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CHEMICAL CONSTITUENTS OF PUNGENT SPICE PEPPER (*Capsicum annuum* L.) FROM MACEDONIAN ORIGIN

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Abstract

In this paper the chemical constituents of the pungent spice pepper *Capsicum annuum* L. *ssp. Microcarpum* from Macedonian origin are estimated. Content of moisture, proteins and soluble sugars is 9.60%, 6.68% and 20.33%, respectively. Color capacity of the pungent spice pepper is 5.60 g capsanthin/kg pepper dry matter.

The influence of organic solvents on the pepper oleoresin extraction and contents of colored components and capsaicin content in it is also studied. The highest quantity of pepper oleoresin (25%) is obtained using ethanol as extraction means. In the pepper oleoresin extracted by diethyl ether the highest concentration of color expressed as a capsanthin is determined. When *n*-hexane is applied during pepper extraction, in the color extracted quantity the red fraction is 93.83% from the total color contents in the pepper oleoresin.

According to the highest capsaicin content of 1.53% in the pepper oleoresin, benzene is most suitable as compared to the other investigated extraction means.

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Key words: *pungent spice paprika, capsaicin, carotenoids, chemical composition, solvent, extraction, pepper oleoresin*

ХЕМИСКИ КОМПОНЕНТИ НА ЛУТАТА ЗАЧИНСКА ПИПЕРКА (*Capsicum annuum* L.) ОД МАКЕДОНСКО ПОТЕКЛО

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Краток извадок

Во овој труд се одредени хемиските компоненти на лутата зачинска пиперка *Capsicum annuum* L. *ssp. Microcarpum* од македонско потекло. Содржината на влага, протенини и растворливи шеќери е 9.60%, 6.68%и 20.33%, соодветно. Капацитетот на боја на лутата зачинска пиперка е 5.60 g капсантин/kg сува материја.

Беа проучувани влијанието на органските растворувачи врз екстракција на олеорезинот од пиперката, како и содржината на обоените компоненти и содржината на капсаицин во него. Највисоката количина на олеорезин (25%) е добиен со употреба на етанол. Во олеорезинот од пиперка, екстрахиран со диетил етер, е одредена највисоката концентрација на боја изразена како капсантин. Кога *n*-хексан е аплициран за време на екстракцијата на пиперка, во количината на екстрахирана оја црвената фракција е присутна со 93.83% од вкупната содржина на боја во олеорезинот. Според највискоката содржина на капсаицин од 1.53% во олеорезинот од пиперката, бензенот е најсоодветен во споредба со сите други испитани екстракциони средства.

Клучни зборови: *лута зачинска пиперка, капсаицин, каротеноиди, хемиски состав, растворувач, пиперкин олеорасин*

1. Introduction

The red pepper (*Capsicum annuum* L.) has been used since ancient times, traditionally used in form of spice paprika, although, today, oleoresins are widely used (Nambudiri *et al.*, 1970; Govindarajan, 1986a).

Spice red pepper is worldwide use as a natural flavour and colorant in food industry, as well as raw material for the pharmaceutical industry.

In addition to the pungency if the spice pepper is pungent depending of the capsaicinoids presence mainly capsaicin, the spice pepper contains

considerable amount of antioxidative components such as the carotenoids and vitamins (Govindarajan, 1986b; Rahman *et al*., 1986; Howard *et al*., 1994; Matsufuji *et al*., 1998). The commercial value of the spice pepper depends on its red colouring power. The components responsible for the colour are the carotenoids pigments (Davies *et al*., 1970; Biacs, *et al*., 1989). The colouring and nutritional capacity of the carotenoids makes them interesting in the food industry as additives and functional food ingredients, in the form of pepper or oleoresin (Gordon and Bauernfeind, 1982).

One of the pepper products is pepper oleoresin extracted from the dried ripe fruits of *Capsicum annum* L. using solvents with the lypophilic characteristics and subsequent solvents removed from the oleoresin extract (Kense, 1970; Rajaraman *et al*., 1981; Cvetkov and Rafajlovska, 1992). Pepper oleoresin basicly contains pigments named capsanthin and carotenes (Mínguez-Mosquera and Hornero-Méndez, 1998). Also, beside the pigments in the pepper oleoresin are present flavours and taste agents, vitamins, fatty oil and capsaicin if pungent pepper is used for pepper oleoresin production (Vinaz *et al*., 1992; Mínguez-Mosquera and Hornero-Méndez, 1993).

In the present work, characterisation of the pungent spice pepper (*Capsicum annuum* L. *ssp. Microcarpum*) is made by determination of the chemical composition. The influences of the extraction solvents on the pungency pepper oleoresin yield and the contents of capsaicin and carotenoids in it are also studied.

2. Materials and methods

Pepper (*Capsicum annuum* L. *ssp. Microcarpum*) was used as experimental material. The morphological characteristics of *Capsicum annuum* L. *ssp. Microcarpum* in the botanical ripening stage are 40 - 45 cm average height, small conical shape fruits with pericarp thickness to 1.2 mm and 2.4 cm length, fruit weight 2.5 - 2.9 g. Drying of the red pepper was made in greenhouse, seven days, on room temperature (~ 25°C). The dried spice pepper was ground in laboratory cutting mill (Retch, Briukmann, Germany). For determination of the chemical composition spice pepper with 0.25 mm particles size was used.

Solvent: pro-analysis-grade solvents such as ethanol, acetone, diethyl ether, *n*-hexane, benzene were purchased from A. D. Alkaloid (Skopje, Republic of Macedonia).

2.1. Procedures for determination of the chemical composition:

***Content of dry matter*:** by drying at 105°C to constant mass achievement (AOAC, 1988).

Content of ash and sand: by burning at a constant temperature of 900°C to constant mass achievement. Sand determination is made by using the ash and 10% HCl (AOAC, 1988).

***Content of total nitrogen, total proteins and protein's nitrogen*:** by *Kjeldahl* method (AOAC, 1988).

***Content of soluble sugars and degree of total reduction*:** by using *Fehling* and *Bertrand* methods (Trajković *et al.,* 1983).

Content of colour components: by using *Benedek* method (Vračar, 2001).

2.2. Extraction procedure

Extraction of the pepper oleoresin is made by using *Soxlet* method. 5g of the grinded pepper (0.25 mm particles size) first is macerated 24h in Erlenmeyer flask with 250 mL extraction solvent. After that, the same system is extracted 5h using *Soxlet* apparatus. Pepper oleoresin extraction is made applying solvents with different polarity degree. The dielectric constant of the used extraction solvents is given in the table 1.

The quantity of obtained pepper oleoresin after drying to constant mass (in vacuum drier, type Heraeus, Vacutherm, Germany, t = 40°C) is weigh. The efficiency of pepper oleoresin extraction is expressed as a yield of pepper oleoresin, according to quantity of extracted pepper sample.

2.3. Determination of colour components and capsaicin in pepper oleoresin:

***Content of colour components*:** Pigments concentration in the pepper oleoresin was calculated using the extinction coefficient of the major pigment capsanthin ($^{1\%}E_{460nm}$= 2300) in acetone. For colour quantification, pepper oleoresin was dissolved in 100 mL acetone (1st dissolution). 5 mL of the 1st dissolution was dissolved in 25 mL acetone (2nd dissolution) and absorbance was measured at 460 nm (UV-VIS spectrophotometer, Cary 50 Varian, Switzerland). Figure 1 shows the visible spectrum of carotenoids of pepper oleoresin in acetone.

For estimation of the fraction of the red carotenoids (C^R) and yellow carotenoids (C^Y) in the pepper oleoresin were used the equations proposed by Hornero-Méndez and Míngues-Mosquera (2001).

$$C^R = \frac{A_{508} \times 2114.0 - A_{472} \times 403.3}{270.9} \ (\mu g/mL) \qquad C^Y = \frac{A_{472} \times 1724.3 - A_{508} \times 2450.1}{270.9} \ (\mu g/mL)$$

To express the results in mg/kg, it is necessary to multiple by the final volume to which the sample was taken and divide it by the weight (grams) of sample. If the sample was diluted by the weight prior to the performance of the spectrophotometric measurement, this factor must be taken into consideraton.

***Content of capsaicin*:** The content of capsaicin in the pepper oleoresin is determined by spectrophotometric reading of the absorbance on 282 nm. 0.5 mL of the 1st dissolution dissolved in 25 mL ethanol (3rd dissolution), than 0.5 mL of 3rd dissolution was dissolved in 10 mL ethanol (4th dissolution) and absorbance was measured at 282 nm (UV-VIS spectrophotometer, Cary 50 Varian, Switzerland). The UV spectrum of capsaicin of the pepper oleoresin in ethanol is presented by figure 2.

The concentration of capsaicin is estimated from the standard curve for capsaicin $y = 9.64 x + 0.005$ ($R^2 = 0.9909$), where x = μg capsaicin/mL extract and y = absorbance. The capsaicin content is expressed in the pepper oleoresin quantity.

3. Results and discussion

The content moisture, ash and sand in the investigated pungency spice pepper *Capsicum annuum* L. *ssp. Microcarpum*, is 9.60%, 16.79 and 0.56%, respectively. The determined colour pepper capacity expressed as capsanthin is 5.60 g capsanthin/kg pepper dry matter. The total amount nitrogen is 2.83%, from which 1.07% belong to the protein's nitrogen (Table 2).

In the literature data variation in the chemical composition with regards to the origin of the spice paprika are presented. The content of proteins is ranging up to 15%. From total sugars 75-98% belongs to the soluble sugars (Rahman *et al*., 1980; Vračar, 2001).

In table 3 the quantity of pepper oleoresin obtained by solvents extraction is presented. The biggest pepper oleoresin of 27.45% expressed as pepper oleoresin yield according to the pepper dry matter is obtained when ethanol is used. By decreasing the solvent polarity, the quantity of extracted pepper oleoresin also decrease. The solvents with lower dielectric constant as are diethyl ether, *n*-hexane and benzene, according to its non-polarity features extracted lower quantity of pepper oleoresin. The quantity of pepper oleoresin obtained with those applied solvents varied from 3.9% to 4.6% (table 3).

The influence of the solvent type on the presence of the colour components in pepper oleoresin expressed as capsanthin is given in table 4.

The pigments that give a red colour of the genus *Capsicum* are the carotenoids. They can be classified by colour into two groups, carotenes and xanthophylls. Depending on their chromophore chain, they give a red or yellow colouration. The red fraction is formed by capsanthin and capsorubin. *β*-carotene, *β*-cryptoxanthin, zeaxanthin, capsolutein and violaxanthin belong to the yellow fraction in paprika.

From the aspect of solvent capability to extract the colour from paprika, the biggest content of colour of 8.43% capsainthin in pepper oleoresin

or 58.95% yield of colour with the respect to the total colour in spice pepper is obtained by using diethyl ether during extraction. Ethanol extracted the lowest quantity of colour in pepper oleoresin, 43.63% from the total colour in the pungent spice pepper (Table 4).

When is compared the possibility of solvent for red and yellow pigments extraction are compared, it can be summarised that *n*-hexane is most suitable to extract the red pigments. In the pepper oleoresin obtained with *n*-hexane is determined 93.83% content of red fraction from the total colour content in pepper oleoresin. In the ethanol pepper oleoresin the content of yellow fraction is the higher (14.14%) than the content of yellow fraction in the oleoresin extracts produced with other investigated solvents as extraction means (table 5).

The literature data show the concentration of carotenoid pigments in pepper oleoresin in the range of 2.3% to 10.3% (Míngues-Mosquera and Pérez-Gálvez, 1998; Hornero-Méndez and Míngues-Mosquera, 2001).

The capsaicin concentration in the pepper oleoresins varied from 0.18% to 1.55% and it depends on the solvent used in the spice pepper extraction. In the pepper oleoresin obtained by benzene highest capsaicin concentration (1.5352%, table 4) is determined.

4. Conclusion

The determined chemical constituents of pungent spice pepper *Capsicum* annuum L. *ssp. Microcarpum* from Macedonian origin contain moisture, proteins, soluble sugars and colour of 9.60%, 6.68%, 20.33% and 5.60 g capsanthin/kg pepper dry matter, respectively.

The highest quantity of pepper oleoresin of 25% is obtained using ethanol during extraction of *Capsicum* annuum L. *ssp. Microcarpum.*

Diethyl ether gave the highest concentration of colour in pepper oleoresin. In the oleoresin extracted from pepper applying *n*-hexane, the red coloured fraction is 98.33% from the total colour determined in pepper oleoresin. The concentration of capsaicin in the pepper oleoresins ranged from 0.18% to 1.55% depend on the solvent used in the spice pepper extraction.

References

AOAO, Standard Methods of Analysis, 15th Ed., Washington, DC, 1988.

Biacs, A. P.; Daood, G. H.; Pavisa, A.; Hajdu F. (1989). Studies on the carotenoid pigments of pepper (*Capsicum annuum* L. var Sz-20). *J. Agric. Food Chem.*, 37 (2), 350-353.

Cvetkov, Lj.; Rafajlovska V. (1992). Choice of suitable solvent for oleoresin extraction from grinded spicy pepper. *Bull. Chem. Technol. Macedonia*. 11, 61-66.

Davies, B. H.; Matthews, S; Kirk, J. T. O. (1970). The nature and biosynthesis of the carotenoids of different color varieties of *Capsicum annuum*. *Phytochemistry*, 9, 797-805.

Gordon, H. T; Bauernfeind J. C. (1982). Carotenoids as food colorants. *CRC Crit. Rev. Food Sci. Nutr.*, 18, 59-97.

Govindarajan, V. S. (1986a). Capsicum - Production, technology, chemistry and quality, Part II. World production and trade. *CRC Crit. Rev. Food Sci. Nutr.*, 23 (3), 207-287.

Govindarajan, V. S. (1986b). Capsicum - Production, technology, chemistry and quality, Part III. Chemistry of the color, aroma and pungency. *CRC Crit. Rev. Food Sci. Nutr.*, 24 (3), 245-533.

Hornero-Méndez D.; Míngues-Mosquera M. I. (2001). Rapid Spectrophotometric Determination of Red and Yellow Isocromic Carotenoid Fraction in pepper and Red Pepper Oleoresins, *J. Agric. Food Chem.,* 49, 3584-3588.

Howard, R. L.; Smith, T. R.; Wagner, B. A.; Villason, B.; Burns, E. E. (1994). Provitamin A and ascorbic acid content of fresh pepper, cultivars *capsicum annuum* and processed jalapenos. *J. Food Sci.*, 59 (2), 362-365.

Kense, W. (1970). Solid-liquid extraction with a Carousel-type extractor. *Chem., Ztg. Chem. Appl.*, 94 (2), 56-62.

Matsufuji, H.; Nakaura, H.; Chino, M.; Takeda, M. (1998). Antioxidant activity of capsanthin and the fatty acids esters in pepper (*Capsicum annuum*). *J. Agric. Food Chem*., 46, 3468-3472.

Mínguez-Mosquera, M. I.; Hornero-Méndez, D. (1993). Separation and quantification of the carotenoids pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reversed phase, HPLC. *J. Agric. Food Chem*., 41, 1616–1620.

Mínguez-Mosquera, M. I.; Pérez-Gálvez, A. (1998)**.** Color quality in pepper oleoresins. *J. Agric. Food Chem.*, 46, 5124-5127.

Nambudiri, E. S.; Lewis, S. Y.; Krishnamurthy, N.; Mathew, A. G. (1970). Oleoresin pepper. *Flavour Ind.*, 1, 97-99.

Rahman, M. M. F.; Buckle, A. K.; Edwards, A. K. (1980). Changes in total solids, ascorbic acid and total pigment content of capsicum cultivars during maturation and ripening. *J. Food Sci.*, 15, 241-249.

Rajaraman, K.; Kuty-Sumathy, A. M.; Sankarikutty, B.; Mathew, G. A. (1981). Ethylacetat as solvent for extraction of spice oleoresins. *J. Food Sci. Technol.*, 18, 101-103.

Trajković, J.; Baras, J.; Mirić, M.; Šiler, S. Analize životnih namirnica, Tehnološko-metalurški fakultet, Beograd, 1983.

Vinaz, P.; Camoilo, N.; Gardia-Lopez, I.; Cordoba-Hernandez, M. (1992). Liquid chromatographic determination of fat-soluble vitamins in pepper and pepper oleoresin. *Food. Chem*., 45, 349-355.

Vračar, O. Lj.; Priručnik za kontrolu kvaliteta svežeg i prerađenog voća, povrća i pečurki i osvežavajuć bezalhoholnih pića, Novi Sad, 2001.

Table 1. Dielectric constant of the solvents
Табела 1. Диелектричка константа на растворувачите

Dielectric constant Диелектричка константа	Solvent type Тип на растворувач				
	ethanol етанол	acetone ацетон	diethyl ether диетил етер	benzene бензен	*n* - hexane *n* - хексан
ε	25.7	21.4	4.34	2.28	2.02

Table 2. Chemical composition of *Capsicum annuum* L.*ssp. Microcarpum*
Табела 2. Хемиски состав на *Capsicum annuum* L. *ssp. Microcarpum*

Moisture (%) Влага (%)	9.60
Ash (%) Пепел (%)	16.79
Sand (%) Песок (%)	0.56
Total nitrogen (%) Вкупен азот (%)	2.83
Protein nitrogen (%) Протеински азот (%)	1.07
Proteins (%) Протеини (%)	6.68
Total reduction (%) Вкупна редукција (%)	22.34
Soluble sugars (%) Растворливи шеќери (%)	20.33
Colour (g capsanthin/kg pepper dry mater) Боја (g капсантин/kg сува материја на пиперка)	5.60

Table 3. Yield of pepper oleoresin
Табла 3. Количество на олеорезин во пиперка

Solvent Растворувач	Oleoresin weight Тежина на олеорезин (g)	Yield of oleoresin Принос на олеорезин (%)	Yield of oleoresin Принос на олеорезин (%*)
ethanol етанол	1.2504	25.00	27.45
acetone ацетон	0.3100	6.20	6.82
n - hexane *n* - хексан	0.2048	4.09	4.52
benzene бензен	0.2087	4.17	4.59
n – hexane : benzene 1:1 *n* - хексан : бензен 1:1	0.1813	3.63	3.99
diethyl ether диетил етер	0.1782	3.56	3.92

*according to the dry matter of paprika
* во однос на сува материја во пиперката

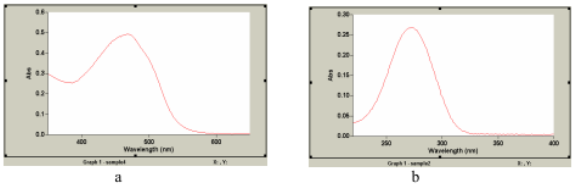
Table 4. Content of capsaicin and colour components in pepper oleoresin
Табела 4. Содржина на капсаицин и обоени компоненти во олеорезин од пиперка

Solvent Растворувач	Capsanthin in oleoresin Капсантин во олеорезин (%)	Yield of colour* Принос на боја* (%)	Capsaicin in oleoresin Капсаицин во олеорезин (%)
ethanol етанол	0.85	43.63	0.1814
acetone ацетон	4.80	58.95	1.0481
n - hexane *n* - хексан	5. 93	48.12	1.3280
benzene бензен	5. 97	49.42	1.5352
n – hexane : benzene 1:1 *n* - хексан : бензен 1:1	6.31	45.35	1.3889
diethyl ether диетил етер	8. 43	59.50	1.4042

*according to the total colour in spice paprika
* во однос на вкупната боја во пиперката

Table 5. Yield of fractions of red and yellow carotenoids in pepper oleoresin
Табела 5. Количество на фракции од црвени и жолти каротеноиди во олеорезин од пиперка

Solvent Растворувач	Content of oleoresin in pepper Содржина во олеорезин на			
	Fraction of red carotenoids Фракција од црвени каротеноиди C^R (%)	Fraction of yiellow carotenoids Фракција од жолти каротеноиди C^Y (%)	$(C^R/ C^R+C^Y)x100$ (%)	$(C^R/ C^R+C^Y)x100$ (%)
ethanol етанол	0.70	0.12	85.86	14.14
acetone ацетон	4.20	0.50	89.21	10.79
n - hexane *n* - хексан	5.34	0.35	93.83	6.17
benzene бензен	5.10	0.57	90.03	9.97
n - hexane : benzene = 1:1 *n* - хексан : бензен = 1:1	5.26	0.80	86.78	13.22
diethyl ether диетил етер	7.27	0.89	89.13	10.87



a b

Figure 1. a) Visible spectrum of capsanthin in pepper oleoresin b) UV spectrum of capsaicin in pepper oleoresin
Слика 1. а) Видлив спектар на капсантин во олеорезин б) UV спектар на капсаицин во олеорезин