60 years of publishing

DE GRUYTER OPEN

herba polonica
Vol. 60 No. 3 2014

DOI: 10.2478/hepo-2014-0016

EXPERIMENTAL PAPER

Capsaicinoid profile of soft-flesh *Capsicum* spp. genotypes according to the purée production method

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Summary

Capsaicin and dihydrocapsaicin concentration was evaluated using the HPLC method in the whole fruit, pericarp, and purée obtained by rubbing fruit through a strainer, purée obtained by fruit expressing, and in the waste following the two above processes. The highest content of each compound was noted in purée and the lowest one in pericarp and waste. Concentration of capsaicin was higher than concentration of dihydrocapsaicin in all of the analysed material. Reciprocal relations between contents of two compounds ranged from 1.4 to 5.3. Capsaicinoid contents in purée obtained by rubbing plus in the waste of the process and in the expressed purée plus the waste of the process were highly similar. Capsaicin and dihydrocapsaicin contents in purée obtained as a result of mechanical separation of soft tissue from the inedible fruit parts was significantly higher than in pericarp and stable, regardless of the processing method.

Key words: *capsaicin, dihydrocapsaicin, soft-flesh Capsicum spp., pericarp, purée*

INTRODUCTION

In 2005, Srinivasan [1] presented a summary of 30-year-long studies on the effect of spices and natural food supplements on the physiological processes in human body. The review pointed to a uniquely advantageous activity of the capsaicin

contained in pepper fruits. Among important properties of this compound, antioxidant, anti-carcinogenic, and lipid metabolism-stimulating activities are particularly important. In another study [2], capsaicin activity was underscored as an angiogenesis inhibitor *in vivo* and *in vitro*. Intake of food enriched with secondary metabolites, such as capsaicinoids, may effectively support the effects of anticarcinogenic therapy. Harada and Okajima [3] observed that topical application of 0.01% capsaicin significantly increased cheek skin elasticity in healthy female volunteers seven days after application.

Analysis of capsaicinoids can be conducted by using spectrophotometric [4] and gas chromatographic [5]. Techniques using high performance liquid chromatography (HPLC) provide accurate and efficient analysis of content and type of capsaicinoids present in the hot pepper. The protocols described for quantitative and qualitative analysis of capsaicinoids are simple, high sensitive as well as time- and cost-effective. Extraction is easy and results in optimum capsaicinoids extraction [6].

The antioxidant property is observed both in hot and sweet cultivars. The study of Marti *et al*. [7] on the sweet pepper revealed that in general, fruit ripening increased levels of lipid peroxidation in mature peppers, with exception of one cultivar. Genotypes characterised with soft pericarp of mature fruits, as an effect of polygalacturonase activity [8] provide raw material for the production of purée or juice with specific capsaicinoid content. Thermal treatment of standard, hard-flesh material as well as drying changes of the chemical and physical characteristics of the product [9-12] while mechanical separation of soft pericarp flesh from inedible parts provides an uniform purée with persisting taste and dietetic properties of fresh fruit. Such a product may be consumed by itself or as an additive to other dishes, fulfilling the function of a nutraceutic agent. In order to develop cultivable forms distinguished by appropriate yield and technological properties, the advantage was taken in the interspecific hybridisation (*Capsicum frutescens* L. x *C. annuum* L.) as a source of genetic variability [13]. Lines developed from the hybrid differed in the pungency of their fruits. The studies aimed at evaluation of the capsaicin and dihydrocapsaicin contents in fruit (FR), pericarp (PE), purée obtained as a result of rubbing (RP) and expressing (EP), as well as in the waste following rubbing (RW) and expressing (EW).

MATERIAL AND METHODS

Research material included the fruit of three soft-flesh genotypes, represented by lines 1/405, 2/405, and 1/905, derived from the hybrids of *Capsicum frutescens* L. and *C. annuum* L., and the products obtained as a result of the mechanical separation of pericarp soft flesh from the inedible parts, as well as the remains that were left following the processes, that is placenta, seeds, peel, and certain amount of pericarp, sticking to the above. The fruits were harvested from culture conducted under an unheated plastic tunnel. Their most important morphological and technological

characteristics, such as average weight, length, width, wall thickness, soluble solids, and dry matter content were examined before the preparation of raw material for the capsaicinoid analysis. After removing the peduncles, the fruit provided the experimental material of 10 kg for each line. Seven samples of approximately 1 kg each were separated for every genotype. Three samples were fragmented in a mixer and rubbed through a sieve (RP) with the eyeholes of 1 mm in diameter. Fruits of the next three samples were subject to expressing in a fruit-squeezer (EP). The processes of purée production were performed in three replications. The share of purée in fruit weight was described as a technological performance. In the seventh sample of every line from half of the fruit, the placenta together with seeds and septa were removed, leaving the pericarp exclusively (PE). The second part of the sample comprised the whole fruit (FR). Both types of purée and waste after its production (RW, EW), pericarp, and the whole fruit were dried at +60°C for three days. Grinding of the dried samples was performed immediately before the extraction of capsaicinoids.

For the analysis of the content of capsaicinoid, the HPLC technique was applied, as described by Collins *et al*. [6]. Samples of the ground material, 1.5 g in weight, were separated in 50 ml glass tubes and submerged in 15 ml of acetonitrile. Extraction was proceeded in a water bath at +80°C for four hours. Tubes closed with teflon foil were manually shaken every hour. After extraction and cooling down to room temperature, 3 to 5 ml of the supernatant were sampled using 10 ml syringes and passed through a 0.45 μm Waters Millex – HN filter to a little tube. The filtrates were closed in tubes and stored in a refrigerator at +5°C up to chromatographic analyses.

Perkin Elmer Series 200 HPLC instrument equipped with Waters S5ODS2 4.6 x 100 mm column, autosampler, and PE Nelson Network Chromatography Interface NC 1900 were used. Standards of 8-Methyl-N-vanillyl-6-nonenaminde (capsaicin) and 8-Methyl-N- vanillylnonenaminde (dihydrocapsaicin) were obtained from Sigma-Aldrich Co. Solutions of 1000, 500, 100, 50, 25, 10, 5, and 1 ppm were prepared in 100% methanol (HPLC) by dilution of a 2000 ppm stock solution. Each sample of the analyzed material was injected in triplicate as a 10 μl aliquot and analysed with 1ml $\cdot$ min^{-1} mobile phase flow and λ excitation 280 nm. The mobile phase was isocratic, with solvent B - 70% (100% methanol) and 30% solvent A (10% methanol – by volume in water). Data on capsaicin and dihydrocapsaicin contents were converted to Scoville Heat Units by multiplying by the factor of 16.1 established by Todd *et al*. [14] for the capsaicinoids mentioned above. The obtained results were subject to the analysis of variance. The values of the smallest significant difference were established using Tukey's test at p=95%. Data in tables marked with the same letters expressed no significant differences.

RESULTS AND DISCUSSION

Plant material presented in the study was obtained as a result of selection among interspecific *Capsicum frutescens* L. and *C. annuum* L. hybrids. The selected

lines brought together appropriate levels of agro- and technological properties and they could provide raw material for the production of capsaicinoid purée. The general characteristics of the lines, presented in table 1, demonstrate the variability in fruit size and dry matter share. The content of the latter one was significantly lower than that in fruits of standard hard-flesh cultivars and their hybrids used in the studies by Ayuso *et al*. [15]. The amount of soluble solids resembled the one noted in various forms of the `Habanero` (*C. chinense* Jacq.) cultivar, grown in Yucatan [16]. The most significant trait, original for the soft-flesh material, is the technological performance of the fruits, which indicates the share of purée in the entire weight of the raw material, that is in the whole fruits with no peduncles. The most effective production of purée was in line 2/405, regardless from the technique applied for the separation of pericarp soft tissues from the inedible parts. Also, in other lines, no effect of the purée production method was noted on the performance of the raw material. The presented results suggest that taking proper advantage of the genetic variability obtained due to interspecific variability provides the opportunity for the creation of genotypes with high technological performance.

Table 1.

General characteristics of fruits of three soft-flesh *Capsicum* spp. lines

Traits	Line		
	1/405	2/405	1/905
Mean weight [g]	13.6 a	14.1 a	12.0 a
Length [mm]	62 a	63 a	48 b
Width [mm]	26 a	26 a	31 b
Wall thickness [mm]	2.48 a	2.12 a	2.66 a
Soluble solids [°Brix]	7.47 a	7.44 a	8.31 a
Dry matter [%]	8.82 a	9.09 b	8.52 a
Technological performance [%]			
Rubbing	59 a	66 b	61 a
Expressing	64 a	69 b	64 a

Data marked with the same letters manifested no significant differences, $p=95\%$.

Great differences in total capsaicinoid content between the studied genotypes were observed (tab. 2). Lines 1/405 and 1/905 may be described as high pungent, although the sum of capsaicin and dihydrocapsaicin in the whole fruit was about five times lower than the one observed by Kurian and Starks [17] in the very hot `Habanero` cultivar. The third line, marked as 2/405, appeared as a low capsaicinoid form. The sum of the compounds was three times lower in comparison with the hard-flesh Polish cultivar `Bronowicka Ostra` [18]. In the evaluated plant material, the highest level of compounds was noted in purée, regardless from the

soft tissue separation method, and the lowest one was found in the pericarp and in the waste after rubbing or expressing. In the whole fruit of two hot lines, capsaicinoid concentration was significantly lower when compared with purée. The lowest level was characteristic for the pericarp and waste, and at the same time there were no statistical differences between the above mentioned fruit parts.

Table 2.

Capsaicin (CAP) and dihydrocapsaicin (DHC) content [$mg \cdot g^{-1}$] in analyzed material of soft-flesh *Capsicum* spp. lines

Line	Analyzed material					
	PE	FR	RP	EP	RW	EW
1/405						
CAP	0.42	1.04	1.63	1.85	0.55	0.39
DHC	0.08	0.21	0.70	0.77	0.24	0.17
Sum	0.50 a	1.25 b	2.33 c	2.62 c	0.79 a	0.56 a
2/405						
CAP	0.09	0.20	0.21	0.18	0.08	0.12
DHC	0.03	0.10	0.12	0.11	0.05	0.06
Sum	0.12 a	0.30 c	0.33 c	0.29 c	0.13 a	0.18 b
1/905						
CAP	0.34	1.18	1.81	1.91	0.46	0.50
DHC	0.17	0.91	0.90	0.63	0.23	0.20
Sum	0.51 a	2.09 b	2.71 c	2.54 c	0.69 a	0.70 a

Data marked by the same letters (for the sum of capsaicinoids within the line) manifested no significant differences, p=95%; PE – pericarp, FR – whole fruit, RP – rubbing purée, EP – expressing purée, RW – rubbing waste, EW – expressing waste.

Reciprocal comparison of analysed material seems to be more interesting: in the pericarp, the whole fruits, purée obtained by rubbing or extracting, and in the waste of the processes, capsaicin content always prevailed over dihydrocapsaicin concentration. In every line, the highest level of the first compound was observed in the extracted purée and the lowest one in the pericarp. The above observation of the content of capsaicin which was higher than that of dihydrocapsaicin is consistent with the results of the studies on standard hard-fruit cultivars [19]. Distinct relations were observed [20] in the vegetative parts of the 'Padron' cultivar. Higher content of dihydrocapsaicin than of capsaicin in leaves and stems may be explained by specific character of compound re-distribution from the places of their synthesis, that is from fruit to peripheral plant parts.

Whole fruit clearly contained more capsaicinoids than the pericarp. This was reflected by the presence of placenta tissues in the samples, the principal place of the synthesis of the studied compounds and their most abundant source. In

the hard-flesh fruit of the Korean pepper cultivar [21], capsaicin content in the placenta was more than twenty-fold higher and dihydrocapsaicin content was thirteen-fold higher than in the pericarp. Similar relations were typical for the 'Habanero' cultivar [22]. In the above context, it is interesting that the content of the two compounds in the waste after rubbing through a sieve and extracting was significantly lower than in whole fruit and in the two types of purée. Conclusion follows that a significant fraction of capsaicinoids was released from the placenta during the separation of the pericarp tissue. Subsequently, they were translocated to the obtained products. As far as the technological performance is concerned (tab. 1), it should be kept in mind that purée comprised 59% to 69% fruit weight. Therefore, higher content of capsaicinoids in purée than in the whole fruits is comprehensible.

Regular relations between capsaicinoids observed in the evaluated material prompted the authors to present detailed calculations (tab. 3). The lowest CAP/DHC coefficient was characteristic for the line with the lowest capsaicinoid content. Lower and more equal coefficients were characteristic for purée and the residues after purée production, unlike the ones noted for the fruit and pericarp. The relations between capsaicin and dihydrocapsaicin contents, established on the basis of the above quoted studies as well as in the previous experiments [22-27] varied within a very broad range, particularly in the placenta of the "Habanero" cultivar. In whole fruit of several cultivars and F_1 hybrids from Spain [15], dried according to specific, traditional principles used in La Vera, the CAP/DHC ratios ranged between 0.87 and 1.63. The data quoted above showed that the reciprocal ratio of the two principal capsaicinoids reflects the genotype and the type of analysed raw material. The ones observed in the present soft-flesh material may be regarded as stable, in particular in purée.

Table 3.

Quantitative relationship between capsaicin (CAP) and dihydrocapsaicin (DHC) in fruit and in purée of soft-flesh *Capsicum* spp. lines

Line	CAP/DHC in analyzed materials					
	PE	FR	RP	EP	RW	EW
1/405	5.3	4.9	2.3	2.4	2.3	2.3
2/405	3.0	2.0	1.8	1.6	1.6	2.0
1/905	2.0	1.3	2.0	3.0	2.0	2.5
Mean	3.4	2.7	2.0	2.3	2.0	2.3

PE – pericarp, FR – whole fruit, RP – rubbing purée, EP – expressing purée, RW – rubbing waste, EW – expressing waste

In order to verify the credibility and reliability of applied sample preparation method and HPLC technique, the sums of capsaicinoid contents were compared for following material: purée obtained by rubbing plus the waste of process versus

purée obtained by extracting plus remains of the process. The list was prepared separately for capsaicin, dihydrocapsaicin, and total capsaicinoids (tab. 4). For each capsaicinoid and its summed content, the values should be similar. In the case of line 2/405, distinguished by the lowest content of capsaicinoids, the sums were almost identical. They were also similar for line 1/405. Slightly less equal sums were characteristic for the last of the genotypes. The small observed differences might have reflected a variable share of parenchyma in the residues after the separation of the pericarp tissue from the inedible parts. It can be suggested that the uniform character of the results increased with the increase in the share of purée in the total mass of the fruit. From the point of view of raw material usefulness in the production of the capsaicinoid nutraceutics, the best are the forms of low capsaicin and dihydrocapsaicin content, although, with high technological performance. The levels of the discussed compounds should not induce an exceedingly spicy taste so that the irritant character would not prevent or restrict the consumption of the nutraceutic product.

Table 4.

Sum of capsaicinoids in the purée and in the waste after separation from pericarp tissue [mg · g^{-1}] according to the method of purée production

Line	CAP		DHC		CAP + DHC	
	RP+RW	EP+EW	RP+RW	EP+EW	RP+RW	EP+EW
1/405	2.18	2.24	0.94	0.94	3.12	3.18
2/405	0.29	0.30	0.17	0.17	0.46	0.47
1/905	2.27	2.41	1.13	0.83	3.40	3.24

CAP – capsaicin, DHC – dihydrocapsaicin, RP – rubbing purée, EP – expressing purée, RW – rubbing waste, EW – expressing waste

Even though processing industry needs reliable, standard, and safe procedures for capsaicinoid analysis, particularly due to their increased use in active food production, in the practical evaluation of pungency, the organoleptic test created by Scoville is commonly used and pepper hotness is expressed in the so-called Scoville Heat Units [14,28]. The scale varies from zero units for sweet pepper to 16,100,000 SHU for pure capsaicin or dihydrocapsaicin. One of the hottest peppers is the group of `Habanero` cultivars (*C. chinense* Jacq.) and pungency gains the mouth-searing level of 300,000 SHU. The conversion of the HPLC data obtained by Kurian and Starks [17] by multiplying by the factor of 15 shows that the taste of whole Habanero Orange fruit is close to 200,000 SHU. On the other hand, using the same conversion factor, the pungency of placenta of this cultivar [22] has been stated at the level of about 1,000,000 units, while for `Habanero White` it was one half smaller. Opposite to the background of the presented data, the hotness of the present genotypes is significantly lower,

additionally depending on the analyzed material (tab. 5). The highest one was observed in purée, and the pungency of lines 1/405 and 1/905 was similar to the one observed in `Süs` hard-flesh pepper cultivar from Turkey [25]. The third line may be described as delicate piquant, and from the sensorial point of view this genotype is more universal raw material for the production of nutraceutics.

Table 5.

Pungency of analyzed material of soft-flesh *Capsicum* spp. lines in Scoville Heat Units

Analyzed material	Line		
	1/405	2/405	1/905
Pericarp	8.050 a	1.771 a	8.211 a
Whole fruits	20.125 b	4.830 c	33.649 b
Rubbing purée	37.352 c	5.168 c	43.631 c
Expressing purée	42.021 c	4.669 c	40.894 c
Rubbing waste	12.558 a	2.093 a	10.948 a
Expressing waste	8.855 a	2.898 b	11.270 a

Data marked by the same letters (within the line) manifested no significant differences, $p=95\%$.

CONCLUSION

Interspecific hybridization between *Capsicum frutescens* L. and *C. annuum* L. gives an opportunity to create soft-flesh genotypes that differ in pungency and are suitable for processing. Capsaicin and dihydrocapsaicin contents in purée obtained as a result of soft tissue mechanical separation from the inedible fruit parts were significantly higher than those of pericarp and stable, regardless of the processing method.

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PROFILE KAPSAICYNOIDOWE GENOTYPÓW SOFT-FLESH *CAPSICUM* SPP. ZALEŻNIE OD SPOSOBU PRODUKCJI PURÉE

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S t r e s z c z e n i e

Za pomocą metody HPLC oceniono zawartość kapsaicyny oraz dihydrokapsaicyny w całych owocach, perykarpie, purée uzyskanym w wyniku przecierania, purée uzyskanym w wyniku wyciskania oraz w pozostałościach po wymienionych procesach. Największą koncentrację każdego składnika zanotowano w purée, najmniejszą w perykarpie i w resztkach. Zawartość kapsaicyny była większa niż dihydrokapsaicyny we wszystkich analizowanych materiałach. Wzajemne relacje między tymi składnikami wahały się w zakresie od 1,4 do 5,3. Suma zawartości kapsaicynoidów w purée uzyskanym przez przecieranie i resztkach po tym procesie była podobna do obserwowanej dla purée wyciskanego i resztek. Stężenie kapsaicyny oraz dihydrokapsaicyny w purée uzyskanym dzięki mechanicznej separacji miękkiej tkanki perykarpu od niejadalnych części owoców była istotnie większa niż w samym perykarpie i stabilna niezależnie od zastosowanych sposobów przetwarzania.

Słowa kluczowe: *kapsaicyna, dihydrokapsaicyna, soft-flesh Capsicum spp., perykarp, purée*