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Effect of Different Ripening Conditions on Pigments of Pepper for Paprika Production at Green Stage of Maturity

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ABSTRACT: The content and composition of pigments and CIELab color properties in fruits ripened in the field were compared with those obtained in ground paprika produced from green pepper fruits after postharvest ripening for 15 days in a greenhouse under different conditions. Obtained data for pigment content, composition, and esterification rate have shown that the processes of pigment biosynthesis in fruits ripened under greenhouse conditions are different from those occurring in fruits naturally matured in the field: the red/yellow pigment ratio (3:1) in greenhouse-ripened fruits is much higher than in naturally ripened pepper in breaker (1:1) and also in faint red (2:1) ripening stages from the field. Additionally, during the postharvest ripening of green pepper in the greenhouse esterification processes are less expressed than during the ripening of the fruits in the field. Postharvest ripening under natural daylight resulted in higher content of red pigments, followed by higher ASTA value.

KEYWORDS: *pepper, ground paprika powder, color, carotenoids, carotenoid esterification, ripening conditions*

INTRODUCTION

One of the main characteristic of ground red paprika is its extractable color. Color, rated on the basis of ASTA,[1] is the base for classification of ground red paprika products worldwide. The color of ground red paprika is also by rule listed among the specific properties in declarations of ground red paprika with protected denomination of origin (PDO) or with protected geographic identification (PGI) such as Pimentón de Murcia (ASTA minimum 120) and Pimentón de la Vera (ASTA minimum 90).

Although widely accepted, the ASTA method has its drawbacks: it requires an equipped laboratory, and it is time-consuming (16 h). Color properties of ground red paprika expressed in the CIELab system have been the subject of investigation of numerous authors using methods for determining color change as a consequence of paprika storage,[2,3] paprika powder diameter,[4] ionizing radiation and steam,[5] and drying rate.[6] Some authors successfully correlated the quick and easy apparent (CIELab) and ASTA methods.[7]

The color of ground red paprika highly depends on the content and composition of carotenoids[8] present in the pepper fruits used in its production, consequently resulting in research investigating the dependence of carotenoid composition on pepper variety,[9,10] maturity stage of pepper fruits,[11−13] and processing conditions.[14,15]

With regard to harvest in traditional, manual production, ripe red fruits are sequentially picked from the plant and left to be dried under natural conditions. High production costs as a consequence of such an approach to the production of ground red paprika are acceptable only in the case of high added value products such as PDO and PGI products. In a large-scale production of ground red paprika, which is commercially very competitive because of its price, there is a tendency to perform harvest mechanically at the moment when the majority of the fruits achieve full ripeness. Nevertheless, at the harvesting moment some fruits on the plants are still not completely ripe, influencing thus the quality of ground red paprika, predominantly by altering its color. Additionally, even when harvesting is performed manually, some pepper fruits remain unripe (green) at the end of the growing season, and the question of the possibility and effects of their utilization in ground red paprika production is still opened.

Nevertheless, there is no sufficient information on changes of pigment content and composition in unripe pepper fruits during their postharvest ripening and their influence on color properties of ground red paprika. The aim of the present research is to analyze the differences in pigment content and composition in green pepper ripened in the field and in the greenhouse under different postharvest ripening conditions and to relate them to the color properties of the produced ground red paprika.

MATERIALS AND METHODS

Sampling and Postharvest Treatment (Ripening) of Pepper Fruits. Green pepper fruits (*Capsicum annuum* L.) variety 'AlevaNK' were picked manually from the field, located in northern Serbia. During the production, good agricultural practice was applied including fertilization, irrigation, and plant protection. Pepper fruits were sampled from the field when 80% of fruits were well developed at a green maturity stage. For fruits analyzed without postharvest ripening picking, measurement, transport from the field to the laboratory, preparation for drying, drying, and grinding were performed in a period not exceeding 3 days. Remaining fruits were subjected to postharvest ripening for 15 days under different conditions: with natural daylight changes and in the dark. For both ripening conditions, treatment was conducted in a greenhouse under semicontrolled temperature (25−45 °C) and humidity (40−60%). During the ripening, pepper fruits were spread on a flat surface. After postharvest ripening, fruits were dried under laboratory conditions in the same manner as fresh fruits (for 10 h with gradual increase of temperature from 40 °C at the beginning to 80 °C at the end of

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experiment). After drying, to obtain powdered pepper, pericarp was separated from seeds and ground briefly, for 10 s, in a laboratory grinder to avoid sample heating, but long enough to obtain powder with >90% of particles with a size under 350 μm.

Simultaneously, the ripening of pepper was observed at the same field, and pepper fruits were picked manually when the majority (approximately 80%) of fruits reached breaker (8 days after picking green fruits) and faint red (another 10 days after picking breaker) stages of ripening. Pericarp of fresh fruits was separated from seeds and used for the analysis. The complete scheme of the conducted experiment is presented in Figure 1.

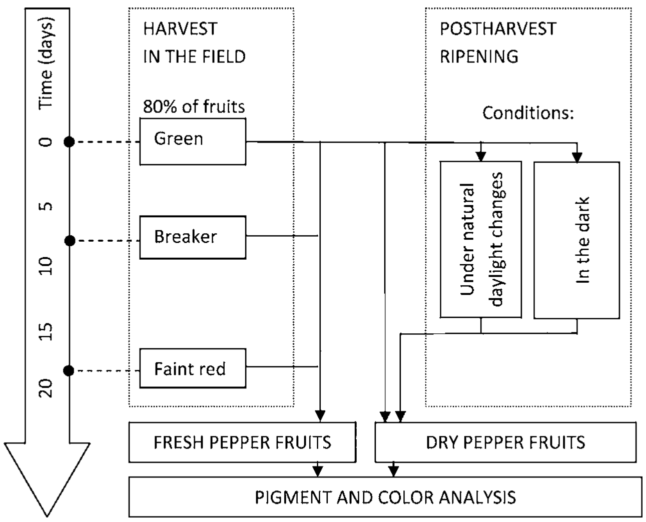

Figure 1. Scheme of conducted experiment.

Analytical Methods. The color of fresh fruits and powder samples was measured as a reflected color in the CIELab color space. Measurements were performed using a MOMCOLOR 101 (MOM, Budapest) and *MOMCOLOR computing unit* was used for calculation of the lightness of color (L^*), the ratios between red and green (a^*) and between yellow and blue (b^*) from measured x_1, x_2, y, and z values. Ten readings were performed for each sample. For fresh fruits color measurements were performed on visually even color characteristic for each maturity stage (green, breaker, and faint red). Powder samples were spread in a 0.5 cm layer, and color was measured at 10 different points. The dominant wavelength was estimated from a chromaticity diagram, whereas the color difference between two samples (ΔE_{ab}^*) was calculated as the square root from the sum of squared differences between measured L^*, a^*, and b^* values of compared samples. The method proposed by the American Spice Trade Association (ASTA)[1] was used for the measurement of extractable red color, and the result is expressed in ASTA units.

In all samples the moisture content was determined by drying the sample to constant mass at 105 °C. Chlorophyll content was determined by extraction of chlorophyll from 10 g of the representative pepper pericarp sample with acetone (p.a. grade, J. T. Baker, Deventer, The Netherlands), under dim light and by measuring absorption of the extract at 662 and 664 nm. Calculation of chlorophyll *a* and *b* content was conducted according to the method of Wettstein.[16]

Extraction of carotenoids from the pepper pericarp was performed according to the method of Mínguez-Mosquera and Hornero-Méndez.[17] Separation of extracted carotenoids was performed according to the method of Morais et al.[18] with minor modifications by Kevrešan et al.[19] An HP1090 (Agilent Technologies, Santa Clara, CA, USA) HPLC system with DAD detector equipped with a Zorbax SB C18 column (3.0 × 250 mm i.d., particle size = 5 μm) and the same precolumn (Zorbax SB C18, 4.6 × 12 mm i.d., particle size = 5 μm) was used. Separation of pigments was carried out at ambient temperature (24 ± 1 °C). Flow rate was 1.5 mL/min. Two eluents were used, (A) acetone/water (75:25, v/v) and (B) acetone/methanol (75:25, v/v), with the following gradient: from 0 to 25% B in 10 min, from 25 to 100% B in 35 min, 100% B for 10 min. All reagents used for separation were of HPLC quality grade purchased from J. T. Baker, whereas water was grade 1 quality according to ISO 3696:1987. After each analysis, eluent B was reduced to 0% in 20 min, and then the system was left running under these conditions for 15 min before the next analysis. Carotenoids were detected at 460 ± 4 nm. For each peak the whole spectrum (from 300−550 nm) was recorded. Peaks were identified by comparing retention time and/or spectra with carotenoid standards (capsorubin, antheraxanthin, zeaxanthin, violaxanthin, and β-carotene). Standards were acquired from Carotenature (Ostermundigen, Switzerland), whereas capsanthin was purchased from Hoffman-La Roche (Basel, Switzerland). All unidentified yellow carotenoids were calculated as β-carotene equivalents. Results for pigment content were expressed per dry weight.

Obtained results were analyzed by analysis of variance (ANOVA), followed by comparison of means with Duncan's test at a significance level of 0.01, using Statistica 10.0 software.[20]

RESULTS AND DISCUSSION

In the first step of analysis of the obtained results, the comparative overview of differences in pepper pigment composition of paprika fruits ripened in the field and paprika obtained from green pepper fruits ripened in the greenhouse under different conditions is presented and related with color properties in the CIELab system and ASTA value of analyzed samples.

Changes in pigment content and composition during the ripening process of investigated pepper (*C. annuum*) variety 'AlevaNK' in the field, from green over breaker to faint red maturity stage are presented in Table 1.

Table 1. Pigment Content (Milligrams per Kilogram Dry Matter) in Fresh Pepper Fruits Ripened in the Field

	fresh pepper picked from the field at different ripening stages[a]		
total content of pigments	green	breaker	faint red
yellow pigments			
β-carotene	ND	22.7b	207.8c
zeaxanthin	28.2a	41.5b	211.6c
antheraxantin	9.4a	9.6a	ND
violaxanthin	50.7a	18.3b	63.4a
nonidentified	268.6b	212.9a	480.7c
red pigments			
capsanthin	ND	293.1b	1841.9c
capsorubin	ND	40.7b	162.4c
green pigments			
chlorophyl (a + b)	126.64a	69.04b	ND

[a]Means within the same row followed by different letters were significantly different at $p \leq 0.01$. ND, not detected.

In green pepper, dominant pigments are chlorophylls and yellow carotenoids, emphasizing photosynthesis as the dominant biochemical process. Red pigments, capsanthin and capsorubin, were not identified in green paprika fruits, but the intermediate products of their synthetic pathways, including zeaxanthin, antheraxanthin, and violaxanthin, were identified, indicating initiation of biochemical changes leading to formation of red pigments (Table 1).

Toward the breaker phase, the content of chlorophyll is reduced to half in green fruits, whereas the total carotenoid content is doubled. Red pigments start to be accumulated, with the content of capsanthin being up to 7-fold higher than the content of capsorubin. More or less equal contents of red and yellow pigments were registered in fruits at breaker maturity phase. Pepper at breaker phase starts to accumulate β-carotene, and the quantity of zeaxanthin is increased in comparison to green fruits. On the other hand, the content of antherxanthin remains at the same level as in the green fruits, whereas the content of violaxanthin significantly decreases. Variation in the content of yellow pigments in pepper ripening stages reported for variety Bola[21] pointed out a gradual but permanent increase of these intermediate products of red pigments during the ripening process in the field. At the same time the findings of Deli et al.,[12] based on the research on the Szentesi Kosszarvu pepper variety, are mainly aligned with our findings. These contrasting results indicate that the stage of development, variety, and ripening conditions influence the dynamic of pigment synthesis and intermediate products equilibrium.

Total carotenoid content in faint red pepper fruits is significantly higher than in breaker stage and especially higher compared to green fruits. In faint red pepper the content of red pigment is twice as high as the content of total yellow pigments, and the content of capsanthin is >10 times higher than the content of capsorubin. In faint red paprika fruits the content of β-carotene and zeaxanthin is multiply increased in comparison to breaker phase, opposite to findings of Mínguez-Mosquera and Hornero-Méndez[22] and Deli et al.;[12,13] antheraxantin was not identified.

Carotenoids synthesized during pepper fruit maturation undergo the esterification process.[21,23] Esterification of carotenoids in investigated fruits of pepper variety AlevaNK is presented in Table 2. It is obvious that in green pepper almost only nonesterified forms of pigments are present; at the breaker stage the quantity of pigments in esterified forms is more or less at the level of nonesterified pigments, whereas in faint red fruits zeaxanthin, capsathin, and capsorubin are present more in esterified than in nonesterified forms with the major quantity being in diesterified form. These results confirm the findings presented by Mínguez-Mosquera and Hornero-Méndez.[21]

Table 2. Contents of Pigments in Esterified Forms (Milligrams per Kilogram Dry Matter) in Fresh Pepper Fruits Ripened in the Field

		fresh pepper picked from the field at different ripening stages[a]		
pigment	esterification	green	breaker	faint red
zeaxanthin	nonesterified	28.2	20.6	60.2
	monoesterified	ND	ND	ND
	diesterified	ND	20.9	151.4
yellow pigments	nonesterified	348	259.0	590.2
	monoesterified	8.9	25.1	221.9
	diesterified	ND	20.9	151.4
capsanthin	nonesterified	ND	111.4	325.8
	monoesterified	ND	72.0	621.9
	diesterified	ND	109.7	894.2
capsorubin	nonesterified	ND	23.4	19.4
	monoesterified	ND	6.6	69.4
	diesterified	ND	10.7	73.6

[a]ND, not detected.

The described changes of pigment content, composition, and esterification in pepper fruits ripening in the field are further compared with pigment composition in ground paprika obtained from pepper fruits picked at green maturity stage and subjected to different postharvest treatments (Tables 3 and 4). Even in green paprika, dried and ground shortly after harvesting, some quantities of β-carotene and capsorubin have been registered, in contrast to fresh green pepper fruit. Another sign indicating instant changes in the pigment composition after picking of green fruits is a complete disappearance of antheraxanthin and violaxanthin, as well as chlorophyll, and a multitude of unidentified yellow pigments. Although the

Table 3. Pigment Content (Milligrams per Kilogram Dry Matter) in Ground Paprika Obtained from Green Pepper Ripened under Different Postharvest Ripening Conditions

	green pepper dried and ground after different postharvest ripening treatments[a]		
total content of pigments	dried without ripening	ripened in the dark	ripened under natural daylight changes
yellow pigments			
β-carotene	14.34a	7.48b	14.50a
zeaxanthin	32.94a	4.56b	8.45b
antheraxantin	ND	3.32b	4.41b
violaxanthin	ND	4.76b	4.79b
nonidentified	4.00a	23.99b	33.79b
red pigments			
capsanthin	ND	146.39b	200.66c
capsorubin	7.27a	10.48ab	17.54b
green pigments			
chlorophyll ($a + b$)	ND	ND	ND

[a]Means within the same row followed by different letters were significantly different at $p \leq 0.01$. ND, not detected.

Table 4. Contents of Pigments in Esterified Forms (Milligrams per Kilogram Dry Matter) in Paprika Ripened in the Field and under Different Postharvest Ripening Conditions

		green pepper dried and ground after different postharvest ripening treatments[a]		
pigment	esterification	dried without ripening	ripened in the dark	ripened under natural daylight changes
zeaxanthin	nonesterified	32.94	4.56	8.45
	monoesterified	ND	ND	ND
	diesterified	ND	ND	ND
yellow pigments	nonesterified	47.28	38.82	55.57
	monoesterified	4.00	5.29	10.37
	diesterified	ND	ND	ND
capsanthin	nonesterified	ND	70.03	83.32
	monoesterified	ND	18.73	41.66
	diesterified	ND	57.63	75.68
capsorubin	nonesterified	3.03	2.89	4.20
	monoesterified	4.24	7.59	13.34
	diesterified	ND	ND	ND

[a]ND, not detected.

complete processing procedure of fresh green pepper fruits was conducted in <3 days and under usual processing conditions used in ground paprika production, it is obvious that significant changes in carotenoid content occurred. Such results can be easily ascribed to changes causing partial or complete destruction of certain pigments even under very mild processing conditions and also to dynamic biochemical processes in the first days after paprika harvesting.[14,15,21,22] Nevertheless, it is quite obvious that the balance of pigments in paprika dried and ground shortly after harvesting is quite different from that in green fresh fruits. This points out that also color changes reflecting more red but less green and yellow tones than fresh fruit can be expected (Table 3). In ground paprika obtained from the green pepper without postharvest ripening, pigments are dominantly in nonesterified form (Table 4).

The majority of green pepper fruits subjected to postharvest ripening during 15 days visually appeared as red fruits. Nevertheless, obtained data for pigment content, composition, and esterification rate (Tables 3 and 4) have shown that the processes of pigment biosynthesis in fruits ripened in postharvest processes are different from those occurring in the naturally matured fruits in the field (Tables 1 and 2).

The quantity of red pigments in paprika produced from green pepper fruits ripened by greenhouse postharvest treatments during 15 days is >3 times higher than the quantity of yellow pigments, providing a red/yellow pigment ratio (3:1) much above that of naturally ripened pepper at breaker (1:1) and also faint red (2:1) ripening stages from the field. Furthermore, in greenhouse-ripened fruits, in contrast to the fruits ripened in the field, no accumulation of β-carotene and zeaxanthin has occurred, but rather the biosynthesis process redirects the pathways to formation of red pigments, capsanthin and capsorubin. These findings are in contrast with the results of Markus et al.,[24] who observed an increase of β-carotene content in paprika spice produced from overipened fruits harvested in later maturity stages. Also, a small quantity of antheraxanthin, which has not been registered in faint red fruit from the field, is present in fruits ripened in the postharvest treatment. The quantity of violaxanthin is multiple times lower than in fruits ripened in the field (Tables 1 and 3). Different stress factors, such as salinity,[25] water stress,[26] and storage temperature,[27] are proven to influence carotenoid biosynthesis in edible plant organs. Harvest, as a process by which fruit is detached from plant, is stress for both the remaining plant and fruit, especially for green, nonmature fruits, and could be one reason for the changing intensity of carotenoid biosynthesis in investigated green pepper fruits and consequently in obtained ground paprika.

By comparing pigment composition of ground paprika obtained from green pepper fruit ripened under natural daylight changes and in the dark, it can be concluded that postharvest ripening under natural daylight changes results in a higher content of red pigments (both capsanthin and capsorubin) and in higher contents of β-carotene and zeaxanthin. At the same time contents of antheraxanthin and violaxanthin are at the same level regardless of applied postharvest ripening conditions. The quantity of capsanthin in paprika produced from the pepper ripened in the dark is >13 times higher than capsorubin content, and for the postharvest treatment under natural daylight this ratio is similar to faint red pepper ripened in the field (Tables 1 and 3).

During the postharvest ripening of green pepper the esterification processes are less expressed than during the ripening of the fruits in the field. Zeaxanthin is not undergoing the esterification processes, and other yellow pigments are present only in monoesterified form. Esterification of red pigments is somewhat more expressed compared to yellow ones, and capsanthin is present in both mono- and diesterified forms. Quantities of monoesterified pigments are aproximately doubled if the postharvest ripening is conducted under natural daylight changes in comparison to dark conditions. Regardless of postharvest ripening conditions the diesterified form is synthesized only in the case of capsanthin with more diesterified form being produced again when postharvest ripening was conducted under natural daylight changes. These findings are in accordance with the results of Markus et al.,[24] who concluded that more sunny days during the ripening period result in more pigments in esterified forms, which are more stable and thus result in better storage properties of produced paprika spice.

Color properties of the surface of fresh pepper fruits from the field at different maturity stages, that is, in fruits of very distinctive visual appearance regarding color when expressed in the CIELab system (Table 5), indicate that there is no significant difference among fruits in different ripening stages regarding the lightness (L^*), whereas the blue/yellow ratio (b^*) only slightly changes to positive values as ripening progresses, indicating domination of yellow over blue color. The main change during ripening that results in visual perception of fruit distinction is related to the change of the green/red ratio (a^*). The values of a^* change from negative values (indicating green fruits) to zero for breaker stage of maturity (indicating balanced ratio of red and green tones) to high positive values for faint red fruits. Differences in the green/red ratio (a^*) result also in significant differences in psychrometric chroma (C^*) among fresh pepper fruits at different ripening stages. The dominant wavelength for green and breaker fruits is in the range of the yellow part of the spectrum, whereas for faint red fruits it inclines more to the orange spectral range.

Table 5. CIELab Color Properties of Surface of Fresh Pepper Fruits from the Field at Different Ripening Stages

	fresh pepper picked from the field at different ripening stages[a]		
CIELab color property	green	breaker	red
L^*	35.80a	33.16a	33.77a
a^*	−17.65a	1.20b	39.23c
b^*	27.00ab	33.16a	22.98b
C^*	30.78b	14.09a	44.05c
dominant wavelength (μm)	562a	579b	615c

[a]Means within the same row followed by different letters were significantly different at $p \leq 0.01$.

Observations regarding the CIELab color properties of ground red paprika obtained from green pepper fruits ripened under different postharvest treatments (Table 6) illustrate that the most intensive red color was obtained for ground paprika produced from green fruits ripened under natural daylight changes. Ground paprika produced from fruit ripened in the dark had less apparently visible red color, whereas in paprika ground from directly dried green pepper the a^* value indicates balanced intensity of green and red tones.

Table 6. ASTA and CIELab Color Properties of Ground Paprika Obtained from Green Pepper Ripened under Different Postharvest Ripening Conditions[a]

CIELab color property	without ripening	ripening in the dark	ripening under natural daylight changes
L^*	41.42c	39.20c	38.67c
a^*	2.22c	7.97b	11.94a
b^*	22.33b	21.88b	25.23a
C^*	20.92c	21.76b	26.40a
dominant wavelength (μm)	579.0c	584.0b	588.5a
ASTA	3.97c	23.10b	42.75a

[a]Means within the same row followed by different letters were significantly different at $p \leq 0.01$.

The positive value of b^* confirms the dominance of yellow tones for all examined samples, with a more intense yellow tone for paprika produced from pepper ripened by postharvest treatment under natural daylight changes than in the dark. This observation can be related to the higher ratio of red/yellow pigment and higher capsanthin content with respect to capsorubin in paprika from pepper ripened in the dark (Table 3). Ground red paprika, regardless of the ripening conditions of the pepper fruits from which it was produced, showed dominant wavelengths in the yellow spectral range.

The ASTA value increased from 3.97 in ground paprika obtained from green pepper dried and ground shortly after harvest to 23.10 in ground paprika obtained from green pepper ripened for 15 days in the dark and even to 42.75 for ground paprika obtained from green pepper ripened for 15 days under natural daylight changes.

On the basis of the L^*, a^* and b^* values from Table 6 color differences (ΔE_{ab}^*) between ground red paprika samples obtained under different ripening conditions were calculated. Color differences between ground paprika obtained from green pepper dried and ground shortly after the harvest and ground paprika obtained from green pepper ripened for 15 days in the dark and under the natural daylight changes were 6.18 and 10.51, respectively, whereas the difference between two postharvest ripening treatments was 5.22. Color difference (ΔE_{ab}^*) indicates the degree of color difference between two samples. ΔE_{ab}^* values in the range of 0−0.5 signify an imperceptible difference in color between two samples, 0.5−1.5 a slight difference, 1.5−3.0 a just noticeable difference, 3.0−6.0 an apparent difference, 6.0−12.0 an extremely apparent difference, and above 12.0 a color of a different shade.[28,29] The obtained results indicate that color differences are apparent to extremely apparent with less expressed difference for paprika which has ripened in the dark.

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Notes

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