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CHEMICAL VARIABILITY OF INEDIBLE FRUIT PARTS IN PEPPER VARIETIES (*CAPSICUM ANNUUM* L.)

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Abstract

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Pepper is an important vegetable widely used for human nutrition and industrial processing. It is used dried or fresh in various pharmacological preparations and as a source of biologically active compounds, such as flavonoids, phenols, carotenoids, capsaicinoids and vitamins. In this paper, the morphological characteristics of the fruit and chemical characteristics of the inedible parts of eight varieties of peppers were studied. To our knowledge, chemical analyses of inedible parts in these varieties, especially of seminal placenta, were not performed. During the processing of the peppers, seeds and seed lodges are separated from a pericarp and dismissed as redundant, and this excludes the precious substances in the human diet. Varietal specificity of the observed parameters was expressed. The seeds of Una variety, and other varieties with 15% oil, could be used to extract edible oil. Seed lodges of the varieties Plamena, Amphora and SM 1 can be used as a food additive rich in cellulose. High concentration of phenolic compounds was found in hot varieties, SM 1 and Plamena, followed by sweet pepper Una. These findings suggest the use of inedible parts of Plamena, Amphora, Una and SM 1 in production of food supplements in order to enrich the human nutrition with these substances. The Vranjska variety had the lowest content of dry matter, cellulose and calcium - pectate, which diminishes its importance in human nutrition. Measurement of fresh mass of fruit parts was performed to illustrate participation of inedible parts in the total fruit mass.

Key words: pepper, cultivar, seed, oil, phenol content, color, cellulose, calcium pectinate

Introduction

Pepper (*Capsicum annuum* L.) is a crop native to South and Central America and represents a very old vegetable crop that was grown during the ancient Incan civilization. Nowadays in the world, pepper is cultivated mostly in Asia, Europe and America, mainly in temperate thermal zone, sometimes in the tropics, while in Europe, it is grown in the southern zone (Markovic and Vracar, 1998). Originally grown as an ornamental plant, and then as a spicy herb, pepper has become one of the most important vegetable species in the world (Gvozdenovic and Takac, 1997). The use of pepper in the human diet is very widespread (in household, industrial, pharmaceutical, cosmetics), primarily because of its high nutritive and biological value. In addition to the use of fresh (raw) state, a large number of foods, pharmaceutical and cosmetic products contain some of the constituents of pepper. There are few plant species in nature, such as pepper, whose usage is so broad (Tepic, 2005).

The nutritional value, as well as a spiciness and aroma, make them popular in ethnic foods in the U.S. (Broderick and Cooke, 2009), and other parts of the world. For instance, annual production of green pepper in the U.S. increased from 467.500 tons in 1990 to 893.610 tons in 2005, while in China from 3.119.220 to 12.523.000 tons during the same time period (Food and Agriculture Organization, 2007).

Capsaicinoids (capsaicin, dihydrocapsaicin) are alkaloid components responsible for distinctive taste of pepper fruits and seeds. Capsaicin accumulates in the fruit placenta and interlocular partitions, from where it is transported into

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seeds, while its level in the pericarp remained low (Markovic and Vracar, 1998). Recent investigations of different pepper tissues using fluorescence microscopy suggested mobilization of capsaicinoids out of epidermal cells of placenta tissues and their deposition out of the cell walls, namely storage in the apoplast (Broderick and Cooke, 2009). A ten fold higher capsaicin accumulation was found in plancenta than in pericarp and seeds (Pandhair and Sharma, 2008). Individual fruits from the same plant exhibit variable capsaicinoid contents (Mueller-Seitz et al., 2008). Consumption of fresh or processed pepper excludes placental tissues and seeds together with these substances valuable for human health. Furthermore, pepper is used dried or fresh in various pharmacological preparations and has a long history as a source of biologically active compounds, such as flavonoids, phenols, carotenoids, capsaicinoids and vitamins (Cantrill, 2008).

Pepper seed quality is determined by biological and agroecological conditions during cultivation, and represents a variety characteristic (Gvozdenovic et al., 1995). Seeds are attached to central and lateral placentas in the fruit. Seed quality is determined by its chemical composition. On the one hand, it is important for the yield stability and level (Bosland, 1996). On the other hand, it seems to be important in the production of pepper powder, since the seeds are included in a technological process along with the pericarp (Minguez-Mosquera et al., 1993). Different pepper varieties differ in their use value. There are varieties that are used fresh, while spicy pepper and hot pepper are used for industrial processing. Capsicum fruits contain colouring pigments, pungent principles, resins, proteins, cellulose, pentosans, mineral elements and low levels of volatile oil (Cantrill, 2008). The biochemical composition of vegetables is very important especially of unprocessed vegetables. Previous studies have shown that the mineral substances in pepper are mostly nitrogen, potassium, calcium, phosphorus, magnesium and iron (Krstic et al., 2010). According to the above, the purpose of our project was to examine the chemical heterogeneity of seeds and seed lodges in eight varieties of peppers. During the processing of the peppers, seeds and seed lodges are separated from a pericarp and dismissed as redundant, and this excludes the precious substance in the human diet. To our knowledge, chemical analyses of inedible parts in these varieties, especially of seminal placenta, were not performed. Measurement of fresh mass of fruit parts was performed to illustrate participation of inedible parts in the total fruit mass.

Materials and Methods

Experimental design and sampling procedure: The study was done using the trial fields at Rimski Sancevi, of the Institute of Field and Vegetable Crops in Novi Sad (Serbia). The following pepper varieties were used: SM 1, Vranjska, Anita, Krusnica, Amfora, Una, Novosadjanka and Plamena. Each variety of pepper was planted in a randomized block design, with three replications of 20 plants. Before transplanting plants, added to the soil was 400 kg of NPK fertilizer (nitrogen:phosphorus:potassium 15:15:15) per hectare and, during the growing season, as well as 100 kg of KAN fertilizer (calcium ammonium nitrate) per hectare. The fields were irrigated nine times, loosened up and turned over soil three times and the trial was once treated with insecticides DECIS + FASTAC. The sampling was done at the stage of technological maturity of the fruit. Five plants from each repetition, of each variety (fifteen in total) were used. After sampling, the weight of the whole fresh fruit, the edible part of the fruit (pericarp) and inedible part of the fruits (seminal placenta and seeds) have been determined. From obtained data, the share of certain parts of the fruit in their total mass was calculated.

Chemical analyses: Dry matter content in the seminal placenta was determined by drying at 105°C to the constant weight. Cellulose content was determined by the method of Kirchner-Ganakova (Vracar, 2001), and is based on treating the sample with a hot acid solution, where oxidation, hydrolysis, and nitration of all substances that accompany cellulose occurred. Determination of pectin in the form of Ca-pectate was performed by the Carre-Haynes method (Ciric et al., 1975) in which pectin was directly deposited with organic agent in a way that pectin underwent saponification into the form of Na-pectate, and then precipitatation with calcium. Total phenol content was determined by the Folin-Ciocalteu (FC) colorimetric method (Waterhouse, 2002) based on the reduction of chemical reagent, a mixture of tungsten and molybdenum oxide. The products of metal reduction had a blue colour that shows a broad absorption spectrum with maxima at 765 nm. Quantitative determination of all lipid components in plant cells can be effectively carried out by extraction with nonpolar solvents. For the extraction of crude lipids and their quantitative determination was used Soxhlet apparatus (Arsenijevic-Maksimovic and Pajevic, 2002).

Fruit color: The surface colour and lightness of the fruit were determined using Minolta CR 400 Chromameter measuring the dominant wavelength (Randjelovic, 2009). Lightness of colour is expressed by design trails Hunter (Hunter, 1960).

Statistical analysis: The obtained results were statistically processed by analysis of variance (ANOVA), and the ranking of mean values of measured parameters was performed by Duncan's test for the significance level of $P<0.05$. Values marked with the same letters are not considerably different for the applied level of significance.

Results and Discussion

Fresh mass of fruit parts

In addition to the number of fruits per plant, an important trait that determines the yield is fruit mass. The largest of the mass comes from the edible parts of fruit and pericarp (Table 1). Studied cultivars differed significantly by mass of the fruit itself and mass of some parts of the fruit. Varieties Amfora, Anita and Vranjska had the highest fruit mass. The lowest fruit mass had varieties Krusnica (40.54 g) and SM 1 (39.22 g).

There can be 70 to 600 seeds in a single pepper fruit and a mass of 1000 seeds ranges from 5 to 7 g (Markovic and Vracar, 1998). The tested varieties differed in seeds mass (Table 1). The highest seeds mass had a variety Amfora (2.47 g), followed by Una (1.91 g) and Anita (1.78 g). The varieties with lowest fruit mass, Krusnica and SM 1, have also the lowest seeds mass. An interesting piece of data is the ratio of total seeds mass to fruit mass (Table 2). The highest percentage of the seeds mass in the total fruit mass had Krusnica (3.35%).

The percentage share of pericarp and inedible parts in total pepper mass varied among the varieties (Table 2). The largest share of non-edible parts was found in the cultivars Novi Sad (21.16%) and Krusnica (21.75%). Interestingly, the cultivar Amfora, which has the largest fruit mass, the ratio of seed mass to fruit mass is the lowest, and is only 13.6%. Among other factors, the mass of the pericarp is partly determined by its thickness. Comparative anatomical analysis of the pericarp of several pepper varieties showed that the pericarp of variety Novosadjanka is thicker than of the varieties Plamena and Krusnica (Merkulov et al., 2000). They suggest that the varieties Plamena and Krusnica have a larger portion of exocarp in relation to the total thickness of the pericarp, which favours their use in industrial processing. Spicy pepper varieties have better developed exocarp in relation to the fleshy fruits (Fischer, 1974).

The values obtained for fruit mass were above the expected when compared with results of other authors for the same variety, which probably can be explained by different environmental conditions during cultivation and harvest time. In this study, fruit mass in the variety Anita was 122.42 g, which is significantly higher than the results from Jozic (2000) who found the same variety of fruit mass 71.03 g. Similar results were obtained when measuring the mass of fruits of other varieties of peppers. The variety of pepper has to meet certain criteria, namely: that is acclimated to certain growing conditions, the fruit mass more than 100 g, the thickness of flesh (pericarp) over 5 mm, to have a good root system, to submit the appropriate circuit plants per unit area, to form a 15-20 market fruits per plant, while fruits must be of a good quality (balanced vitamins, sugars and amino acids) (Gvozdenovic et al., 1996). Peppers require a large amount of water due to poorly developed root systems and high overhead of organic production, and have high requirements in terms of light intensity, with more response to the intensity of light than the length of day (Gvozdenovic and Vasic, 1997). Environmental conditions in the year of the experiment were extremely favorable for the development of pepper, which suggests the data about fruit mass.

Fruit color

The colour of pepper depends on the stage of maturity. At the technological maturity, the colour of the fruit can be: white, pale yellow, light green, green, dark green or purple. At the stage of physiological maturity, the fruit colour can be: light-red, burgundy, orange or yellow. Of all the coloured substances found in pepper, the most important are carotenoids. Carotenoids, the largest group of plant pigments, function as antioxidants and as vitamin A precursors (Guzman et

Table 1
Morphological characteristics of fruit in pepper cultivars

	Fresh mass of (g per fruit)			
Variety	Fruit	Pericarp	Placenta	Seeds
Anita	122.42 ^{b}	103.35 ^{b}	16.57 ^{b}	1.78 ^{bc}
Vranjska	121.92 ^{b}	97.92 ^{b}	19.57 ^{a}	1.58 ^{bc}
Novosadjanka	65.57 ^{d}	50.19 ^{d}	12.46 ^{c}	1.42 ^{cd}
Amfora	157.91 ^{a}	135.86 ^{a}	18.27 ^{ab}	2.47 ^{a}
Una	82.73 ^{c}	69.70 ^{c}	10.42 ^{cd}	1.91 ^{b}
Plamena	67.88 ^{d}	56.12 ^{d}	9.60 ^{de}	1.51 ^{bc}
SM 1	39.22 ^{e}	32.63 ^{e}	4.90 ^{f}	1.03 ^{d}
Krusnica	40.54 ^{e}	30.78 ^{e}	7.48 ^{e}	1.36 ^{cd}

Means within a column followed by the same letter are not significantly different at $P<0.05$

Table 2
The share of pericarp and inedible parts in fruit total fresh mass, %

Variety	Share of seminal placenta	Share of seeds	Total (seeds + seminal placenta)
Anita	13.5	1.45	14.80
Vranjska	16.0	1.29	17.34
Novosadjanka	19.0	2.16	21.16
Amfora	11.5	1.56	13.06
Una	12.6	2.30	14.90
Plamena	14.1	2.22	16.30
SM 1	12.5	2.62	15.12
Krusnica	18.4	3.35	21.75

al., 2010). All carotenoid pigments present in red peppers are isoprenoids of 40 carbon atoms, with nine conjugated double bonds in the central polyenic chain, with different terminal groups that change the chromophore properties of each pigment (Ayuso et al., 2008). Fruit color changes during ripening are due to the difference in the distribution of individual carotenoids, while the ripe fruit colour varies among cultivars (Ha et al., 2007). The green color of pepper is derived from chlorophylls and carotenoids. When the maturation begins, the fruit gets orange, and finally the intense red color. This change is mainly due to the newly synthesized oxygenated carotenoids, including beta-carotene, capsanthin and capsorubin, which make up to 65-80% of the total colour of the red peppers, and are unique to the fruits of this genus (Mínguez-Mosquera and Hornero-Méndez, 1994; Guzman et al., 2010). Carotenoid composition of pepper depends on weather conditions during ripening, growing conditions, harvesting time (stage of maturity during harvest) and from cultivars or varieties of peppers (Tepic, 2005). The specific red colour of pepper makes it an attractive addition to food to enhance taste or colour (Mínguez-Mosquera et al., 2008). Ground powder of dried pepper has traditionally been used as food colorant, while concentrated oleoresins are more widely used today in the food industry (as natural flavouring and as colouring agent for as spicy culinary, meat products, cheese, food coatings, popcorn oil) (Cantrill, 2008).

Between studied pepper cultivars, there was a little variation in wavelength (λ) and thus the colour of pepper (Table 3). The largest wavelength was observed at cultivar Plamena (615 nm), which corresponds to a pink colour in the chromaticity diagram CIELAB system. The smallest wavelength is measured in the cultivar Anita (594 nm) and thus the color is yellow-green. In other varieties of pepper the colour is orange-red, and there is a great variability of wavelengths. Recent investigations suggest variability of phenolic compounds, ascorbic acid and carotenoid content in differently coloured (green, red, yellow) peppers. Green pepper has the highest level of phenolics, while red pepper the highest level of ascorbic acid (Zhang and Hamauzu, 2003). Differences in the lightness of the fruit (L) were also observed. A variety SM 1, which has an orange-red colour, has the highest brightness of pepper, followed by Anita with yellow - green colour, Una with lightness of 32.64, Vranjska, Novosadjanka, and finally the darkest Plamena with lightness of 28.23 and pink colour.

Table 3
Wavelength, colour and lightness of the fruit of different varieties of peppers

Variety	Wavelength (λ)	Fruit colour	Lightness of fruit (L)
Vranjska	603 ^{abc}	Orange-red	31.95 ^{abc}
Krusnica	607 ^{ab}	Orange-red	30.87 ^{bc}
Novosadjanka	600 ^{bc}	Orange-red	31.41 ^{bc}
Una	608 ^{bc}	Orange-red	32.64 ^{abc}
SM 1	597 ^{bc}	Orange-red	38.71 ^{a}
Plamena	615 ^{a}	Pink	28.23 ^{c}
Anita	594 ^{c}	Yellow-green	35.98 ^{ab}

Means within a column followed by the same letter are not significantly different at $P<0.05$

Chemical characteristics of stalk and seminal placenta

Dry matter content

The dry matter content defines the biological productivity and is very important for the yield of certain varieties. The dry matter content in the stalk and seminal placenta significantly varied among cultivars of pepper, from 7.43 do10.38% (Table 4). The highest percentage had varieties Plamena, Krusnica and SM 1, and the lowest Vranjska. It is interesting to note that Plamena and SM 1 are hot varieties. Our results are in agreement with previously reported dry matter levels in hot and sweet pepper varieties (Perucka and Materska, 2007). The authors found higher dry matter content in hot varieties, ranging from 8.18 to 16.14%, while sweet peppers contained about 8% of dry matter.

In addition, hot varieties are pointed out by the highest content of cellulose (Table 4), and the values ranged from 2.06% (Plamena) to 1.25% (Vranjska). Cellulose has no nutritional significance for human consumption, because there is no enzyme that can degrade β 1,4 - links, but is useful because it encourages and accelerates intestinal peristalsis (Varga, 2008). Ruminants use the cellulose in the diet because their digestive tract contains symbiotic micro-organisms (bacteria) that secrete cellulase. The results obtained in this study indicate that the seminal placenta of varieties Plamena, Amfora and SM 1 can be used as a food additive in order to enrich the cellulose material.

The content of calcium pectate

Variability of calcium pectate concentration in the pepper cultivars is shown in Table 4. The highest values were found in the varieties Krusnica and Novosadjanka. Of the examined chemical characteristics, the content of calcium pectate showed the greatest variability between different varieties (CV = 8.43%). The only currently known structural role of calcium in the metabolism of plants is to increase rigidity of cell walls by forming calcium pectates. Pectic substances in the middle lamella may be in free form or associated with calcium. The enzyme pectinesterase demethyloxylates pectins in plant tissues and this reaction allowed interaction of carboxylic groups with calcium ions to produce calcium pectates (Villareal-Alba et al., 2004). Reaction between two pectin carboxylic groups and one calcium ion results in increased firmness of the cell wall. This seems to be an important mechanism involved in rigidity improvement in processed vegetables (Bartolome and Hoff, 1972). The amount of calcium pectate affects the strength of the pericarp, which determines the use of certain varieties. As noted above, because of the pericarp structure, the Krusnica variety is mainly used for industrial processing. Temperature is important during industrial processing for pectinesterase enzyme that enhances firmness of the pepper (Villareal-Alba et al., 2004). Pectic substances are also within the dietary fiber, which is important from the aspect of nutrition. Pepper seeds could be considered as a good source of dietary fiber (El-Adawy and Taha, 2001).

Total phenol content

Chemical analysis of pepper cultivars showed differences in the phenol content (Table 4). Studied cultivars had contained 11.47 g kg^{-1} of phenol. The hot varieties were distinguished by the content of these compounds, SM 1 and Plamena, with 13.55 and 13.51 g kg^{-1}, respectively, followed by sweet pepper Una. Our investigations revealed significant differences between hot and sweet cultivars in the phenol compounds content. Contrary to our results, clear differences in phenolic concentrations in sweet and hot pepper cultivars were not found in previous work (Perucka and Materska, 2007). Furthermore, phenolic levels obtained in our work were over two-fold higher than those of Perucka and Materska (2007). The authors suggested importance of industrial processing conditions in preservation of antioxidant activity of pepper extract, in order to improve quality of human diet. Phenolic compounds in plants have a protective antioxidant role (Zhang and Hamauzu, 2003), along with other antioxidants (vitamins C and E, carotenoids and xanthophylls). However, differences in the levels of phenolic compounds in edible fruit parts were reported previously, suggesting relation with successive stages of fruit development (Estrada et al., 2000). Although capsaicinoids content increase with development, the maximal levels of free phenolics and lignin are observed during the early stages of development.

Oil content

Capsicum seeds contain fixed, non-volatile oil (Cantrill, 2008). The total lipids content in fresh pepper fruits is about 0.40% while seeds contain approximately 10.00 – 12.00% (Kinsella, 1971; Ciric et al., 1973). Pepper seeds contain higher protein (24.40%) and oil (25.91%) contents than many legumes (El-Adawy and Taha, 2001). The content of seed oil in studied cultivars ranged from 8.45 to 20.00% (Table 4), and similar results (values from 10.78 to 21.00%) were reported in our previous work (Krstic et al., 2010). Clear differences in oil content between hot and sweet cultivars were not found. However, the lowest values were recorded for the hot cultivar Plamena seeds, while significant differences were not found between another hot pepper SM 1 and sweet cultivars. The highest oil content recorded in cultivar Una is in the range of values obtained in other works (Krstic et al., 2001). Distribution of oils in pepper fruits is not uniform. Previous works showed the highest oil accumulation in seeds. In the Greek pepper, the average oil content of pericarp, seed, and stem and calices was 6.8, 25.4 and 1.7%, respectively (Tsatsaronis and Kehayoglou, 1971). Furthermore, authors reported higher oil content in a «hot» pepper (15.6%) than in «sweet» variety (12.9%). Oil content of Pimiento pepper was higher in seeds (20.6%) than in fruit wall and placenta (0.17 and 0.18%,

Table 4
Differences in dry matter content, crude fiber, Ca-pectates and phenol in the stalk and seminal lodge, as well as oil in pepper's seeds

Variety	Stems and seeds lodge				Seeds oil %
	Dry matter %	Cellulose %	Ca-pectates %	Total phenols g kg^{-1}	
SM 1	9.23 ^{c}	1.99 ^{ab}	0.46 ^{d}	13.55 ^{a}	14.70 ^{d}
Vranjska	7.43 ^{e}	1.25 ^{e}	0.13 ^{f}	9.85 ^{c}	14.44 ^{d}
Anita	7.46 ^{e}	1.31 ^{de}	0.26 ^{e}	9.40 ^{c}	15.94 ^{bc}
Krusnica	9.46 ^{b}	1.89 ^{b}	1.91 ^{a}	10.89 ^{b}	16.72 ^{b}
Amfora	8.56 ^{d}	1.98 ^{ab}	0.52 ^{d}	10.78 ^{b}	14.09 ^{d}
Una	8.67 ^{d}	1.76 ^{c}	1.33 ^{b}	12.74 ^{a}	20.00 ^{a}
Novosadjanka	8.62 ^{d}	1.37 ^{d}	1.85 ^{a}	11.06 ^{b}	14.93 ^{cd}
Plamena	10.38 ^{a}	2.06 ^{a}	0.92 ^{c}	13.51 ^{a}	8.45 ^{e}

Means within a column followed by the same letter are not significantly different at $P<0.05$

respectively) (Marion and Dempsey, 1964). Qualitative analysis of the pepper seed oil identified linoleic and oleic acids as the main unsaturated fatty acids, while major saturated fatty acids of crude oil of paprika were palmitic and stearic (Marion and Dempsey, 1964; El-Adawy and Taha, 2001; Krstic et al., 2001). The linoleic acid is important in human diet due to its ability to reduce cholesterol, suggesting the use of pepper seed oil in human nutrition (El-Adawy and Taha, 2001). However, in cultivars used for seed production, the oil content and fatty acid composition are important for seed viability and nutritive capacity (Domokos et al., 1993).

Conclusions

In the present study, genotype specificity of dimensions and chemical characteristics of inedible portion of pepper fruits was reported. During the processing, the seminal placentas and seeds are being separated from pericarp and dismissed as redundant, leading to exclusion the substances precious in the human diet. The seeds of Una variety, and other varieties with 15% oil, could be used to extract edible oil. Inedible parts of Plamena and SM 1 were the richest in phenols and and cellulose among other varieties. These findings suggest their usage in production of food supplements in order to enrich the human nutrition with these substances.

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