetable products during cultivation, storage and manufacture. Quality control as well as quality and shelf-life prediction are of prime importance to guarantee an optimal product quality. In practice, quality analyses must be fast, simple, robust, reproducible and reliable. Ideally, they should simul-

taneously describe chemical and physical parameters and, of course, should be non-destructive. Chlorophyll fluorescence is a technique with a great potential to fulfill these requirements. It enables non-destructive measurements to determine a plants' physiological state, its vitality and its 'stress history'. It describes not only visible damages but also an eventual early stage of deterioration a few days before visible damage symptoms occur. In addition, chlorophyll fluorescence is able to identify effects of potentially toxic substances such as pesticides that have been applied to a plant a few days before harvest. To summarize, the chlorophyll fluorescence technique can be used to record the effect of biotic and abiotic stress factors (KEUTGEN 2000, RUIZ-ALTISENTA et al. 2010, PAPAGEORGIOUA, GOVINDJEE 2011), to predict changes of nutritive characters, the maturity state of the harvested or stored plant organ and thus shelf-life (DEELL et al. 1999, KEUTGEN 2000). Chlorophyll fluorescence potentially allows studies that range from maturity assessment to the prediction of quality attributes during the postharvest period and effects of postharvest stress on product quality.

Contemporary research into postharvest physiology is focusing on the development of instrumental methods for non-destructive quality assessment. This kind of research is empirical, correlating analytical parameters with quality properties for single products under variable conditions. This approach is quite helpful in developing new methods to monitor and control production and storage processes. However, in many cases a better understanding of the plant's physiological and metabolic basis for quality degradation is needed to identify relationships that are determined by vegetable quality but not by pre- or postharvest environmental conditions. It is the aim of the proposed research project to develop objective quality criteria for spinach and broccoli using non-destructive methods for vegetable quality assessment.

MATERIAL AND METHODS

Spinach plants (*Spinacia oleracea* L. cv. San Felix F1) were grown on experimental fields at Marhof Research Station near Bonn at two sowing dates: 2nd and 16th April of two consecutive years. Two different forms of nitrogen were applied as fertiliser (220 kg N ha^{-1}) with 27% of nitrogen, where ammonium nitrate (KAS) consisted of 13.5% nitrate and 13.5% ammonium, whereas basammon-nitrogen (BAS) consisted of 6.8% nitrate, 18.2% ammonium and 2% nitrification blocker Dicyandiamid (DCD), respectively. Plants were cultivated on brown soil (70-78 soil points), with a thickness of 0.75 to 1.20 m, situated on sand and gravel, of middle water permeability and of middle to high water storage capacity, with the ground water level at 13 m depth. The soil (0-30 cm) was characterized by an pH_{KCl} – value of 6.6, P_2O_5 0.43 g kg^{-1} soil, K_2O 0.24 g kg^{-1} soil, and MgO 18 g kg^{-1} soil. On

average the content of nitrate (V) and ammonia before fertilization were 39.36±12.87 and 17.4 9.0 kg N ha^{-1}, respectively. No additional fertilization with phosphorus and potassium were applied. During the growing period from April to June the mean temperatures of the air was 15.7°C and of the soil in the depth of 10 cm of 13.8°C (depth of mean root mass of spinach). The mean precipitation in the vegetation time was on average 221 mm. Depending on spinach requirements, automatic watering from a computer controlled pouring car at the tensiometer value of -120 mb was applied (150 ml of water per plant). Spinach was harvested twice from each experimental field (22nd and 27th May, 4th and 9th June, respectively) and kept under simulated sale conditions for 5 days. Prior to the experiments, harvested spinach was washed in cool tap water at about 12°C and drained using a hand centrifuge. Clean spinach was placed in Euro-Pool-System-Boxes D. Over night (16 h) plants were stored in darkness in closed 400 l containers at 2-4°C and 96-98% relative humidity. To establish high humidity in the containers, air was forced with a pump through distilled water. During the day (8 h), spinach was kept in diffuse daylight at room temperature of about 22°C and 65% relative humidity similar to conditions on the fresh market. Beside the kind of fertilisers, other experimental factors were different leaf applications during the growth period: Previcur 0.2% (active substance: propamocarb-HCl 66.5%) against *Peronospora* sp., $CaCl_2$ water solution (15, 30, 45, 60 mmol dm^{-3}) for quality improvement (Perucka et al. 2007), and Cerone as a precursor of ethylene (50, 100, 150, 200 mmol dm^{-3}) in the amount of 2 dm^{-3} m^{-2} each. Samples of 80 spinach plants were used for each combination (4 replicates with 20 plants each). Analyses were carried out during storage to investigate quality changes continuously. Samples for chlorophyll fluorescence measurement and for chemical analyses were taken from the same plant (rosette). Water loss was determined for all plants within a single Euro-Pool-System-Box.

Chlorophyll fluorescence measurements were conducted in a photosynthesis laboratory at the temperature of 25°C and atmospheric CO_2 between 400-600 ppm, using a portable pulse-amplitude-modulation Fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). The plant tissues were dark-adapted for 30 min prior to the analyses. Fo (minimal fluorescence), Fm (maximal fluorescence), Fv (= Fm-Fo, variable fluorescence) and optimum quantum yield (= Fv/Fm) were determined three times on each representative of fully expanded and old leaves, with at least four replicates per treatment. For setting of Fo, the leaf was illuminated with low modulated light (650 nm, <0.1 µmol m^{-2} s^{-1}) and then for Fm after a light pulse (665 nm, 3200 µmol m^{-2} s^{-1}) (Keutgen 2000).

To detect colour changes and discoloration, the colour attributes were determined daily, recorded in L*, a*, b* CIE co-ordinates of the spinach leaves using a Chroma-Meter II Reflectance, Minolta, Japan. On each leaf blade, three measurements were taken between the main veins of a leaf.

The leaf samples for intrinsic compounds were freeze-dried and ground for chlorophyll, total N and carbohydrate analyses. Chlorophyll content (Chl a, Chl b and total Chl) was determined according to WELLBURN (1994) from methanol extracts and measured at 649 nm and 665 nm using a spectrophotometer Lambda 5/15 (Perkin-Elmer). Residues of the methanol extractions were used for starch analyses. Starch was enzymatically hydrolysed to glucose, which was detected by HPLC (Knauer, Berlin, Germany). Starch content was calculated according to LOPEZ-HERNANDEZ et al. (1994). Soluble carbohydrate content was determined from water extracts using HPLC as described by KEUTGEN (2000). Total N was determined by a modified Kjeldahl method through reduction of the organic and inorganic N into ammonium by acid hydrolysis (CHEN et al. 1997). Content of total N was measured colorimetrically in a SFAS - 5100 Skalar Auto-Analyser (Skalar GmbH, Erkelenz, Germany).

The experimental design was a completely randomised one. The data were analysed using correlation and multiple regression procedures of the statistical program SPSS for Windows, release 9.01. standard version, Copyright SPSS Inc., 1989-1999. Significances of differences were defined at P=0.05.

RESULTS AND DISCUSSION

The application of chlorophyll fluorescence for the estimation of concentration of intrinsic parameters in vegetables is a less investigated issue. Investigations on different plants indicate a correlation between chlorophyll fluorescence and content of nitrogen, ascorbic acid, carotenoids or soluble sugars and starch (PAPAGEORGIOUA, GOVINDJEE 2011). In spinach, quality changes during storage could at least partially be detected using chlorophyll fluorescence of dark-adapted leaves. During the storage under simulated sale conditions, a decrease of the optimum quantum yield Fv/Fm (Figure 1) was detected. On the third day of the storage, a drop of Fv/Fm below the value of 0.80 was observed. For comparison, values between 0.82 and 0.80 are typical of healthy, fresh plants. A decrease of Fv/Fm may also indicate the occurrence of cold stress during the storage time and/or the loss of membrane integrity due to senescence processes. The optimum quantum yield Fv/Fm was identified as a meaningful parameter, because the relationship between Fv/Fm and fresh mass losses was significantly linear (Figure 2; r=0.77). The fresh mass loss represents an important, if not the main parameter for the quantification of spinach freshness in practice. By definition, a fresh mass loss of 3% during the storage represents a critical value. With higher losses, spinach is regarded as non-marketable. In the present experiment, wilting symptoms and deterioration of spinach quality did not

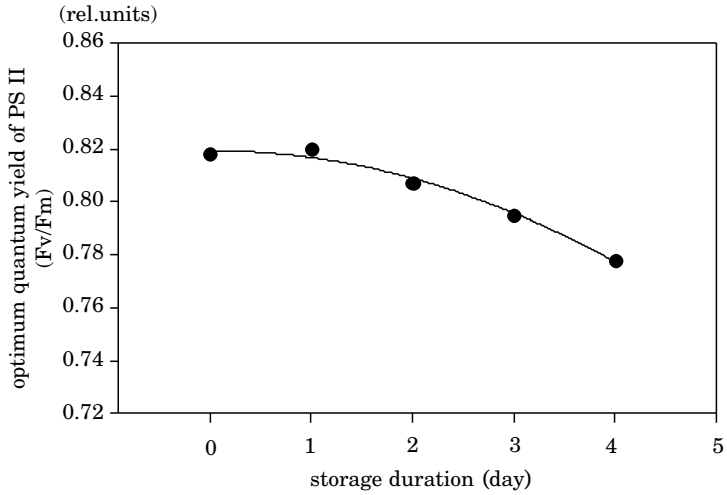

Fig. 1. Changes of optimum quantum yield of PS II (Fv/Fm) in spinach under simulated sale conditions (mean values of 4 experiments)

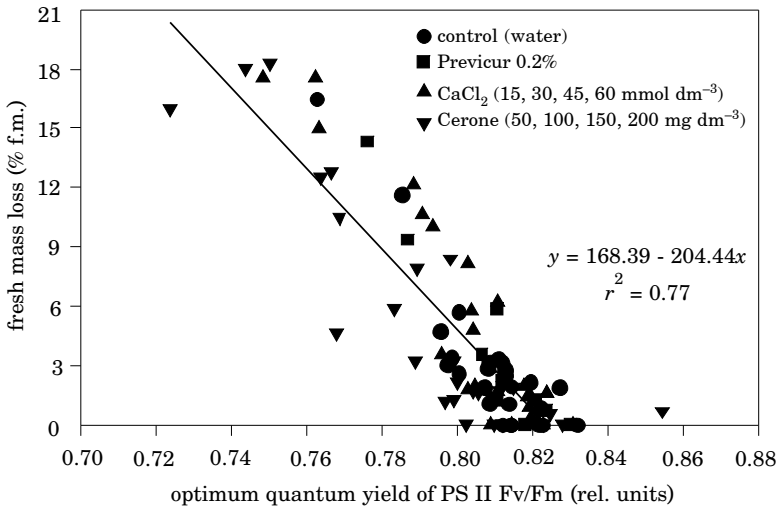

Fig. 2. Linear relationship between the optimum quantum yield of PS II (Fv/Fm) and fresh mass losses in spinach leaves during storage under simulated sale conditions (values are means of 4 experimental replications)

become evident before the third day, when the fresh weight loss reached 5-6%. During the storage of spinach under simulated sale conditions (16 h cool storage/8 h sale keeping), a loss of 4-5%, which is close to the critical value of 3%, corresponded with values for the optimum quantum yield at or below 0.80.

The correlation between optimum quantum yield of PS II and calcium content was positive and high as well as independent on used cultivation technique (Figure 3). A low content of calcium coincided with a low value of Fv/Fm. Spinach plants with the calcium content below 9 g kg^{-1} of dry matter were already non-marketable during the storage under simulated sale conditions. This relation may be explained by the significance of calcium in cell walls and cell membranes, where it supports the anti-senescence reactions. Ca^{2+} is important for the selective permeability and membrane integrity (PALTA 1996). Furthermore, Ca^{2+} stabilizes the cell membranes by linking the phosphate- and carboxylic groups of phospholipids present on the membrane surface. The fact that the lower optimum quantum yield coincides with a low content of calcium may indicate an impairment of membrane integrity.

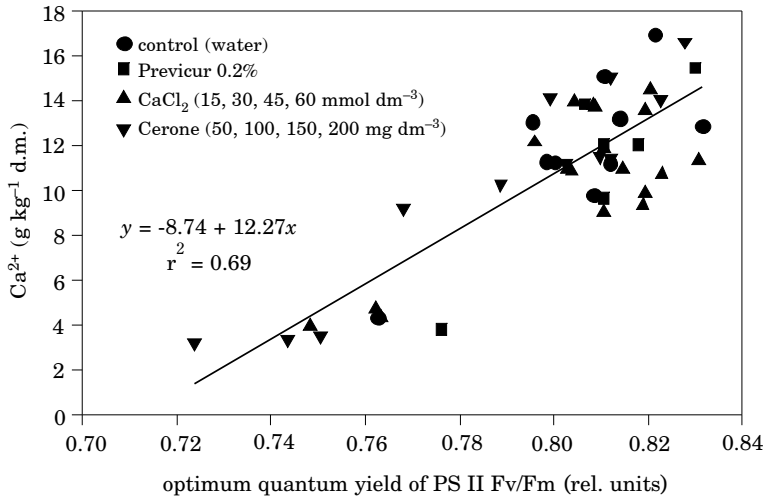

Fig. 3. Correlation between optimum quantum yield of PS II (Fv/Fm) and content of calcium in spinach leaves stored under simulated sale conditions (mean values of 4 experiments)

Considering all measurements of the investigated variants, the relationship between Fv/Fm and starch content in leaves was best explained by a non-linear function (Figure 4). In the case of a starch content up to 10 g kg^{-1} of dry matter, the relationship showed linear character. Based on these results it can be concluded that spinach leaves with a starch content higher than 5 g kg^{-1} of dry matter will exhibit good shelf-life behavior. However, the content of starch in spinach is quite low compared to the other carbohydrates. Nevertheless, this parameter seems to be suitable for the prediction of shelf-life.

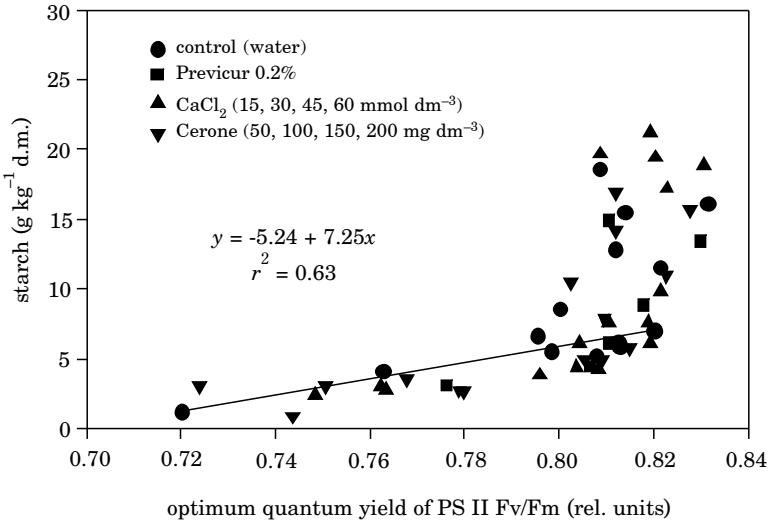

Fig. 4. Linear relationship between optimum quantum yield of PS II (Fv/Fm) and starch content in spinach leaves stored under simulated sale conditions (values are means of 4 experimental replications)

The investigations did not reveal any kind of relationship between chlorophyll fluorescence parameters and respiration intensity, ethylene emission, changes of leaf colour and intrinsic parameters such as total nitrogen, soluble carbohydrate and chlorophyll content. Indeed, chlorophyll fluorescence depends on the chlorophyll content. However, the optimum quantum yield is not directly correlated with the latter. Primarily, a high chlorophyll loss causes a decrease of Fv/Fm (KEUTGEN 2000). This can at least partly explain why no correlation between the chlorophyll content and Fv/Fm was observed in the presented investigations. In the present work, chlorophyll losses during storage were quite low.

CONCLUSIONS

The investigations showed that the quality of spinach during the storage under simulated sale conditions can be evaluated quickly and easily by chlorophyll fluorescence. In commercial practice, freshness is quantified by the water loss during storage. At a fresh mass losses of 3%, spinach is classified as non-marketable. The chlorophyll fluorescence, especially the optimum quantum yield Fv/Fm, is a good tool for the determination of spinach quality state independently from cultivation techniques. Fv/Fm < 0.80 corresponded to a loss of fresh mass of 4 to 5% and more under simulated

sale conditions. The Fv/Fm value can be lowered by cold stress, pesticides or infections, which can be used for the protection of interests of consumers and retailers. However, it is important to mention that successful application of chlorophyll fluorescence as an indicator of quality requires very good and broad knowledge of postharvest physiology, especially of the quality parameter changes combined with the maturity and senescence of the investigated plant.

REFERENCES

CHEN K., HU G., LENZ F. 1997. *Effects of CO_2 concentration on strawberry.* V. *Macronutrient uptake and utilization.* Angew. Bot., 71: 189-194.

DEELL J., VAN KOOTEN O., PRANGE R., MURR D. 1999. *Application of chlorophyll fluorescence. Techniques in postharvest physiology*. Hortic. Rev., 23: 69-107.

FERRANTE A., MAGGIOREA T. 2007. *Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables*. Postharvest Biol. Tech., 45(1): 73-80.

KEUTGEN A. 2000. *Modification on spinach quality by selected pre- and postharvest treatments.* Beiträge zu Agrarwissenschaften 24, Witterschlick/Bonn: Verlag P. Wehle.

LOPEZ-HERNANDEZ L., GONZALEZ-CASTRO M. J., VAZQUEZ-BLANCO M. E., VAZQUEZ-ODERIZ M. L., SIMAL-LOZANO J. 1994. *HPLC determination of sugar and starch in green beans*. J. Food Sci., 59(5): 1048-1049.

PALTA J.P. 1996. *Role of calcium in plant responses to stress: linking basic research to the solution of practical problems*. Hort Sci., 31: 51-57.

PAPAGEORGIOUA G.C., GOVINDJEE 2011. *Photosystem II fluorescence: Slow changes – Scaling from the past*. J. Photoch. Photobio. B, Special Issue on Recent Progress in the Studies of Structure and Function of Photosystem II, 104 (1-2): 258-270.

PERUCKA I., NURZYŃSKI J., OLSZÓWKA K. 2007. *Influence of $CaCl_2$ on mineral nutrients content in lettuce leaves*. J. Elementol., 12(3): 181-190.

RUIZ-ALTISENTA M., RUIZ-GARCIAA L., MOREDAA G.P., LUB R., HERNANDEZ-SANCHEZA N., CORREAA E.C., DIEZMAA B., NICOLAĎC B., GARCÍA-RAMOSD J. 2010. *Sensors for product characterization and quality of specialty crops-A review*. Comput. Electron. Agric., 74(2): 176-194.

WELLBURN A. R. 1994. *The spectral determination of chlorophylls a and b, as well as carotinoids, using various solvents with spectrophotometers of different resolution*. J. Plant Physiol., 144: 307-313.