THE COMPARATIVE EFFECT OF FAR-RED LIGHT AND UV-C LIGHT ON SOME PHYSICAL-CHEMICAL ATTRIBUTES IN RED BELL PEPPERS (*Capsicum annuum* L.) DURING STORAGE

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Abstract. Red bell pepper fruits were subjected to six weeks storage in the darkness, ultraviolet light (UV-C) and far red light (FRL), respectively at 8°C and 55% relative humidity. Fresh and stored fruits were sampled and analyzed in terms of wax specific amount and chemical composition, wax-cuticle wettability, barrier properties of cuticle against transpiration and weight loss. Wax biosynthesis occurred in both UV-C and FRL – exposed fruits and moreover in a higher hydrophobic ratio, results in an enhanced wax-cuticle compatibility and adhesion. On contrary, the cleavage of hydrophobic compounds in some more hydrophilic occurred into the wax of the darkness-stored peppers, increases the hydrophilic nature of wax, weakens the wax-cuticle adhesion and reduces the wax spreadability over the cuticle. As results, the barrier properties against water loss are debilitated to a larger extent in the darkness-exposed peppers and to a lesser extent in the FRL-exposed peppers. The water loss rate is positively correlated with the wax specific amount and its hydrophobic nature, the fruits exposed to the FRL displaying the lowest water loss rate followed by the fruits exposed to UV-C.

Keywords: far red light, UV-C, red bell peppers, wax specific amount and composition, weight loss

INTRODUCTION

The cuticle has the pivotal role in the limitation of the transpiration from the primarily plant surface [12]. This thin and flexible layer is covered in wax mainly made of aliphatic compounds on which different functional groups are attached (alkanoic acids, alkanols, aldehydes, alkanes, alkyl, esters) as well as non-aliphatic components such as pentacyclic triterpenoids and sterol derivatives originating from the isoprenoid biosynthetic pathway [12, 17]. The cuticular wax plays a vital role in the protection against injury, air pollution, microorganisms attacks, water repellency, moisture loss and appearance. If the papers addressed to the influence of genetic factors, climate conditions and development stage on the wax quantitative and qualitative composition are numerous [3, 1], those addressed to the changes in the wax specific amount and composition occurred under different storage parameters is rather scarce. Also, the correlations between wax characteristics (amount and chemical composition) and barrier properties against transpiration are poorly studied and understood [27, 31]. Mihaly Cozmuta et al. [19] found that the storage of red tomatoes in the FRL results in the wax biosynthesis not only into a larger specific amount but also into a higher hydrophobic composition and thus the barrier properties against internal water loss and protection against yeasts and molds proliferation on the surface are improved.

In this end, the aim of this paper was to investigate the changes occurred in the red peppers wax characteristics (specific amount and chemical composition) when they are stored in the darkness, FRL and UV-C and to screen the changes in the cuticle barrier properties against transpiration in relation with the wax characteristics.

MATERIALS AND METHODS

Biological material

Red bell pepper fruits (*Capsicum annuum* L. cv. Bogdan) were purchased from a local produced, inspected to remove the damaged and contaminated fruits and delivered to the laboratory no later than 1 h after they were harvested. The fruits were washed in distilled water, air-dried and stored.

Storage and sampling

The bell peppers fruits were divided in three batches of fifty fruits each and stored for 6 weeks at 8°C and relative humidity (RH) of 55±5% in three different designs. The bell peppers in the first batch were exposed 12 hours/day to FRL (740 nm) to LumiBulb-Far Red LED lamp (LumiGrow, USA) at the light density of 5 Watts and another 12 hours/day to darkness. The peppers in the second batch were subjected to UV-C irradiation provided by a germicidal lamp (TUV, 30W, Philips) under the same lighting cycle as above presented. The fruits in the third design were stored in the darkness. During storage, all fruits were manually rotated to assure the equally exposure. Samples of peppers were collected from each batch and analyzed in terms of wax characteristics (specific amount, speciation and wettability) barrier properties against transpiration and weight loss. Similar analyzes were conducted on the fresh red bell pepper fruits.

Red bell pepper fruits wax specific amount and composition

The wax specific amount in the pepper fruits was quantified and fractionated by using the method proposed Bauer et al. [4]. According to it, the fruits were kept in contact for 5 minutes in ultrasonic bath with tert-butyl methyl ether (TBME) and the resulted liquid was filtrated. The solid residue was evaporated to dryness and weighed. Two chemical fractions in the composition of bell peppers were separated by mixing the removed wax 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°C, Merck). The hydrophobic fraction was extracted in a solution made of hexane/toluene (1+2, v/v) while the hydrophilic fraction was eluted in a solution containing hexane/TBME (3+1, v/v). Before each fraction weighing, the solvents were evaporated. To calculate the specific wax amount, the total area of fruits was measured based on immersion method. Each fruit was immersed and kept for 1 min into a film forming solution made of distilled water, gelatin and glycerol (100:4:0.6 w/w/v) at 40°C [19]. The dried coated fruit was conditioned for 24 h at 20°C and 45% RH into a desiccator, the solidified film was gently peeled and measured in term of thickness (T444.1XRL-1, micrometer, Starrett, USA) in ten randomly selected points. The mean thickness was calculated as average. The surface area of bell pepper was calculated according to the equation:

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

where: A – the surface of the bell pepper fruit, cm^2; g – 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 pepper fruits 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 wax-cuticle adherence regulates the water loss process from fruits in the environment and subsequently their firmness. Its intensity can be assessed in the basis of the wettability parameters.

Determination of wax-cuticle contact angle (θ)

The bell pepper fruits were coated in an aqueous dispersion of gum arabic (120% w:w, mass ratio), drying for an hour [6] and peeling. The extraction was repeated three times, the gum arabic films were gathered together and put in contact with in a mixture made of chloroform-water 1:1 (v/v) for 5 min under stirring. The solvent was evaporated from the organic phase and the extracted wax was fluidized by heating at 35°C and used to measure the contact angle by employing the sessile drop method [21]. According to it, 5 µL of reconstituted wax was dispensed on the skin of dewaxed bell pepper peeled from the equatorial region. The images were captured by using a Fujifilm FinePix S3200 camera (4288 x 2416 pixels) and processed by following the goniometer algorithm [33]. To avoid the wax adsorption by the cuticle the images were captured no later than 30 s after the wax deposition. Distilled water, glycerol and toluene were used as reference liquids to calculate the wettability parameters [25, 26]. Ten replicates of contact angles were obtained for each measurement and expressed as mean±standard deviation.

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

The stalagmometric method proposed by Lee et al. [11] was used to calculate 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 weighed and the value of γ_{LV} was calculated according the equation [25]:

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

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 capillary in stalagmometer, mm; and ψ is the correction factor, adimensional; 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 fluid wax density (digital densimeter Mettler Toledo, Spain).

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

The surface tension (γ_{SL}) expresses the unbalanced molecular cohesive forces that appear at the wax-cuticular layer interface. It expresses the contribution of polar and non-polar (dispersive), respectively groups [25, 26]:

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

where: γ_{SL} is 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 the wax-cuticle system were extracted by plotting the polar and dispersive, respectively components of reference liquids against the cosine of their contact angles on the peppers skin and by knowing the contact angle of fluid wax on pepper skin.

The surface tensions of the reference liquids (distilled water, glycerol, toluene) were plotted against the cosine of the contact angles of liquids on dewaxed cuticle and the intercept of the straight line of dependency with cos $\theta \rightarrow 1$ results in the critical surface tension, γ_C [32]. It represents the surface tension value of a liquid above which it spread on a solid surface is complete [7].

Wax-cuticle wettability

By knowing the wax-air interfacial force, wax-cuticular surface tension and wax-cuticle contact angle, the wettability or spreading coefficient (W_s) is calculated [9] as the work needed to split solid and liquid from the solid/liquid interface [18]. In our study, it describes the ability of fluid wax to spread on the bell pepper skin. The equation proposed by Kurek et al. [18] was used to calculate the wettability of 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}).

Barrier properties against transpiration of bell peppers cuticle (WVP)

Respiration and diffusion across cuticle are the main mechanisms involved into the postharvest water loss from pepper fruits [10] in both cases the cuticle permeability being the key factor. The evolution in the cuticle permeability of pepper fruits in relation with theirs storage was assessed in the basis of the method proposed by Sobral et al. [24] adapted to our study. Cuticle portions were peeled from equatorial, shoulder and blossom end areas of each bell pepper sample and fixed on top of a cell (permeation area of 1 cm^2) filled with 10 mL of distilled water. The cell was stored in a desiccator with silica gel (25±5% RH) at 8±0.5°C 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 in range of 0.96-0.98) 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 vapor 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 6 weeks-stored stored red bell peppers were weighed (0.0001 g accuracy) and the results were expressed as weight loss (%) relative to the initial value±standard deviation.

Statistical analysis

One-way ANOVA analysis (Tukey HSD Test $p < 0.05$) with Statistica 7.0 software (StatSoft, Inc., Tulsa, USA) was used to assess the significance differences between the experimental.

RESULTS

Red bell pepper fruits wax specific amount and composition

The wax specific amount in the fresh and 6-weeks stored pepper fruits as well as its composition are presented in the Table 1. A decrease in the wax specific amount can be observed in the fruits stored in the darkness as compared to those stored in the light. The ratios of hydrophobic and hydrophilic fractions in the total wax amount of 37.6% and 62.4%, respectively are also significantly changed during storage.

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

The values of wettability parameters associated to the fresh and 6 weeks stored peppers are indicated in the Table 2. In all samples, the wax-cuticle contact angles are significantly lower than 90° and indicate a spontaneous wetting of cuticle. During storage, different trends in the evolution of wax-cuticle contact angles can be noticed (Figure 1). Thus, the contact angles in the fruits stored in UV-C and FRL, respectively were reduced while in the darkness-exposed peppers the increased.

Barrier properties against transpiration of bell peppers cuticle (WVP)

As shown in Figure 2, a continuous increase trend is observed in the cuticle permeability in all stored fruits in different extent depending by the storage time and design. At the end of storage, the darkness-stored fruits seem to have the most permeable cuticle, followed by the UV-C- and FRL-, respectively stored fruits. Thus, the cuticle permeability in darkness-exposed peppers increased 2.52-fold in the darkness-exposed peppers, 2.26-fold in the UV-C – exposed peppers and 2.19-fold in the FRL-exposed peppers. The evolution in cuticle permeability is in line with the weight loss trend (Figure 3), the most intense water loss occurring in the darkness-stored peppers followed by the UV-C and FRL, respectively.

Table 1. The specific wax amount and composition in the fresh and 6-weeks stored red bell pepper fruits

Storage time	Storage design								
	Darkness			UV-C			FRL		
	Wax specific amount, μg/cm^2	Hydrophobic fraction, %	Hydrophilic fraction, %	Wax specific amount, μg/cm^2	Hydrophobic fraction, %	Hydrophilic fraction, %	Wax specific amount, μg/cm^2	Hydrophobic fraction, %	Hydrophilic fraction, %
Fresh fruits	82.6±4.12	37.6±2.11	62.4±3.87	82.6±4.12	37.6±2.11	62.4±3.87	82.6±4.12	37.6±2.11	62.4±3.87
6 weeks	79.3±1.24^{a*}	34.8±1.78^{a*}	65.1±3.87^{a*}	84.78±3.74^{a*}	40.1±1.95^{a*}	59.9±2.56^{a*}	89.45±2.78^{a*}	43.8±2.06^{a*}	56.2±4.14^{a*}

Results are presented 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 comparison with the other storage designs.

Table 2. Wettability parameters associated to the fresh and 6 weeks-stored red bell pepper fruits

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 fruits	37.14±1.68	42.37	22.11	32.07	54.18	91.37	119.34	-27.97
6 weeks in darkness	39.88±2.44		25.76	29.04	54.80	91.76	121.04	-29.28
6 weeks in UV-C	34.61±2.75 a*		20.74	35.29	56.03	89.35	112.39	-23.04
6 weeks in FRL	31.33±1.07 a*		19.29	38.02	57.31	87.96	110.08	-22.12

Results are presented 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 comparison with the other storage designs.

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

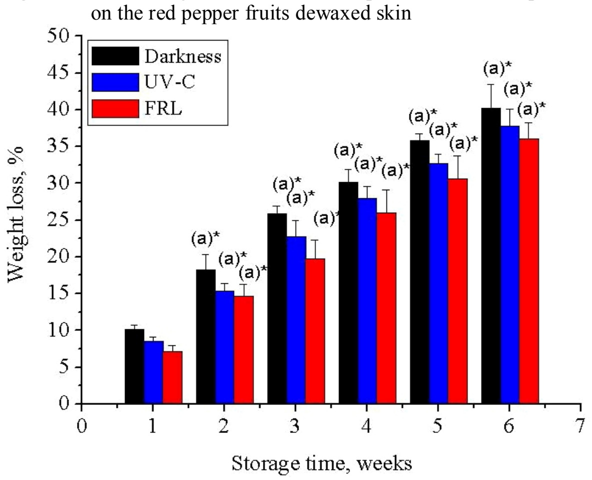

Figure 2. Barrier properties of red bell pepper fruits cuticle against transpiration during storage in the darkness, UV-C and FRL; results are expressed as mean ± standard deviation; a–significant difference at p<0.05 reported to the initial moment within the same storage design; *- significant difference (p<0.05) reported to the corresponding value in the other storage designs; the error bars represent standard errors of the means (n=3)

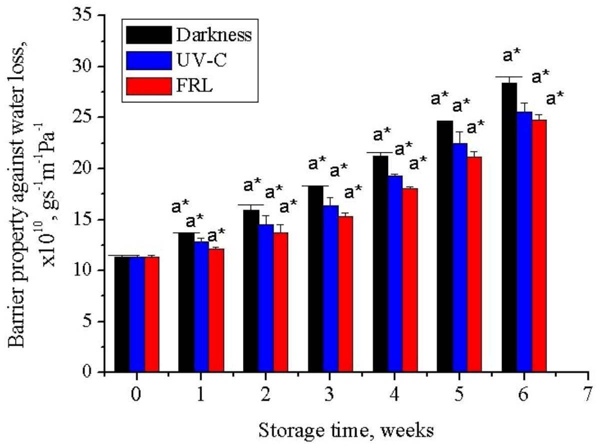

Figure 3. Weight loss in the red bell pepper fruits during storage in the darkness, UV-C and FRL; results are expressed as mean ± standard deviation; (a)- significant difference at p<0.05 reported to the first week of storage within the same storage design; *- significant difference (p<0.05) reported to the corresponding value in the other storage designs; the error bars represent standard errors of the means (n = 3)

DISCUSSIONS

Red bell pepper fruits wax specific amount and composition

Our value of wax specific amount in the fresh pepper fruits (82.6 µg/cm^2 – Table 1) is lower than that for the Keystone cultivar (113 µg/cm^2) and higher than the values of NuMex N Raky (76.4 µg/cm^2) and Sante Fe Grande (55.5 µg/cm^2), respectively indicated by Lownds et al. [14]. The ratios of hydrophobic and hydrophilic fractions in the total wax amount of 37.6% and 62.4% are close to the values 39% and 61%, respectively indicated by Bauer et al. [4]. According to them, the hydrophobic fraction is mainly made of n-alkanes (up to 84.7%) while the triterpenes are the major components (57.1%) in the hydrophilic fraction. During storage, changes occurred in both wax specific amount and composition in a storage design-depending manner. As Table 1 indicates, in the case of the darkness-stored fruits, the wax specific amount significantly decreases in relation with the fresh sample, explained by the absence of wax biosynthesis in the darkness [5]. The weight of fractions in the darkness exposed fruits significantly changed, the fractionation yielded 34.8% hydrophobic fraction and 65.1% hydrophilic fraction corresponding to an increase of 1.04-fold and a decrease of 1.08-fold, respectively. It suggests that during darkness storage the chemical compounds in the hydrophobic fraction split into more hydrophilic compounds. Dong et al. [8] reported that the wax specific amount in "Red Fuji" apples decreased sharply when they were stored at 3°C for seven months along with the decrease in the ratio of alkanes (mainly n-nonacosane, n-heptacosane and n-nonacosene). In opposition to darkness-stored pepper fruits, the wax biosynthesis occurred in the UV-C and FRL – respectively, stored pepper fruits probably as response to the light stress. After 6 weeks, the wax specific amount in the fruits exposed to UV-C increased 1.02-fold while in the FRL-exposed fruits 1.08-fold. Its chemical composition has also modified during UV-C and FRL exposure, the hydrophobic fractions gaining larger ratios, especially in FRL-exposed fruits. Thus, at the end of storage, the hydrophobic fraction covers 40.1% of the total wax in the UV-C-fruits and 43.8% in the FRL-fruits, higher than in the darkness-fruits (34.8%). The esters hydrolysis which led to the accumulation of free fatty acids can explains the reduction in the hydrophilic fraction along with the increase in the hydrophobic fraction in the UV-C and FRL – exposed fruits. The hydrophobic fraction enlarged to varying extents in the light-exposed fruits, by 1.06-fold in UV-C-fruits and 1.16-fold in FRL-fruits. The reduction in the alkanes chain length occurred in the UV-C pepper fruits [5, 2, 29] could explain the difference.

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

The values of γ_L in range γ_C-100 mNm^{-1} (Table 2) suggest that the bell pepper skin can be included into the low-energy category [26] and justify the application of Zeismann method in calculation of the wettability parameters. At the end of storage, the contact angle in the darkness exposed fruits is 1.07-fold higher as compared to the fresh fruits. A reduction in the contact angles by 1.07-fold and 1.18-fold can be observed in the UV-C and FRL-, respectively exposed pepper fruits. A possible explanation targets the chemical changes occurred in the wax composition during storage. The increase in the hydrophilic components in the darkness exposed fruits alters the wax-cuticle compatibility, the non-polar nature of the wax being well known. On the contrary, the larger proportion of hydrophobic compounds in the wax of the fruits exposed to UV-C and FRL, respectively strengths the wax-cuticle bonds. The evolution of polar and dispersive components (Table 2) is in line with this assumption. The polar component, representing the hydrophilic compounds in the wax, rises during darkness storage along with the lowering of the dispersive component. In the fruits exposed to light, regardless its nature, the evolutions of polar and dispersive components are positive correlated with the hydrophilicity-hydrophobicity balance of the wax. In the same time with the enlargement in the hydrophobicity of wax the increase in the dispersive component values occurred. The evolution in the spreading coefficients values are in good agreement with that in the contact angles, polar and dispersive components. The best (the highest) spreading coefficient is obtained in the case of FRL-stored peppers followed by the one corresponding to the fruits stored in the UV-C light.

Barrier properties against transpiration of bell peppers cuticle (WVP) and weight loss

Our results concerning the positive influence of UV-C on mass loss are consistent with those of Vincente et al. [30] who reported a mass loss 4.40% in the peppers exposed to UV-C (in a dose of 7 kJ m^{-2}) for 12 days at 10^{o}C, as compared to 5.41% in the not exposed fruits. The work of Rodoni et al. [28] shows that after 5 day of storage at 4^{o}C , the water loss in the control samples was two-fold higher that in the UV-C (dose of 10 kJ m^2) exposed red bell peppers sticks. No information regarding the effect of FRL on bell pepper attributes were found in the literature. Multiple factors can be considered responsible for such evolution in the cuticle impermeability and subsequently in the water loss rate [10]. A higher was specific amount in association with a larger ratio of hydrophobic fraction in the wax of UV-C and FRL – exposed pepper fruits (Table 1) result in a better coverage of cuticle, strengthening in the wax-cuticle adhesion and low rate of water loss. A rearrangement of the epicuticular wax layer and minimization the cracks and fissures [13] is also possible. In the case of pepper fruits stored in the darkness, the change in the hydrophobic-hydrophilic balance in the hydrophilic meaning weakens the wax-cuticle adhesion, favors its permeabilization and results in high rate of water loss. Kissinger et al. [16] and Lownds et al. [14] also have demonstrated that the pepper fruits with high amount of epicuticular wax lost weight at significantly lower rates. The presence of so called “aqueous pores” [22, 23, 15] to a larger extent in the darkness-fruits due to a larger ratio of hydrophilic fraction is also possible. They intensify the extraction of water molecules across the cuticle and implicitly the transpiration of fruits. The chemical composition of cuticular wax also mediates the water loss. Nadakuduti et al. [20] demonstrated that a lower proportion of long-chain alkanes in waxe increased cuticular permeability in the tomato mutants. In our study, as compared to fresh fruits, a higher proportion of hydrophobic fraction mainly contains n-alkanes (Table 1) in the UV-C and FRL-, respectively exposed fruits reinforces the cuticle function as barrier against water loss, while a higher proportion of hydrophilic fraction in the darkness-exposed fruits impairs this attribute.

In the basis of above presented results, it may be concluded that the FRL enhances to a larger extent in relation to UV-C the wax specific amount and its hydrophobic nature, barrier properties against transpiration and water loss. Further investigations will be addressed to the influence of UV-C and FRL on chemical and microbiological attributes in the red bell pepper fruits.

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