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ORIGINAL RESEARCH PAPER

THE INFLUENCE OF FAR-RED LIGHT ON THE ATTRIBUTES OF GREEN BELL PEPPER FRUITS (*Capsicum annuum* L.) DURING STORAGE

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Green bell peppers fruits, stored for 1 month at 8°C and 45±5% relative humidity in Far-Red light (FRL) and darkness respectively, were investigated in terms of physical, chemical and microbiological parameters. The exposure to FRL slows down the water loss from fruits by stimulating the surface wax biosynthesis into a higher specific amount and hydrophobic nature and reducing the apertures, diameters of the pericarp cells, intercellular walls and dermal layer thicknesses. The higher level of catalase enzyme in the FRL-exposed fruits resulted in lower chilling injury index in comparison with the one in fruits exposed to darkness. FRL has favorable effect on chlorophyll and carotenoids accumulation rates. The multiplication of yeasts and molds on the surface of FRL-exposed bell peppers was significantly delayed as compared to the multiplication on the surface of the darkness-exposed bell peppers.

Keywords: bell peppers storage, far-red light, cuticle wettability and barrier properties, SEM bell pepper skin, chlorophyll and carotenoids, catalase

Introduction

Due to its high nutritional value, *Capsicum annuum* L. is widely cultivated worldwide. Like all fruits and vegetables, bell pepper fruits are susceptible to spoilage during improper storage, the most encountered problems being the loss of nutritional value, shriveling and pathogenic disorders (Özden and Bayindirli, 2002). In this line we can mention their storage under 7°C which results in seed browning, tissue discoloration and depressions in the pericarp which evolve into scalds at advanced stages (González-Aguilar *et al.*, 2000; Cuvi *et al.*, 2011).

Different methods were proposed to extend the shelf-life of pepper fruits. Beaulieu *et al.* (2009) studied oilseed-derived lipid films from soapstock and observed a significant reduction in the moisture loss across the produce epidermis. The edible coating made of chitosan with lemongrass oil is effective in the control of anthracnose of bell pepper in vitro, due to the presence of numerous secondary compounds (Ali *et al.*, 2015). The UV-C exposure of red peppers prevents chilling injuries and weight loss probably due to the increased activity of antioxidant enzymes (Cuvi *et al.*, 2011). Modified atmosphere containing O_2 and CO_2 in range of 2-5% was proven to be effective in the preservation of bell pepper fruits (Saltveit, 1990) at 8°C as result of slowing down the respiration and retaining of the initial green color. Treatment with ozone is also recommended due to the reduction of microbial contamination and it extends the shelf-life of the product without having an adverse effect on the product's visual, textural and nutritional quality (Allende *et al.*, 2008). Far Red light is one portion of the solar spectrum that plants work with. The action of FRL on plants has beneficial effects: promoting the flowering and seedling, regulation of defense responses against microorganisms` attacks, stimulation of pathways related to secondary metabolism, increasing the yield, synthesis of high value compounds (Kuo *et al.*, 2015).

Starting from the above discussed aspects, the aim of this study is to investigate the potential beneficial effects of monochromatic Far-Red light on the physical, chemical and microbiological attributes of green bell pepper fruits in order to propose an effective method meant to extend their shelf-life.

Materials and methods

Biological material

Dark green bell pepper fruits (*Capsicum annuum* L. cv. Anaheim) grown in open field with average weight of 150 g were purchased from a local producer. After the removal of contaminated and damaged fruits, they were washed in distilled water, dried in atmosphere and stored.

Storage and sampling

The bell peppers fruits were divided into two batches of ninety fruits each and stored for 1 month in the refrigerator at 8±0.5°C and relative humidity (RH) of 45±5% in two different designs. The bell peppers in the first batch, equally split over three gratings, were exposed for 12 hours/day to FRL (740 nm) from LumiBulb-Far Red LED lamps (LumiGrow, USA) at the light density of 5 Watts and then for 12 hours/day more to darkness. The peppers in the second batch, used as control, were stored in darkness. By periodical rotation, the equal exposure of fruits was ensured. Weekly, from each batch were sampled fifteen fruits and equally divided into five groups. The fruits in the first group were investigated for weight loss, the fruits in the second group for microbiological charge, the fruits in the third group for barrier property against transpiration, those in the fourth group for chilling injuries and those in the fifth group for catalase, chlorophyll and carotenoids contents. Moreover, two groups made of thirty and ten bell peppers,

respectively were characterized at the end of storage in terms of wax characteristics (specific amount, composition and wettability) and electronic microscopy. Similar analyses were conducted on fresh green bell pepper fruits.

Physical investigations

Green bell pepper fruits wax specific amount and composition

The method proposed by Bauer *et al.* (2005) was used to quantify the wax amount and to separate the fractions. Briefly, the wax was removed by rinsing the surface of each green bell pepper fruit with tert-butyl methyl ether (TBME) for 5 minutes in ultrasonic bath. The residue remained after filtration was evaporated to dryness and weighed. The fractions in the residue were separated by mixing it with Celite 545 (Fluka Chemie AG) (1:2 w/w) and placing the mixture on top of a Supelco column (Sigma Aldrich) containing 100 mg silica gel (60 Å, 200-425 mesh particle size, dried for 24 h at 150^{o}C, Merck). A solution made of hexane/toluene (1+2, v/v) was used to elute the hydrophobic fraction and a solution made of hexane/TBME (3+1, v/v) was used to extract the hydrophylic fraction. After solvents evaporation to dryness, each fraction was weighed. The total area of the bell peppers was assessed based on the immersion method. Briefly, each investigated bell pepper was immersed into a film forming solution made of distilled water, gelatine and glycerol (100:4:0.6 w/w/v) at 40°C and prepared according to the method described by Mihaly Cozmuta *et al.* (2015). After 1 minute, the coated fruit was suspended into a desiccator with silica gel and allowed to dry for 24 hours at 20°C and 45% relative humidity. The solidified film was gently peeled, its thickness was measured with a micrometer (T444.1XRL-1, Starrett, USA) in thirty points and the mean value was calculated as average. The surface area of the bell pepper was calculated according to the equation:

$$A = \frac{m}{d \cdot \delta} \quad (1)$$

where: A – the surface of the bell pepper fruit, cm^2; m – the weight of the film forming solution adherent to the bell pepper fruit, calculated as the difference between the initial weight of the film forming solution and the weight after the fruit immersion, g; d – the density of the initial film forming solution, g/cm^3; δ – the thickness of the film removed from the surface of the bell pepper, cm.

Wax specific amount was calculated as the ratio of the total amount of extracted wax and the total area of investigated bell peppers and expressed as mean±standard deviation.

Contact angle (θ), liquid vapor interfacial force (γ_{LV}), surface tension (γ_{SL}), critical surface tension (γ_C) and wettability measurements

The changes which occurred in the wax-cuticular layer adherence were investigated with the aim of establishing the influences of FRL and darkness storage on the skin permeability to internal water. Briefly, the epicuticular wax previously collected from peppers skin was dropped on the dewaxed peppers surfaces, the wax-cuticle contact angles were measured and, subsequently, the

wettability parameters were calculated. The initial and the final moments of storage were considered in sampling.

Determination of wax-cuticlar contact angle (θ)

The epicuticular wax from bell peppers was collected by their coating in an aqueous dispersion of gum arabic (120% w:w, mass ratio), dried for an hour (Chatterjee *et al.*, 2012) and peeled. The procedure was repeated three times and the gum arabic layers were gathered together. The wax retained in the gum arabic fractions was dissolved in chloroform-water 1:1 (v/v) mixture for 5 min under stirring. Two phases were separated and the solvent evaporated. The extracted wax was fluidized by heating at 55ºC and used to measure the contact angle by employing the sessile drop method (Newman and Kwok, 1999). Around 5 µL of fluid reconstituted wax was dispensed with a syringe needle on the skin of the dewaxed bell pepper peeled from the equatorial region. A Fujifilm FinePix S3200 camera (4288 x 2416 pixels) was used to capture the images that were further processed based on goniometer algorithm (Golden Ratio Image Analyzing Software, Welz, 2008). For each image, the contact angles on both sides of the drop were considered. The captures were made no later than 30 s after the drop was dispensed in order to avoid the wax adsorption by the skin. In order to calculate the wettability parameters distilled water, glycerol and toluene were used as reference liquids (Ramirez *et al.*, 2012; Ribeiro *et al.*, 2007). Ten replicates of contact angles were obtained for each measurement at (25±1)°C and expressed as mean±standard deviation.

Determination of wax-air interfacial force (γ_{LV})

The stalagmometric method (Lee *et al.*, 2008) was used to quantify the wax-air interfacial force. The weight of five drops of fluid wax dripped from the stalagmometer (outer radius of 3.2 mm) was 0.0001 g accurately quantified and on the basis of equation 2 (Ramirez *et al.*, 2012) the value of γ_{LV} was calculated:

$$\gamma_{LV} = mg/(2\pi r\psi) \quad (2)$$

where: γ_{LV} is the wax-air interfacial force (mNm^{-1}); m is the weight of the falling droplets, g; g is the gravitational acceleration, m/s^2; r is the outer radius of the capillary in stalagmometer, mm; and ψ is the dimensionless correction factor; it depends on the ratio $r/V^{1/3}$, in which V is the volume of the wax droplet at the work temperature (mm^3) calculated as a ratio between the droplet weight and the fluid wax density (digital densimeter Mettler Toledo, Spain).

Wax-cuticle surface tension (γ_{SL}) and wax-cuticle critical surface tension (γ_C)

In our case, the surface tension (γ_{SL}) quantifies the unbalanced molecular cohesive forces that appear at the wax-cuticular layer interface. It is made of two components, namely the polar and dispersive ones (Ribeiro *et al.*, 2007; Ramirez *et al.*, 2012) which consider the nature and intensity between the wax and the cuticular layer on which the tension is dispensed:

$$\gamma_{SL} = \gamma_{SL}^{p} + \gamma_{SL}^{d} \quad (3)$$

where: γ_{SL} is the wax-cuticular surface tension of the wax (mNm^{-1}), γ_{SL}^{p} is the polar component (mNm^{-1}) and γ_{SL}^{d} is the dispersive component (mNm^{-1}).

The polar and dispersive components of reference liquids were plotted against the cosine of their contact angles on the bell pepper skin and, by knowing the contact angle of the fluid wax on the pepper skin, the polar and dispersive components of the wax-cuticle system were extracted.

Critical surface tension (γ_C), represents the surface tension value of a liquid above which its spread on a solid surface is complete (Cerqueira *et al.*, 2009). The surface tensions of the above-mentioned reference liquids were plotted against the cosine of the contact angles of liquids on the dewaxed cuticle and the intercept of the straight line of dependency with $\cos\theta \to 1$ results in the critical surface tension (Zisman, 1964).

Wax-cuticle wettability

On the basis of wax-air interfacial force, wax-cuticular surface tension and wax-cuticle contact angle, the wettability (W_s) or spreading coefficient (Hershko and Nussinovitch, 1998) is calculated as the work needed to split solid and liquid from the solid/liquid interface (Kurek *et al.*, 2013). In our study, it describes the ability of the fluid wax to spread on the bell pepper skin. The equation proposed by Kurek *et al.* (2013) is used to calculate the wettability of the wax-cuticle system:

$$W_s = W_a - W_c \quad (4)$$

$$W_a = \gamma_{SL}(1+\cos\theta) \quad (5)$$

$$W_c = 2\gamma_{LV} \quad (6)$$

where: W_a is the adhesion coefficient (work of adhesion per unit area), which promotes the liquid spreading on a solid surface (mNm^{-1}), and W_c is the cohesion coefficient (work of cohesion per unit area), which promotes liquid contraction (mNm^{-1}).

Scanning electron microscopy (SEM)

The changes which occurred during storage in the bell pepper skin topography were observed in a high resolution of FEI DualBeam Quanta 3D FEG scanning electronic microscope (USA) under high vacuum and accelerating voltage of 30 KV. Images were collected from outer pericarp peeled from equatorial, shoulder and blossom end of the bell pepper as well as from its cross-sections. At least one hundred complete cells and one hundred sectioned cells from twenty SEM captures of exocarp from fresh and four weeks-stored peppers, respectively were examined and measured to calculate the average diameter and aperture of pericarp cells and the thickness of the dermal system, respectively.

Barrier property against transpiration of bell peppers skin (WVP)

The skin permeability governs the internal water loss during storage and significantly influences the vegetables and fruits shelf-life. The method of Sobral *et al.* (2001) adapted to our study was used to measure the evolution of permeability

in bell pepper skin during storage. Skin portions were peeled from equatorial, shoulder and blossom end areas of each bell pepper sample. The excised tissue was fixed with inner face on top of a cell (permeation area of 1 cm^2) filled with 10 mL of distilled water (air gap at approximately 0.5 cm between the skin and water). The cell was stored in a desiccator with silica gel at 8±0.5°C and 45±5% RH and daily weighed to the nearest 0.0001 g until the steady state was reached. The weight loss was plotted versus time and the linear regression analysis (correlation coefficient range 0.97-0.99) was applied to obtain the time required to reach the steady state. The water vapour permeability (WVP) was calculated according to the equation:

$$WVP\ (gs^{-1}m^{-1}Pa^{-1}) = (wx)/(tA\Delta P) \quad (7)$$

where: WVP is the water vapour permeability, ($gs^{-1}m^{-1}Pa^{-1}$); w is the weight lost at the steady state, g; x is the skin thickness, m; t is the time required to reach the steady state, s; A is the permeation area, m^2; and ΔP is the vapour pressure differential across the pericarp (2652 Pa at 8°C).

A micrometer T444.1XRL-1 (Starrett, USA) was used to measure the thickness of peeled tissue in ten random positions.

Weight loss

Fresh and stored bell peppers were weighed (with an accuracy of 0.0001 g) and the results were expressed as weight loss (%) relative to the initial value±standard deviation.

Chilling injury (CI)

Chilling injury index was assessed according to the method described by Cuvi *et al*. (2011). Twenty fruits were inspected every week in terms of tissues discoloration, hardness, pitting, decay and surface shriveling. Their external CI symptoms have been reported to a scale ranging from 1 to 4 where: 1 – no damage; 2 – low damage; 3 – moderate damage; 4 – severe damage. The CI index was calculated according to the equation:

$$\text{CI index} = (\sum \text{injury level x number of samples inspected})/\text{Total number of fruits considered} \quad (8)$$

and expressed as mean±standard deviation.

Chemical parameters

Green bell pepper fruits powder was prepared by removing the seeds and drying the fruits to constant weight at 50°C in a vacuum oven (Fistreem International Ltd.). After milling, the resulted powder was passed through a 60 mesh sieve and submitted to analysis.

Assay of catalase level activity (CAT)

The level of catalase in the fresh and stored green bell peppers was measured by using the method proposed by Wang *et al.* (2012). According to it, 4 g of fresh

pulp were mixed with 0.1 g PVPP (polyvinylpolypyrrolidone) and homogenized in 10 mL ice-cold phosphat buffered saline solution (25 mM) containing 1 mM EDTA (ethylenediaminetetraacetic acid). The mixture was centrifuged at 12,000 x g for 20 min at 4°C and the supernatant was analyzed for the catalase content (Xing *et al*., 2011). A mixture made of 2 mL sodium phosphate buffer (50 mM, pH 7.0) and 0.5 mL H_2O_2 (40 mM) was contacted with 0.5 mL enzyme extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm (UV/VIS Lambda 35 Spectrometer, Perkin Elmer). CAT specific activity was expressed as U/kg of fresh sample, where $U = \Delta A_{240}$ nm/s.

Chlorophyll and carotenoids contents

The levels of chlorophyll fractions and carotenoids in the investigated bell peppers were assessed by using spectrophotometric method (Mohr and Schopfer, 1979) adapted to our study. Around 50 mg of powder sample, accurately weighted, were mixed with 4 mL of amoniacal solution (80% acetone v/v, 15% distilled water and 5% ammonium solution of 25%) and centrifuged for 20 minutes at 4800 rpm. The absorbance of the supernatant was red at 480 nm, 645 nm, 647 nm, 652 nm, 663 nm, 664 nm and 750 nm (UV/VIS Lambda 35 Spectrometer, Perkin Elmer). The chlorophyll and carotenoid contents were calculated by using equations:

$$CChla = (11.78 \times Abs_{664} - 2.29Abs_{647}) \times FD \times m \quad (9)$$

$$CChlb = (20.05Abs_{647} - 4.77Abs_{664}) \times FD \times m \quad (10)$$

$$CChl(a+b) = (20.20Abs_{645} + 8.0Abs_{663}) \times FD \times m \quad (11)$$

$$CCar = (Abs_{480} + 0.114Abs_{663} - 0.638Abs_{645}) \times FD \times m \quad (12)$$

where: CChla, Chlb – the content of chlorophille a and b, mg/g; Chl(a+b) the content of total chlorophyll, mg/g; CCar – the content of carotenoids, mg/g; m – the weight of dried sample, g; Abs_{480}, Abs_{645}, Abs_{647}, Abs_{652}, Abs_{663}, Abs_{664}, Abs_{750} – the absorbance at 480 nm, 645 nm, 647 nm, 652 nm, 663 nm, 664 nm and 750 nm, respectively; FD – dilution factor

and expressed as mg/g of dried sample±standard deviation.

Microbiology

Green bell pepper fruits were subjected to microbiological investigations in terms of yeasts and molds total load during storage in FRL light and darkness, respectively. Fresh green bell pepper fruits were washed with 0.1% sodium hypochlorite, rinsed in distilled water, dried in ambient air and stored in respect of the indicated parameters. Every day during storage, the refrigerator door was kept open three times for 15 seconds each, to allow the contamination of samples with environmental microorganisms. Every week, three samples were extracted and each immersed in 9 ml of saline solution, homogenized for 1 h and serial 10-fold dilutions were prepared and inoculated on Sabouraud Dextrose Agar W/Chloramphenicol (Scharlab, Germany) selective medium (STAS 6349/6-80 and STAS ISO 7954-2001). Following the incubation for 5 days at 25°C, the colonies

were counted and the results were expressed as log of colony forming unit per gram of green bell pepper (log CFU/g) ±standard deviation.

Statistical analysis

The significant differences between the experimental data were assessed by using one-way ANOVA analysis (Tukey HSD Test $p < 0.5$) with Statistica 7.0 software (StatSoft, Inc., Tulsa, USA).

Results and discussion

Green bell pepper fruits wax specific amount and composition

The wax amount in the fresh green bell peppers was 90.01 µg/cm^2 (Table 1), close to the value of 96 µg/cm^2 indicated by Schreiber and Schönherr (2009).

Table 1. Surface wax average specific amount and composition in green bell pepper fruits in relation with storage conditions

Time of storage, weeks	Wax specific amount, µg/cm^2	Wax composition, %	
		Hydrophobic fraction	Hydrophylic fraction
Initial moment	90.01±1.48	36.12±2.14	63.88±2.46
4 weeks in darkness	87.34±5.21 c*	30.43±1.48	69.57±4.61
4 weeks in FRL	94.58±2.19 a*	38.43±4.21	61.57±2.01

Results are expressed as mean ± standard deviation; a – significant difference at $p < 0.05$ reported to the initial moment; c- not significant difference at $p < 0.05$ reported to the initial moment; * - significant difference at $p < 0.05$ reported to the corresponding value in the opposite storage design.

The study of Bauer *et al.* (2005) indicates two fractions in the wax composition of 12 bell pepper cultivars. The first one, which represented up to 39% from total amount, consisting of *n*-alkanes $C_{20} – C_{35}$ (C_{31} being prevalent), *iso*-alkanes C_{25}-C_{35} and some aldehydes, can been seen as the hydrophobic fraction. The 61% second fraction, consisting of 15 triterpenes, *n*-alkanoic acids and traces of *n*-alkanols and phytosterols, is the hydrophilic fraction. In our study, the wax composition in the fresh bell peppers fruits consisted of 36.12% of the hydrophilic fraction and 63.88% of the hydrophobic fraction. After a four-week storage, the wax amount and chemical composition have changed in opposite directions and to different extent. Thus, in the case of fruits stored under darkness not significant reduction in the wax amount and hydrophobic fraction was noticed as compared to the initial status. The absence of wax biosynthesis in darkness (Charles *et al.*, 2008) allows us to assume that the hydrophylicity increase is due to the cleavage of initial components in wax into more hydrophilic compounds. The exposure of fruits to FRL results in the significant ($p<0.05$) rises for both wax amounts by 1.05-fold and hydrophobic fraction by 1.06-fold. It can be viewed as a wax biosynthesis initiated by the healthy epidermal cell (Sheperd and Griffiths, 2006) in response to light stress. By increasing the wax amount and its hydrophobic ratio, a better coverage of the cuticle and an enhanced wax-cuticle adhesion are ensured (as discussed

below) and thus the protection against FRL is enhanced (Juniper and Jeffree, 1983).

Wax-cuticle system wettability

The skin of bell peppers can be included into the low-energy category due to the value of γ_L in range of γ_C-100 mNm^{-1} (Ribeiro *et al.*, 2007) and thus the application of Zisman (1964) method in the calculation of wettability parameters is justified (Table 2). The wax-cuticle contact angles in both fresh and four weeks-stored samples are lower than 90^0 corresponding to a spontaneous wetting of pepper fruits cuticle (Figure 1). Modifications in the hydrophobic-hydrophilic ratio result in changes in the values of wax-cuticle contact angles and implicitly in the wax-cuticle compatibility. Different trends in the evolution of wax-cuticle contact angle can be noticed at the end of storage depending on the storage design. Thus, a significant decrease ($p<0.05$) by 1.07-fold in the wax-cuticle contact angle of FRL-exposed peppers suggests the strengthening in the wax-cuticle adhesion. The reduction in the polar component along with the increase in the dispersive one indicates an enlargement of the hydrophobic fraction in the wax composition. The non-polar bonds between wax and cuticle are favored and result in the reinforcement of wax-cuticle adherence. On the contrary, in the samples exposed to darkness, the 1.06-fold increase in the wax-cuticle contact angle can be noticed and results in higher value of the polar component and lower value of the dispersive component as compared to the values in the fresh sample. In this line, we can assume that the debility in the wax-cuticle adhesion which occurred during storage in darkness is probably due to the rise of hydrophilic components in the wax composition to the detriment of hydrophobic ones. The 1.069-fold increase in the contact angle of darkness-stored samples suggests the reduction in the cuticle-wax compatibility expressed in debility of the cuticle-wax adhesion. In the same line, the rise of polar component value (from 23.12 mNm^{-1} to 26.88 mNm^{-1}) shows a more pronounced ability of wax to establish polar bonds. The values of wettability coefficients support the evolution of contact angles. The best (the highest) value is displayed by the wax-cuticle system in FRL-exposed sample and confirms the enhanced wax-cuticle compatibility. No data was found in the literature regarding the wax-cuticle wettability in bell peppers.

Scanning electron microscopy (SEM)

Changes occurred in the green bell peppers pericarp during storage can be observed in Figure 2. The well-individualized contours of the cell walls in the fresh samples are preserved in the FRL-exposed samples while in the darkness-exposed samples they are blurred. The changes which occurred in the chemical composition of cuticular wax can explain the difference. The higher ratio of the hydrophobic fraction strengthens the cuticle-wax compatibility and enhances not only the wax dispersion over the cuticle and its coverage but also the cuticle-wax adhesion.

Table 2. Values of contact angles and wettability components of extracted wax dispensed on the dewaxed green bell pepper fruits cuticle (by Zisman plot method)

Time of storage, weeks	Contact angle of reconstituted wax on cuticle, θ	Critical surface tension γ_C (mNm^{-1})	Polar component γ_L^p (mNm^{-1})	Dispersive component γ_L^d (mNm^{-1})	Surface tension γ_L (mNm^{-1})	Work of adhesion Wa (mNm^{-1})	Work of cohesion Wc (mNm^{-1})	Spreading coefficient W_s (mNm^{-1})
Fresh sample	48.56±0.71	54.71	23.12	33.60	56.72	94.25	113.44	-19.19
4 weeks in darkness	51.93±1.124 a*		26.88	31.82	58.70	94.89	117.4	-22.51
4 weeks in far-red light	45.21±0.85 a*		20.60	34.79	55.39	93.72	110.78	-17.06

Results are expressed as mean ± standard deviation.
a – significant difference at $p < 0.05$ reported to the initial moment.
* - significant difference at $p < 0.05$ reported to the corresponding value in the opposite storage design.

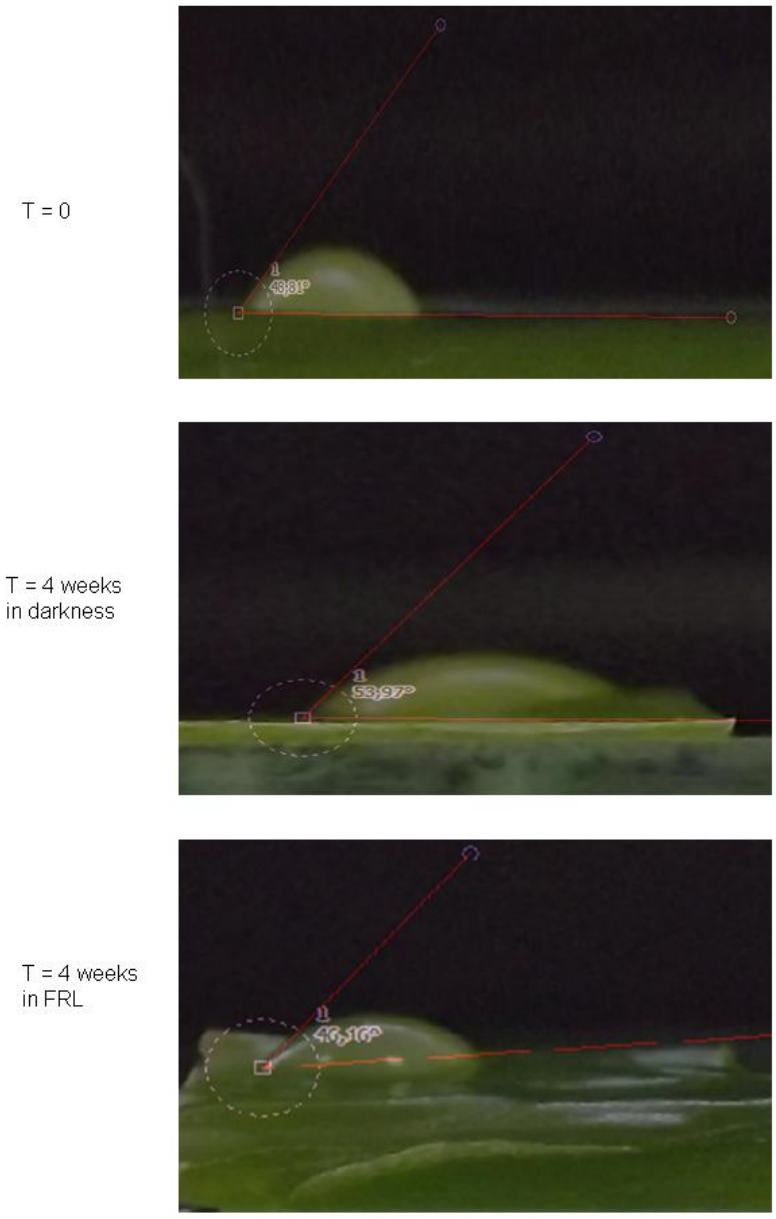


Figure 1. Contact angles of reconstituted epicuticular wax dispensed on the pepper fruits dewaxed skin

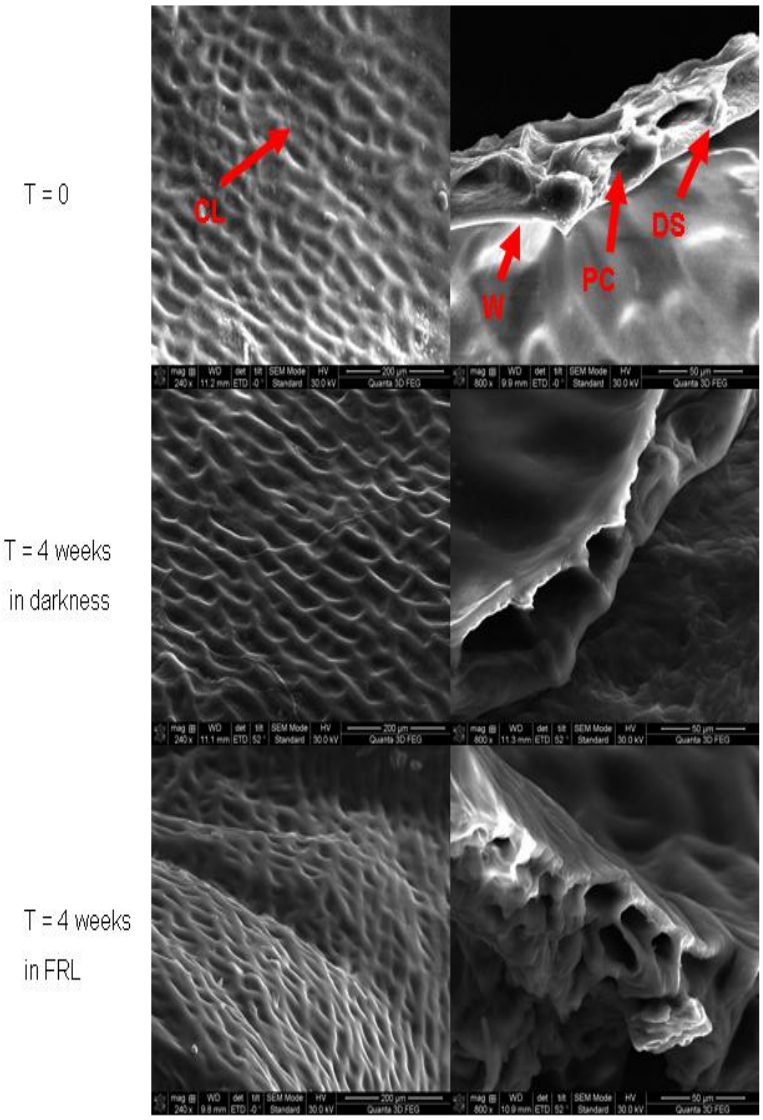


Figure 2. SEM images of outer pericarp tissue and dermal system in bell peppers fruits stored under FRL and darkness, respectively; DS – dermal system; PC- pericarp cell; CL – cuticular layer; W-wax.

On the contrary, the higher ratio of hydrophilic fraction in the darkness-exposed samples lowers the cuticle-wax compatibility and weakens their adhesion. As a result, the contour of cell walls looks rather attenuated. Measurements of some structural parameters (apertures and diameters in the pericarp cells, intercellular walls and dermal layer thicknesses) indicate their rescaling (in average) during storage.

As compared to the fresh samples, the cells aperture in the darkness-exposed samples was widened 2.24–fold (from 9.27 μm to 20.76 μm) along with the broadening 1.63-fold of their diameter (from 18.34 μm to 29.89 μm). In opposition, the aperture and diameter of pericarp cells in FRL-exposed samples were reduced 2.11-fold (from 9.24 μm to 4.38 μm) and 1.79-fold (from 18.34 μm to 10.24 μm), respectively. The reduction in the intercellular walls thickness during storage was noticed in both samples but to a different extent. Thus, the walls were thinner 1.34-fold in the FRL-exposed samples (from 10.54 μm to 7.86 μm) and 1.11-fold in the darkness-exposed samples (from 10.54 μm to 9.49 μm). The dermal layer displays a descending trend in all samples during storage. It became 2.94-fold smaller in FRL-samples (from 6.16 μm to 2.09 μm) and 3.81-fold smaller in darkness-exposed samples (from 6.16 μm to 1.61 μm), respectively.

Barrier property against transpiration of bell pepper fruits cuticle

The permeability to water of bell peppers skin has risen during storage in time- and in a storage design-dependent manner, respectively (Figure 3).

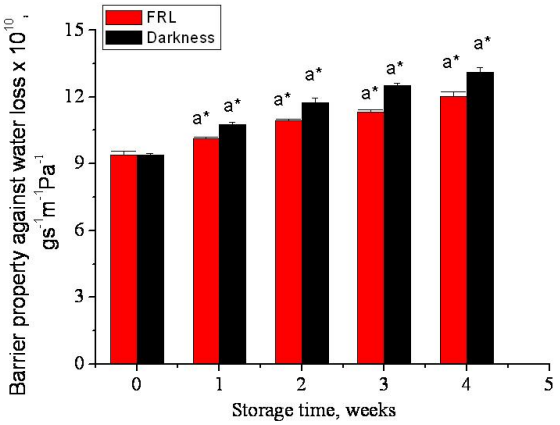


Figure 3. Barrier properties of green bell pepper fruits cuticle against transpiration during storage; results are expressed as mean ± standard deviation; a–significant difference at $p<0.05$ reported to the initial moment within the same storage design; *- significant difference ($p<0.05$) reported to the corresponding value in the opposite storage design; the error bars represent standard errors of the means (n=3)

Thus, regardless of the storage design, the significant ($p<0.05$) deterioration in the skin permeability is observed every week. The increase in the

hydrophobic fraction of the surface wax, the lowering in cells sizes of pericarp tissue and the thickening of the dermal layer diminished the deterioration of the fruits stored under FRL. Thus, at the end of storage, the WVP is 1.27–fold and 1.39-fold higher in FRL-exposed and darkness-exposed fruits as compared to the initial sample. Changes in the cuticle permeability influence the level of water loss. A larger wax amount in combination with a larger hydrophobic fraction in the FRL-exposed fruits ensures a better coverage of cuticle and an improved cuticle-wax adherence. A thicker dermal layer in the FRL-exposed peppers increases the tortuosity of the channels through which the free water inside the fruits is transported to the cuticle surface. Moreover, the narrowing of the pericarp cells in terms of aperture and diameter reduces the specific surface area available for water evaporation. By increasing in hydrophilic fraction in the darkness-exposed fruits, the cuticle permeability to water is enhanced due to weakening the cuticle-wax adhesion. The enlargement in the pericarp cells also favors the water transportation from inside to the surface. The hydrophilic changes in the wax composition which occurred in the darkness stored peppers favor the formation across the cuticle of so-called "aqueous pores" which accelerate the water extraction through the cuticle (Schönherr, 2006; Schreiber, 2005; Kerstiens, 2006) and intensifies the transpiration rate (Figure 4).

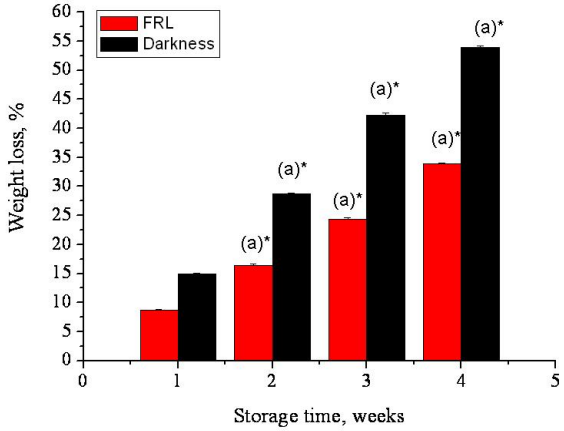


Figure 4. Weight loss in green bell pepper fruits during storage; results are expressed as mean ± standard deviation; (a)- significant difference at $p<0.05$ reported to the first week of storage within the same storage design; *- significant difference ($p<0.05$) reported to the corresponding value in the opposite storage design; The error bars represent standard errors of the means (n = 3).

Chilling injury index (CI)

Regardless of the storage design, no significantly damaging symptoms (CI around 1) were noticed after the first week of storage, but after that a significant rise in the CI can be noticed (Figure 5). The samples exposed to darkness were scored as low damaged (CI = 2) after a storage between 1 and 2 weeks while those exposed to

FRL obtained the same score after 3 weeks of storage. At the end of storage, the darkness-exposed samples were severely damaged (CI over 4) and FRL-exposed samples were moderately damaged.

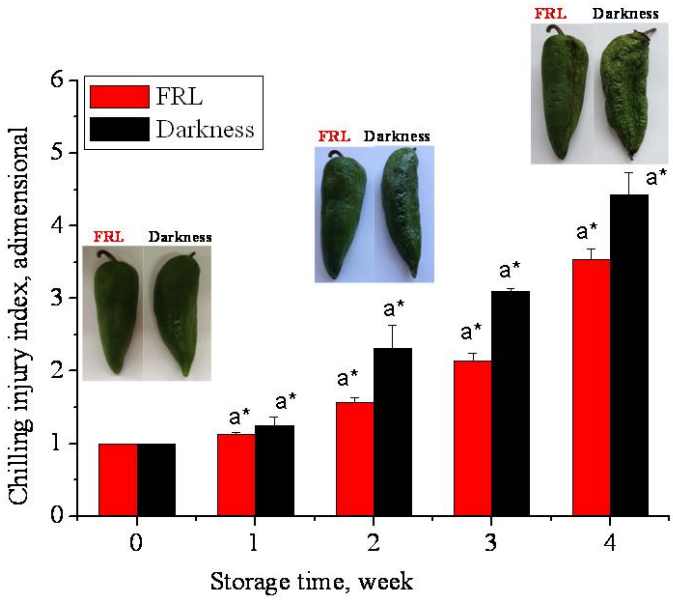


Figure 5. The evolution of the firmness index in green bell pepper fruits during storage; results are expressed as mean ± standard deviation; a– significant difference ($p<0.05$) reported to the initial moment within the same storage design; *- significant difference at $p<0.05$ reported to the corresponding value in the opposite storage design; the error bars represent standard errors of the means (n=3)

According to Wang *et al.* (2012), the chilling injury is often associated with the oxidative damage of the plant tissues and the presence of antioxidant enzymes contributes to the adaptation of plants to cold stress. The CAT activity in both our samples displays an initial increase followed by a decline (Figure 6). The CAT activity in the darkness-exposed fruits reached the highest level at 7 days while in the FRL-exposed fruits at 14 days. Moreover, the highest CAT content was 1.35-fold higher in the FRL-exposed fruits than the one in the darkness-exposed fruits. The higher level of catalase enzyme in the FRL-exposed samples as compared to the darkness-exposed samples (Figure 6) could explain the lower values in CI. Our results are supported by the work of Wang *et al.* (2012) who found that the levels of catalase, peroxidase, ascorbate peroxidase and glutathione reductase are higher in the peppers treated with brassinolide as compared to untreated peppers, supporting thus their improved cold resistance expressed in low values of CI. Similarly, Cuvi *et al.* (2011) founded that the activity of superoxide dismutase, catalase and ascorbate peroxidase is higher in UV-C treated pepper fruits and prevents chilling injuries. The chemical composition of wax also plays an important role in the fruits resistance to cold stress. As the increase in the

hydrophobic ratio occurs, the thermal conductivity of surface wax decreases and the cold flow transfer from environment to the cuticle is delayed.

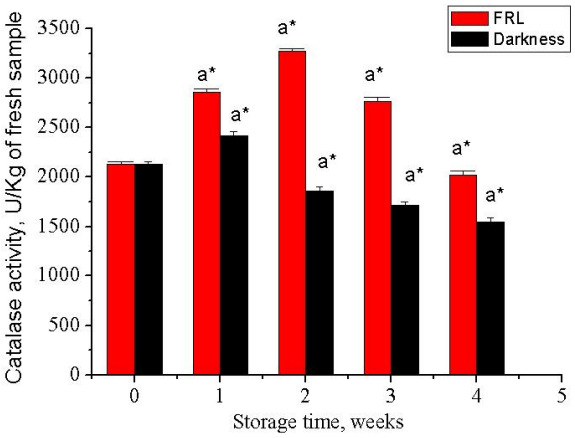


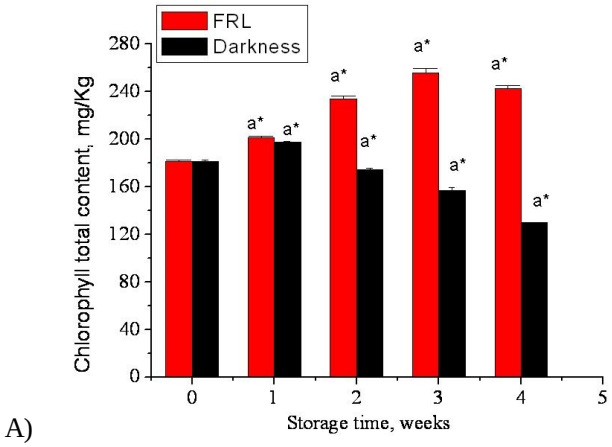
Figure 6. Changes in the catalase content in the FRL and darkness-exposed pepper fruits during storage; results are expressed as mean ± standard deviation; a– significant difference ($p<0.05$) reported to the initial moment within the same storage design; *- significant difference at $p<0.05$ reported to the corresponding value in the opposite storage design; the error bars represent standard errors of the means (n = 3)

Chlorophyll and carotenoids contents

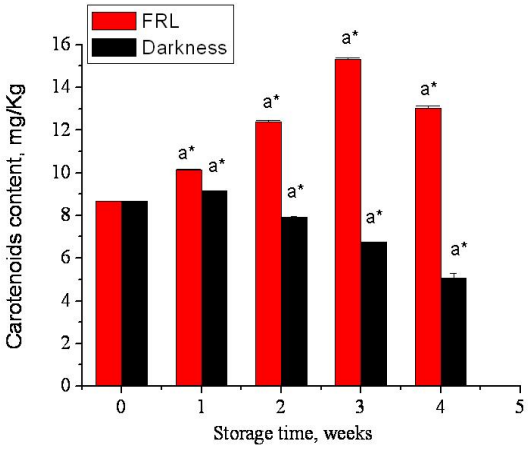
The chlorophyll and carotenoids levels in the investigated peppers evolved in different patterns in a storage parameters-depending manner (Figure 7 A, B). The exposure to darkness results in slow accumulation rates in chlorophyll and carotenoids in the first week of storage by 1.09-fold and 1.05-fold, respectively probably due to a prolongation of the biosynthesis processes initiated when they were attached to the mother-plant.

Beginning with the second week, the decrease in the chlorophyll and carotenoids contents occurs. At the end of storage, the chlorophyll and carotenoids total contents were reduced by 1.39-fold and 1.71-fold, respectively. Unlike darkness, the exposure to FRL has a favorable effect on chlorophyll and carotenoids accumulation rates in the first three weeks of storage. At this moment, the chlorophyll content is 1.41-fold higher that in the fresh peppers while the carotenoids content is 1.76-fold higher. In the fourth week, the chlorophyll and carotenoids levels decreased by 1.05-fold and 1.17-fold, respectively but still remained above the levels in the fresh fruits. The favorable effect of FRL on the accumulation of chlorophyll was also observed by De Greef *et al.* (1971) who reported an upward trend in the greening of bean leaves exposed during 1 week to FRL for 12 hours/day and to darkness for additional 12 hours/day. The activation of the photosynthetic system which requires the photo-transformation of protochlorophyll into the chlorophyll is mentioned as being responsible for the process. The degradation of chlorophyll (and subsequently of carotenoids) results

when hydrolysis processes occurred with the aim of preventing the cells damage due to the chlorophyll phototoxic potential as well as for the protein nitrogen and lipid carbon recovery as valuable resources in the cells metabolism (Matile *et al.*, 1999). The trend in the formation and breakdown of chlorophyll and carotenoids in our study is supported by the results reported by Frosch and Mohr (1980).



A)



B)

Figure 7. Changes in the chlorophyll (A) and carotenoids (B) contents in the green bell peppers fruits affected by storage in FRL and darkness, respectively; results are expressed as mean ± standard deviation; a– significant difference ($p<0.05$) reported to the initial moment within the same storage design; *- significant difference at $p<0.05$ reported to the corresponding value in the opposite storage design; the error bars represent standard errors of the means (n = 3)

Microbiology

As Figure 8 displays, the multiplication of yeasts and molds over the surface of the FRL-exposed peppers is significantly slowed down as compared to the

multiplication on the surface of the darkness-exposed peppers. Direct and indirect action pathways of FRL reduce the microorganisms invasion. FRL, which is detected by an evolved photosensory system developed in plants, inhibits the germination and growth of some fungi by acting against membrane lipids, cytoplasmic enzymes and nucleic acids (Fuller *et al.*, 2013). Furthermore, the changes in the wax chemical composition, produced by the FRL exposure, influence the skin colonization with yeasts and molds. The better coverage of cuticle in wax and enhanced cuticle-wax adhesion, as in the FRL-exposed fruits, act as a barrier against microorganisms penetration into the tissues. The compatibility of hydrolytic enzymes secreted by the yeasts and molds to digest the surface in their way to the substrate is also an important factor. In the case of the FRL-exposed fruits, the larger proportion of hydrophobic fraction in the wax composition hampers the enzymes action and their access into the cuticle. The poor spreadability of wax over the skin surface in the darkness-exposed fruits due to the larger ratio of hydrophilic fraction reveals opening areas through which the microorganisms have access to nutrients and the fruits spoilage is favored.

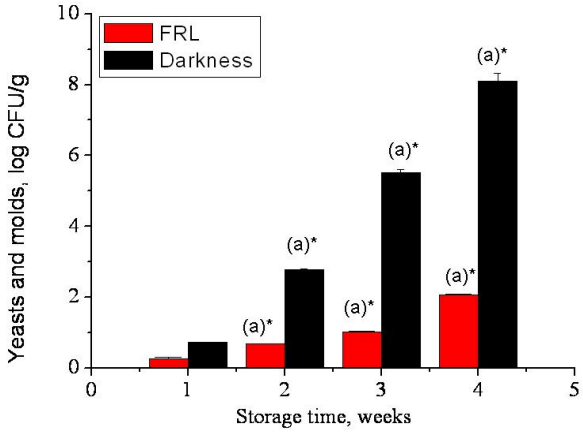


Figure 8. Evolution of total molds and yeasts on peppers skin during storage; results are expressed as mean ± standard deviation; (a)- significant difference ($p<0.05$) reported to the first week of storage within the same storage design; *- significant difference at $p<0.05$ reported to the corresponding value in the opposite storage design; the error bars represent standard errors of the means (n = 3)

The increase of the fruits resistance to microbiological contamination due to changes in their surface which occurred during monochromatic light exposure was also reported by Charles *et al.* (2008). They demonstrated that the physical and chemical modifications in tomatoes fruits topography stored under UV-C provide them with resistance against the infection with *Botrytis cinerea.*

Conclusions

The storage in Far-Red light proves to be more effective in the extension of the shelf-life of green bell pepper fruits as compared to the storage in darkness. By

favoring the wax biosynthesis in a more hydrophobic composition, the cuticle-wax adhesion was strengthened. It results in a significant reduction in water loss and a better protection of fruits membrane against cold. Nutritional parameters in terms of chlorophyll and carotenoids contents were also at a higher level in the FRL-exposed fruits than in the darkness-exposed ones. The multiplication of yeasts and molds on the surface of FRL-exposed peppers is significantly slowed down as compared to their multiplication on the surface of the darkness-exposed peppers. These factors depend on the direct action of FRL against microorganisms as well as on the green bell peppers morphology. Further detailed investigations are required in order to elucidate the mechanisms involved in the responses of green peppers fruits to FRL.

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