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QUANTIFICATION OF CAROTENOIDS IN SOME VARIETIES OF RED PEPPERS *(CAPSICUM ANNUM* L.) AND THEIR PROCESSED PRODUCTS

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ABSTRACT

The red pepper (*Capsicum annuum* L.) is one of the most essential vegetable crops due to multiple ways it can be processed, as well as for the content of the nutritional phytochemicals, such as carotenoids, which are important for human health. Carotenoids are responsible for the pepper's colour, as natural antioxidants. They are present in relatively high amounts in the ripe fruit and they play a positive role to ensure the colour stability of the final product.This research examined the carotenoids composition of the red pepper varieties: *kurtovska kapija, palanechko chudo* and *horgosh*. For each of the varieties, the following preservation technologies have been applied: pasteurization, freezing, and drying. The extraction method of carotenoids was performed by using methanol-dichloroethan and anhydrous Na_2SO_4. The changes in the carotenoid pigments of examined varieties during processing have been investigated quantitatively by usage of HPLC technique. The HPLC method was performed on stationary phase Nucleosil 100-3 C18, 250 x 4,6 mm, with a flow rate: 0,7 mL/min, and the gradient program with following eluents: methanol, distilled water and a mixture of isopropanol, acetonitrile and methanol. Carotenoids have been detected between 200 and 700 nm. In all of the analyzed samples, 47 peaks of carotenoids were detected, of which 5 were unknown, one was mixed, and 41 carotenoids were identified. The red pepper variety *horgosh* was found to contain the highest content of total carotenoids in fresh pepper fruits (690,15 µg/g), frozen pepper (587,88 µg/g), pasteurized pepper (575,76 µg/g) and dried pepper (493,94 µg/g).

Key words: carotenoids composition, HPLC method, red pepper varieties.

INTRADUCTION

The genus *Capsicum*, belongs to the *Solanaceae* family and includes peppers of important economic value. *Capsicum* are one of the oldest and most popular vegetables and spices in the world. Its popularity derives from a combination of different factors such as colour, taste and pungency (Giuffridaet al., 2013). Fruits of *Capsicum* can vary tremendously in colour, shape, and size both between and within the species (Ranjith et al., 2014). There is a wide range of production of peppers (over 200 varieties) that are used for different processing. It is of particular importance that the pepper that is used fresh, as well as for processing, has a larger fleshy part, the so-called useful part, also to have the higher content of dry matters and with more expressed sensory characteristics (Marković & Vračar, 1998). In Republic of North Macedonia, there is almost no region where pepper is not grown, and in some regions, due to the favorable agro-ecological conditions, there is a long tradition of growing this crop (Jankulovski, 1997). In recent years, there has been an increase in the areas planted with pepper, which is especially characteristic for those regions where the adequate processing facilities have been built.

The ripe fruits of the different varieties of peppers have been traditionally widely used as natural food colourants. Although generally, the colour of each *Capsicum* variety is variable, starting from green, yellow or white for the unripe fruit, and turning to red, dark red, brown and sometimes almost black in the ripe stage, ripe pepper (*Capsicum* sp.) fruits can display a range of colours from white to deep red. The mature fruit of the red pepper is consumed worldwide with an even increasing demand, in the fresh form and as processed natural colourants, in the form of paste, paprika and oleoresin (Giuffridaet al., 2013).

The fruit of the *Capsicum* species, which is also known as pepper, is one of the common ingredients used in various cuisines worldwide for its unique flavors, either spicy, hot, sweet or sometimes sour. These unique tastes made some of the general population, especially those who are living around Asia, the Mediterranean and Southern America, to have them in their daily diets either in raw, cooked or processed products. *Capsicum* species are also used traditionally as medicine by the Asian cultures, especially the Chinese and Indian together with the Native Americans (Norazianet al., 2019).

During ripening of the pepper fruits, the synthesis processes are dominant, which condition the storage of characteristic ingredients: coloured matters, sugars, acids, etc. At the end of pepper fruit ripening, there is an intense synthesis of various aromatic substances that are characteristic of different pepper varieties. In the period of full maturity, the pepper fruits acquire the best taste, aroma, color and have the most delicate consistency (Niketić-Aleksić, 1994).

The chemical composition of pepper is particularly complex and variable. It depends on several factors, the most important of which are: the type and variety, climatic conditions, pedological properties of the soil, applied agrotechnical measures, protective measures, degree of maturity, individual stages of development and growth, etc., (Marković & Vračar,1998).

The time of the pepper harvesting is usually determined according to the colour of the pepper, but also based on the shape, the size, the weight of the pepper fruit, the texture, which are characteristic of a given variety (Gvozdenović & Takač, 2004; Ilić et al., 2009).

The degree of ripeness of the pepper fruits after harvest is a significant influencing factor on the changes of properties in the period from harvest to processing. After harvesting and during storage, various processes continue in the fruits, the most important of which are: respiration, evaporation of water and ripening. In doing so, complex chemical processes of decomposition take place, similar to the processes in the stage of overripening, due to which the content of the characteristical and often necessary components for quality is reduced. Due to these processes, peppers mainly lose in quality, changing the taste, smell, colour, consistency and composition, and the intensity of these processes is closely related to the conditions in which the harvested pepper fruits are stored (Niketić-Aleksić, 1994).

The pepper is a carotenoids-rich non-leafy vegetable. Carotenoids are lipophilic yellow-orange-red pigments found in photosynthetic plants, algae and microorganisms. Carotenoids are subgroup of isoprenoid compounds and currently comprise more than 700 characterized structures. They are commercially exploited as food colorants and feed additives and are being used in pharmaceutical, nutraceutical and cosmeceutical products (Ranjith et al., 2014). According to the chemical structure, the carotenoids are tetraterpenoids comprising of a central polyenic chain of nine conjugated double-bonds and a variety of end groups as the chromophore that confers each pigment's properties. Usually, these compounds are divided into two groups: hydrocarbons (commonly known as carotenes) and oxygenated compounds (generally named xanthophylls), that usually occur in esterified forms with fatty acids (Norazianet al., 2019). To further increase the natural variability of these compounds, it has to be considered that the carotenoids can be present in nature as free carotenoids or in a more stable form esterified with fatty acids, in the case of the oxygenated compounds. Moreover, esterification greatly increases during the fruit ripening process (Giuffrida et al., 2013). Hydroxy-carotenoids in fruits and vegetables are predominantly found as esterified with fatty acids. Variations in the number and the position of hydroxyl groups in carotenoids coupled with the availability of variety of fatty acids in nature, results in a wide spectrum of carotenoid esters. In some fruits during ripening, the degree of esterification increases. The esterification is believed to provide a high stability to carotenoids against thermal, photo and enzymatic oxidation reactions (Ranjith et al., 2014).

At the beginning, the fruit is green in colour, which is full of chloroplast containing approximately 68 % chlorophylls, whereas carotenoids at 32 % is at the lowest level. At this stage, the typical chloroplast carotenoids like lutein, violaxanthin, neoxanthin and β-carotene co-exist with and are masked by chlorophylls. As the fruit ripens, the chromoplast carotenoids synthesis occurs through transformation of the existing chloroplast carotenoids and de novo synthesis. Throughout the ripening process, the chloroplast is differentiated into chromoplast containing mixed carotenoids, which contribute collectively to different fruit colours from green to brown, then to yellow, orange, red and/or dark red at the final maturity stage, depending on the cultivars (Norazian et al., 2019).

Based on their biological significance, they are also classified as primary and secondary carotenoids. Primary carotenoids are directly involved in the photosynthesis (β-carotene, zeaxanthin, lutein etc.) and secondary carotenoids (lycopene, α-carotene, capsanthin etc.), do not have direct role in photosynthesis. Interactions with light, heat, oxidants and other reactive chemical species alter the isomerization pattern of carotenoids and thus impart significant changes in their properties (Ranjith et al., 2014).

Niketić-Aleksić (1994) conducted research on the content of carotenoids in green and red pepper fruit (*Capsicum annuum*). They determined that the green pepper fruits contain: lutein (0.276 mg/100 g), β-carotene (0.095 mg/100 g), violaxanthin (0.042 mg/100 g) and cryptoxanthin (0.027 mg/100 g). The red pepper fruits contain: capsanthin (9.60 mg/100 g), β-carotene (2.35 mg/100 g), zeaxanthin (1.75 mg/100 g), capsorubin (1.46 mg/100 g), cryptoxanthin (1.10 mg/100 g) and violaxanthin (0.70 mg/100 g). According to Minguez-Mosquera & Hornero-Mendez (1994), the content of total carotenoids can be increased from 34.76 mg/kg in green pepper fruit up to 962.50 mg/kg in red pepper fruit.

Most of the vitamins from group A (retinol) are in the form of precursors. In 2001, the US Institute of Medicine determined that 1 mg of retinol is equivalent to 12 mg of β-carotene or 24 mg of other provitamin A carotenoids (Coultate, 2011).

The presence of the vitamin A in peppers is conditioned by the high content of carotenes (β-carotene), as well as cryptoxanthin. It has also been established that there is almost the same amount of vitamin A in a ripe fruit as in a carrot. The following pigments are represented in the pepper: capsanthin, capsorubin, zeaxanthin, lutein, cryptoxanthin, α-carotene and β-carotene (Gvozdenović & Takač,2004).

The processed peppers include the following products: quickly frozen, sterilized, marinated (in marinade-vinegar), biologically preserved, dried, and pepper sauce and other peppers products. During the production of these products, various preservation technologies are applied, including the following: freezing, application of high temperature in tin or glass packaging, bottles and other packaging, drying (dehydration) and other innovative technologies. The processed peppers are produced in accordance with the Regulations of the requirements regarding the quality of processed products from fruits and vegetables, as well as mushrooms and their processing (Official Gazette of the Republic of Macedonia, number 69/2014) and the Regulations of the special requirements for the safety of quick-frozen food (Official Gazette of the Republic of Macedonia, number 16/2013), whose regulations mainly correspond to international norms.

During the production of pasteurized pepper, if the pasteurization process is not performed at the appropriate temperature or if the jar of the finished product is not hermetically sealed, it may get spoiled after a few days. At the same time, under the action of microorganisms, changes in the composition and sensory properties of the product may occur, due to the decomposition of individual ingredients of the product and the creation of new ones, with which the finished product becomes sensorially unacceptable and may be harmful to human health (Cvetkov, 1982).

According to Jašić (2007), the colour evaluation is often important, especially for the final products, because the food spoilage is associated with colour change. The liquid solution of the pasteurized pepper should be mostly clear, with a beautiful light-yellow color, with a slightly acidic taste, a pleasant smell, characteristic of pepper. The appearance of a slightly opalescent pouring solution in some samples is related to the degree of maturity of the pepper (Marković & Vračar, 1998).

According to Marković & Vračar, (1998), during the quality control, the frozen pepper that is put on the market is necessary to meet certain conditions, which mainly refer to sensory properties: to be clean, healthy and without foreign admixtures; the aroma, the color and the taste are characteristic of the variety from which it is produced; in the case of cut pepper, it is necessary that the parts have equal dimensions: size and shape. Vereš (2004) indicates that it is normal for the frozen products to deteriorate in quality during storage. It is considered that in practice is acceptable a frozen product that retains at least 70 % of the initial quality.

During the drying process, the product must not have significant changes and losses of the important components in relation to the fresh raw material (sugars, proteins, minerals, vitamins, etc.), but also of the sensory properties, smell, taste, appearance, colour, i.e. the product after drying should preferably retain its natural properties (Cvetkov, 1982).

Drying is a vital phase in the processing of the spice red-coloured peppers, and it usually has a negative impact on the finished product’s qualitative features and nutritional content. As a result, there have been a several research attempts to adopt a drying strategy that causes minimum quality loss while also improving the crop stability and safety (Se Souza et al., 2022).

Vereš (2004) states that changes can occur due to the action of oxygen, which is found in the atmosphere. Also, traces of metals often cause undesirable changes in color, taste and aroma. If there is a mechanical damage to the living tissues, some oxidation-reduction reactions are initiated. Namely, the oxidation reactions lead to the deterioration of the sensory properties of the products. Oxidative changes during the storage of the dried products can be minimized by procedures such as: appropriate storage conditions (temperature), packaging in air-tight packaging, packaging in an inert gas atmosphere or by applying antioxidants.

The colour is one of the most important indicators of the quality of the dried peppers, and in many products it surpasses even the importance of the taste. In general, it can be said that the dried pepper is required to have a specific intense and uniform colour. Pale colour, dull colour, "dead" colour, or improper colour change due to manufacturing defects are serious drawbacks when evaluating the quality of the dried pepper. The presence of yellow, brown or terracotta colour is undesirable for red pepper. For high-quality dried pepper, it is necessary to introduce colour monitoring during the individual processing operations, starting from the storage of the fresh pepper, and ending with the storage conditions of the final product (Marković & Vračar, 1998).

According to Vereš (2004), maintaining the quality of the product implies that it preserves the nutritional ingredients and properties, such as colour, smell and taste of the preserved food, during the prescribed period of use, and, at the same time, meets the requirements for a health-safe product.

The primary goal of this study was to determine the differences and the changes of carotenoids composition in the three varieties of the red pepper, fresh and processed, when using traditional procedures often used in the large-scale production. We also aimed at evaluating the content and composition of carotenoids, by using HPLC-DAD methods developed by the research group from Institute of Horticultural Sciences, Hungarian University for Agricultural and Life Science, Godollo″, Hungary.

MATERIALS AND METHODS

In this research were used are three varieties of sweet pepper (*Capsicum annuum* L.). The varieties *kurtovska kapija* and *palanechko chudo* (Figure 1.) are used in the processing industry of freezing or canning vegetables, while the spicy variety of *horgosh* (Figure 1.) is used for drying and obtaining ground or milled red peppers. The peppers were harvested at technological maturity, when the pepper fruits are characterized by an intense red color and have reached the maximum varietal characteristics.

Figure 1. Examined varieties of fresh peppers: *kurtovska kapija, palanechko chudo, horgosh*

The carotenoids content was analyzed in all examined varieties of fresh peppers, as well as in their processed pepper products, obtained by drying, freezing and pasteurization. For this purpose were used standard laboratory methods for preparation, extraction and quantification.

Sampling is the first and very important step in analysis. For proper analysis it is important to have representative sample. Homogenization and subsampling may be done simultaneously or sequentially in an interchangeable order. Depending on the sample condition, physical operations are carried out, such as chopping, cutting into pieces, mixing, milling, blending, and sieving. The process can be done manually or through commercially available mills, blenders, grinders, riffle cutters, etc.

The varieties of fresh peppers, as well the pepper products obtained by drying, freezing and by pasteurization was prepared by following procedure: 2.5-3 g representative samples were grind (cut), blended or milled to get a homogenized sample. Where necessary, the samples were crushed in a crucible mortar in presence of quartz sand. Then was gradually added 20 mL of methanol with continuous crushing and decanted the supernatant into conical flask with a stopper carefully and quantitatively. The crushing of the residues was continued with gradual addition of a mixture of methanol-dichloroethane (10 mL + 50 mL) and was poured the supernatant carefully into the flask with the previous one. The samples were transferred to the separation funnel, where the lower solvent layer contains the carotenoids. Then, the water from the pigment-containing solvent was removed by dropping through anhydrous Na_2SO_4 in a filter paper. The samples were evaporated under vacuum at max. 40 °C and the residues were dissolved in 5 mL of HPLC eluent or suitable solvent (Figure 2).

Figure 2. Extraction method of carotenoids

Carotenoids should be detected between 200 and 700 nm. For quantification, spectrum of each peak should be drawn and manual integration must be done at the maximum wavelength for each peak. The calibration procedure is also applicable, by the following steps: weigh 5 mg of standard carotenoids available; dissolve it in 10-25 mL of hexan or chlorophorm depending on the component; make four different dilutions (working solutions) with acetone or HPLC eluent; inject these solutions onto the HPLC column under the same conditions of sample and record peak area and make the calibration curves. Using calibration data calculate the concentration of a selected carotenoid component by relating peak area in the samples to that of standard calibration.

The determination of carotenoids was performed by using HPLC method, with photodiode-array detector. For separation and quantification of the carotenoids was used HPLC Column, Nucleosil 100-3 C18, 250 x 4,6 mm, flow rate: 0,7 mL/min and gradient program with eluent A (7 % water in methanol); and eluent B (55 % isopropanol+35 % Acetonitrile+10 % methanol). The run was performed for 45 minutes, with detection at 470 nm (Figure 3).

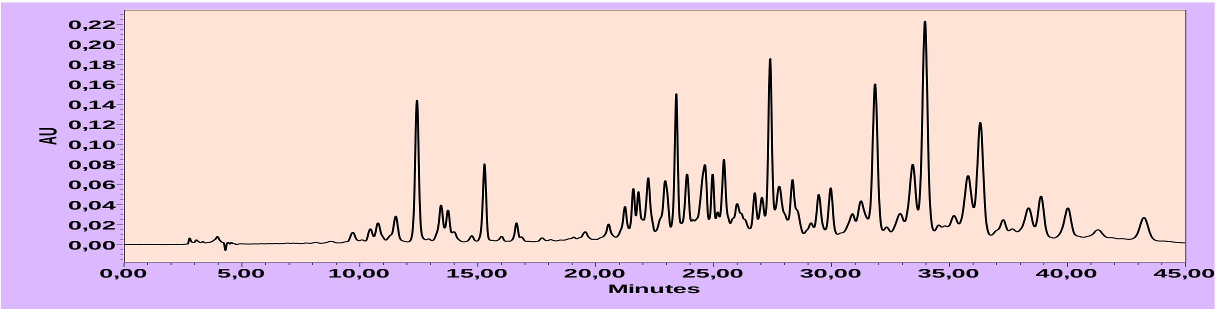


Figure 3. HPLC profile of carotenoids

Based on the obtained results, a comparison was made of the changes in the content of carotenoids that occur during the application of freezing, pasteurization or drying technology. Carotenoids are important bioactive components, especially β-carotene, as a provitamin of vitamin A, which is of great biological value for humans. Carotenoids are components sensitive to light and high temperature. Therefore, the influence of the applied technology (application of low or high temperatures) on the preservation of these components in the finished product is of great importance.

Based on the obtained results, a statistical analysis of the results was done by using statistical methods ANOVA. Standard statistical operations were applied in the program Microsoft Excel, 2010, as well as a statistical software package for statistical analyzes (IBM SPSS Statistics 21), for determination of LSD (Least Significant Difference), at the level of statistical significance $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

The processing industry has a series of requirements regarding the quality of fresh pepper, for which some minimum conditions need to be met (Tijskens, 2000). It is especially important for processing to use appropriate varieties of pepper as raw material, which will be in the stage of technological maturity, with appropriate properties, in order to obtain quality products. Of particular importance for the pepper fruits are their mechanical characteristics (randomness, shape and size), also, they should be healthy, without physiological, mechanical or any other damage (Niketić-Aleksić, 1994). Also, it is important to conducting appropriate production procedures and conditions.

According to the international and national regulations, the peppers are primarily sorted - classified, according to variety, colour and size. It is preferable that the pepper fruit varieties intended for processing to be harvested in full maturity, when the peppers are red in colour. The market value of the red pepper depends mainly on the red color (Chen et al. 1999).

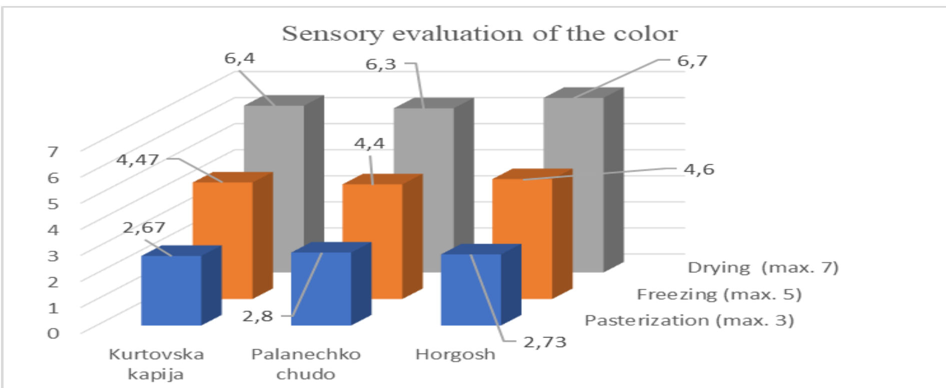
When purchasing and receiving the pepper, the visual appearance is evaluated first. During the sensory analysis of the colour, the intensity, the shade, the uniformity of the colouris particularly significant for the quality, in term of the sensorial properties of the processed products. The term colour refers to the presence of coloured matters in products, where the colour intensity directly depends on the content of the pigments in the product itself (Karakashova et al., 2013).

In our research, a sensory analysis was made regarding the color of the examined varieties, according to which: the *kurtovska kapija* and p*alanechko chudo* varieties were characterized by a red color with several shades, that are characteristic of the variety, from light to dark red, acceptable for the purpose; the *horgosh* variety had an intense red colour, characteristic of the variety.

Karakashova et al., (2013) point out that the sensory quality of the pepper are important quality characteristics. The changes of the different sensorial properties: appearance, color, taste, firmness and texture, as well as the changes that can occur during production itself and under certain conditions of storage until processing, need to be monitored and maintained within acceptable quality limits. Therefore, sensory analysis is needed, which is an effective instrument for measuring and monitoring the sensory quality of food.

Regarding the individual sensory properties, all examined processed peppers products were included in sensory analysis of their colour, according to the scoring by points method. When evaluating the colour, different number of maximum points were used in different processed pepper products (Karakashova et al., 2013). With the frozen pepper products, it can be noted that the best colour (4.67 - average number of points, out of max. 5) have the frozen products of the *horgosh* variety; according to the results, in the tested varieties of dried pepper, the best colour (6.73 - average number of points, out of max. 7) has the dried pepper of the *horgosh* variety. For the pasteurized products, the best colour (2.80 – average points, out of max. 3) has been estimated for the pasteurized pepper of the *palanechko chudo* variety. These results from the sensory evaluation of the colour are presented in the Graph 1.

The obtained results from the sensory analysis of the colour in the different processed pepper products were statistically evaluated with the ANOVA statistical method, in order to determine the effects of the factors variety and technology on the number of points. It was determined that the variety and the technology as factors, as well as the interaction variety - technology, have a statistically significant influence on the average number of points for colour, as a variable. At the same time, it was determined that the lowest loss of colour, was determined in the *hrogosh* variety, after applying the processing methods with pasteurization, freezing and drying.



Graph 1. Average points for colour, obtained by sensory analysis of processed peppers varieties *kurtovska kapija*, *palanechko chudo* and *horgosh*

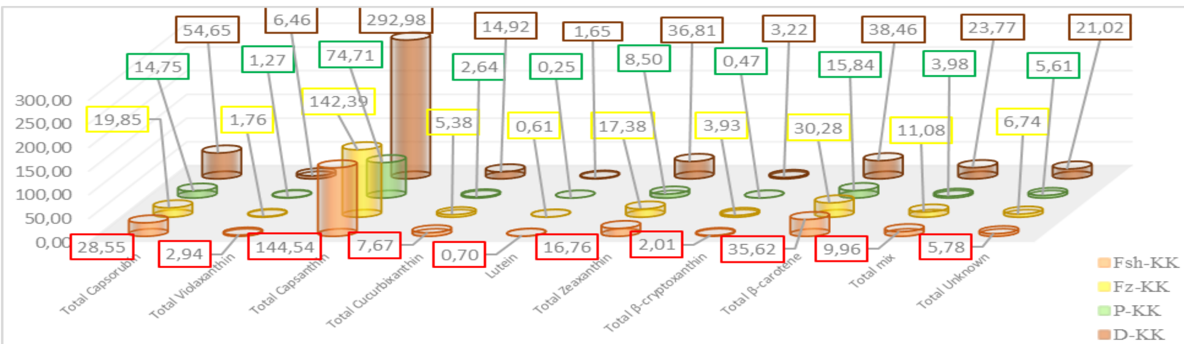
The pepper has a specific and rich chemical composition. The high nutritional value is due to the content of carbohydrates, proteins, fibers, oils, organic acids and mineral matters. The high biological value is also due to the content of vitamins, capsaicin, coloured matters and essential oils (Niketić-Aleksić, 1994; Marković & Vračar, 1998).

The most important pigments capsanthin and its isomer capsorubin, are represented from 30 to 60 % and 6 – 18 %, respectively, in relation to the total amount of carotenoids in the fruits (Weissenberg et al., 1997; Nadeem et. al., 2011). Jarén-Galán & Mínguez-Mosquera, (1999) determined that the capsanthin and capsorubin constitute 65-80 % of the total amount of the red pepper.

Marković & Vračar, (1998) indicate that the percentage content of the individual color matters is as follows: capsanthin about 70 %, capsorubin about 14 %, carotene about 6 %, zeaxanthin 5 %, cryptoxanthin, violaxanthin and the others about 5 %. The capsanthin is one of the main carotenoids in ripe pepper fruits besides carotene (Hoshi et al., 1998).

In this research, during the analysis of the carotenoids content in fresh and processed pepper by using the HPLC method, 47 carotenoids were separated and quantified, of which 5 were unknown and other 42 were in the form of free, monoesters (ME), and diesters (DE) bearing distinct fatty acid moieties. According to the chemical structure of carotenoids that were detected and quantify, they were grouped into the following groups: total capsorubin, total violaxanthin, total capsanthin, total cucurbixanthin, lutein, total zeaxanthin, total β-cryptoxanthin, total β-carotene, total mix and total unknown carotenoids. Based on the obtained results, the total values for each group of carotenoid compounds were calculated, where by their representation in each variety of pepper, fresh and processed, are presented in Graph 2, Graph 3 and Graph 4.

From the Graph 2 it can be notice that the highest values of mostly all groups of carotenoids are present in the dried pepper variety *kurtovska kapija*. Also, it was determined that the highest values of total capsanthin in fresh and all processed peppers. According to the determined content of carotenoids, as the second group of carotenoids that are represented in significant quantities in all analyzed peppers are total β-carotene.



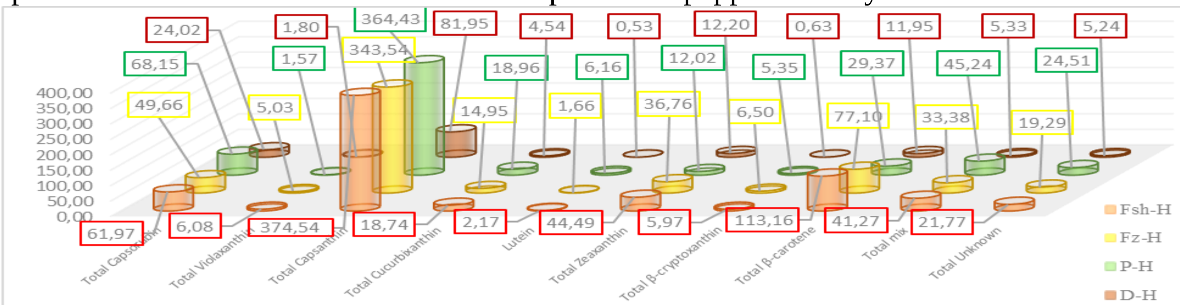
Graph 2. Total values for each group of carotenoids in fresh (Fsh) and processed (Fz-frozen, P-pasteurized, D-dried) variety of pepper *kurtovska kapija* (μg/g)

Based on the obtained results, the percentage representation was calculated of each group of carotenoids, in relation to the total amount of carotenoids, determined in the analyzed peppers varieties *kurtovska kapija*, fresh and processed, what is presented in the Table 1. It can be noted that the contents of total capsanthin have the highest values and these are in range from 56.78 % in fresh peppers, up to 59.48 % in frozen peppers. The second highest values were determined for total β-carotene, ranging from 7.79 % for the dried peppers to 14.00 % for the fresh peppers of the *kurtovska kapija* variety.

Table 1. Total values (%) for each group of carotenoids in fresh and processed pepper, *kurtovska kapija* variety

Kurtovska kapija	**Fresh**	**Frozen**	**Pasteurized**	**Dried**
Total capsorubin(%)	11.22	8.29	11.52	11.06
Total violaxanthin (%)	1.16	0.73	1.00	1.31
Total capsanthin (%)	**56.78**	**59.48**	**58.35**	**59.32**
Total cucurbixanthin (%)	3.01	2.25	2.06	3.02
Lutein (%)	0.28	0.25	0.20	0.33
Total zeaxanthin (%)	6.58	7.26	6.64	7.45
Total β-cryptoxanthin (%)	0.79	1.64	0.37	0.65
Total β-carotene (%)	**14.00**	**12.65**	**12.37**	**7.79**
Total mix (%)	3.91	4.63	3.11	4.81
Total unknown (%)	2.27	2.82	4.38	4.26

In the Graph 3 are presented the total values for each group of carotenoids in variety of pepper *horgosh*, fresh and processed. It can be noted that the highest values of the total capsanthin were determined in fresh and all processed peppers variety.



Graph 3. Total values for each group of carotenoids in fresh (Fsh) and processed (Fz-frozen, P-pasteurized, D-dried) variety of pepper *horgosh* (μg/g)

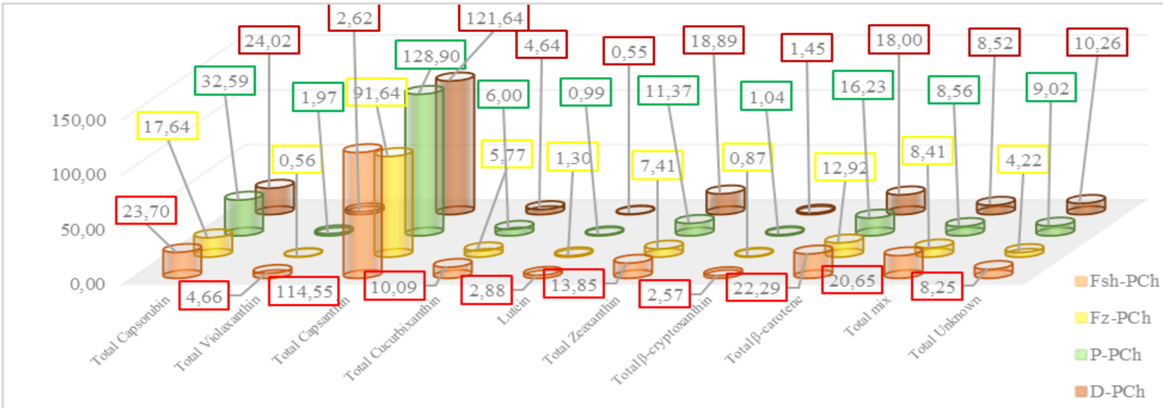
Also, it was determined the higher content of total capsorubin in all forms of analyzed peppers, as well as the high content of total β-carotene in all analyzed peppers, of the variety *horgosh*.

The determined values (%) of each group of carotenoids, in relation to their total amount, in the analyzed peppers varieties *horgosh*, fresh and processed, are presented in the Table 2. It can be noted that the highest values of the content of total capsanthin were between 54.27 % in fresh peppers, up to 63.29 % in pasteurized peppers. For this variety, the higher values were determined for total β-carotene, ranging from 5.10 % for the pasteurized peppers up to 16.40 % for the fresh peppers, as well as for total capsorubin, what was between 8.45 % in the frozen peppers and 17.33 % in the dried peppers.

Table 2. Total values (%) for each group of carotenoids in fresh and processed pepper, *horgosh* variety

Horgosh	**Fresh**	**Frozen**	**Pasteurized**	**Dried**
Total capsorubin (%)	8.98	8.45	11.84	17.33
Total violaxanthin (%)	0.88	0.86	0.27	1.30
Total capsanthin (%)	**54.27**	**58.44**	**63.29**	**59.11**
Total cucurbixanthin (%)	2.71	2.54	3.29	3.27
Lutein (%)	0.31	0.28	1.07	0.38
Total zeaxanthin (%)	6.45	6.25	2.09	8.80
Total β-cryptoxanthin (%)	0.87	1.11	0.93	0.45
Total β-carotene (%)	**16.40**	**13.11**	**5.10**	**8.62**
Total mix (%)	5.98	5.68	7.86	3.84
Total unknown (%)	3.15	3.28	4.26	3.78

The total values for each group of carotenoids in variety of pepper *palanechko chudo*, fresh and processed are presented in the Graph 4. The content of total capsanthin was with the highest values in fresh and all processed peppers. Also, it was determined the higher content of total capsorubinand total β-carotene in all analyzed peppers.



Graph 4. Total values for each group of carotenoids in fresh (Fsh) and processed (Fz-frozen, P-pasteurized, D-dried) variety of pepper *palanechko chudo* (µg/g)

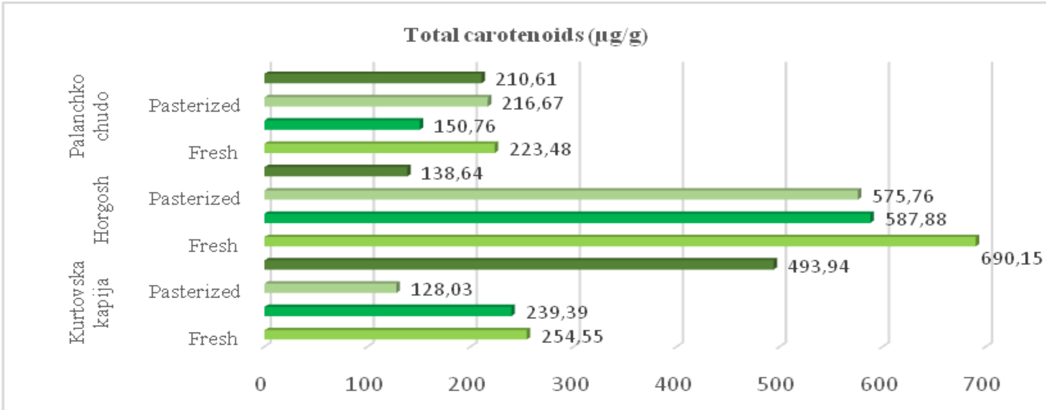
In the Table 3 are presented the total values (%) for each group of carotenoids, in relation to their total amount of carotenoids, in fresh and processed variety of pepper *palanechko chudo*. The highest percentage representation was determined for total capsanthin, what was 51.26 % in fresh peppers, up to 60.78 % in frozen peppers. The highest percentage representation was determined for total capsanthin, what was 51.26 % in fresh peppers, up to 60.78 % in frozen peppers. For this variety, the second highest values were determined for total capsorubin, ranging from 10.60 % for the fresh peppers up to 15.04 % for the pasteurized peppers.

Table 3. Total values (%) for each group of carotenoids in fresh and processed pepper, *palanechko chudo* variety

Palanechko chudo	**Fresh**	**Frozen**	**Pasteurized**	**Dried**
Total capsorubin (%)	**10.60**	**11.70**	**15.04**	**11.40**
Total violaxanthin (%)	2.09	0.37	0.91	1.24
Total capsanthin (%)	**51.26**	**60.78**	**59.49**	**57.76**
Total cucurbixanthin (%)	4.51	3.83	2.77	2.20
Lutein (%)	1.29	0.86	0.46	0.26
Total zeaxanthin (%)	6.20	4.92	5.25	8.97
Total β-cryptoxanthin (%)	1.15	0.58	0.48	0.69
Total β-carotene (%)	9.97	8.57	7.49	8.55
Total mix (%)	9.24	5.58	3.95	4.05
Total unknown (%)	3.69	2.81	4.16	4.88

It should be noted that all obtained results for the content of carotenoids, individually or as a group, were not calculated on dry matter.

In the Graph 5 are presented the content of total carotenoids (µg/g) in fresh and processed peppers, by freezing, pasteurization and drying, for all examined varieties, *kurtovska kapija*, *palanechko chudo* and *horgosh*.



Graph 5. Content of total carotenoids in fresh (Fsh) and processed (Fz-frozen, P-pasteurized, D-dried) peppers, for all examined varieties (µg/g)

From the Graph 5 it can be notice that the highest values of total carotenoids content are present in all analyzed peppers products of the variety *horgosh*, as fresh (690.15 µg/g), as well as in pasteurized (575.76 µg/g), in frozen (587.88 µg/g) and in dried 575.76 µg/g.

CONCLUSION

From this research it was determined that the variety and the technology as factors, as well as the interaction variety - technology, have a statistically significant influence on the average number of points for colour, as a variable. At the same time, it was determined that the lowest loss of colour, was determined in the *hrogosh* variety, after applying the processing methods with pasteurization, freezing and drying.

From the obtained results, it was estimated that the highest values of mostly all groups of carotenoids, are present in the dried pepper of the variety *kurtovska kapija*. Also was found, that the highest percentage representation was determined for total capsanthin (%) in all pepper varieties *kurtovska kapija*, *horgosh* and *palanechko chudo*, as in fresh, as well in all processed peppers.

The contents of total capsanthin were in range from 56.78 % in fresh peppers, up to 59.48 % in frozen peppers, variety *kurtovska kapija*. For the varieties of peppers *kurtovska kapija* and *horgosh*, it can be noted the higher contents of total capsorubin and β-carotene in all analyzed peppers. For the pepper variety *horgosh*, the content of total capsanthinwas from 54.27 % in fresh peppers, up to 63.29 % in pasteurized peppers. In the variety of pepper *palanechko chudo*, the highest values were determined for total capsanthin (%), what was 51.26 % in fresh peppers, up to 60.78 % in frozen peppers.

With the analysis of variance (ANOVA) for the examined fresh and processed pepper, it can be concluded that the processing technology has a statistically significant influence on the content of total carotenoids. When the interaction between the pepper variety and the technology was included as a factor, it showed a statistically significant difference for the content of the total carotenoids.

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