Relationships between capsaicinoids in the soft-flesh genotypes of *Capsicum* spp.

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Summary

The studies aimed at determining the levels and reciprocal proportions of capsaicinoids (capsaicin and dihydrocapsaicin) in the pericarp and puree obtained by the mechanical separation of the soft tissue from the inedible parts of fruits. The studied material involved a few lines, selected from the interspecies hybrid of *Capsicum frutescens* L. x *C. annuum* L., manifesting a soft-flesh tissue of pericarp in mature fruits. The quantitative estimation of capsaicinoids was conducted using HPLC. The capsaicin content in the studied lines ranged from 9 to 27 and from 20 to 387 $mg \cdot kg^{-1}$ in the pericarp and puree, respectively. For dihydrocapsaicin, the values ranged from 3 to 26 and from 6 to 155 $mg \cdot kg^{-1}$, respectively. Depending on the genotype group, the content of each of the compounds in the puree was 2.5- to about 10-fold higher than in the pericarp tissue. Both in the pericarp and in the puree the level of capsaicin was around 2.5-fold higher than that of dihydrocapsaicin.

Key words: *capsaicin, C. annuum L. , C. frutescens L., dihydrocapsaicin, HPLC, pericarp, puree*

INTRODUCTION

The *Capsicum* genus comprises 30 species and the modern taxonomists recognize five major characterized by cultivated varieties [1]. Certain genotypes of *Capsicum frutescens* L. are characterised by soft tissue of the mature pericarp. The tissue of the fruit wall can be sufficiently soft in order to be easily separated from inedible and ballast parts, such as the placenta, seeds, septa and skin. The results of current investigations [2] indicate that the softening of ripening fruits is induced by polygalacturonase activity. Mechanical rubbing through a sieve requires no thermal maceration of the tissues. Therefore, in the obtained puree all biological and taste values persist. The formation of hybrids between the capsaicinoid forms of soft-flesh *Capsicum frutescens* L. and the high-yield cultivars of *C. annuum* L. makes it possible to develop a new genetic variability within the range of size and quality of the yield and its various content of capsaicinoids. The materials obtained in this way may provide a raw material for nutraceutics producing, while their technological efficacy and content of capsaicinoids determine their usefulness [3].

Both in hard- and in soft-tissue fruits, their variable pungency depends on the concentration of capsaicinoids. The most important of them include capsaicin and dihydrocapsaicin. They may account for over 95% of total content of capsaicinoids, but their reciprocal shares are different in various species of *Capsicum* genus [4]. According to earlier opinions of Kosuge and Furata [5], capsaicin and dihydrocapsaicin account for 90% of the total taste pungency.

The results of studies on *C. frutescens*, *C, chinense*, and *C. annuum* [6, 7] indicate that capsaicin represents the principal component. However, depending on species, cultivar and conditions in which studies on its content are performed, its content varies and may comprise between 40% and 68% of the sum of the two mentioned capsaicinoids.

Until now, no studies were performed on soft-flesh hybrids of *C. frutescens* L. x *C. annuum* L. which would attempt to evaluate them as raw material in the production of nutraceutics. Therefore, our experiments aimed at an attempt to evaluate the capsaicinoids' relationship in the pericarp and in the puree, obtained by the mechanical separation of the soft tissue from the inedible parts of the fruits.

MATERIAL AND METHODS

The investigated fruits originated from plants cultivated under unheated foil tents. The plants represented lines selected from an interspecies hybrid. Development of the hybrid took advantage of the fine-fruit, spicy soft-flesh form of *C. frutescens* L. and the sweet, large-fruit line of hard-flesh *C. annuum* L. – ATM1 (the registered primary component of commercial hybrids in Poland). The selection of a few years yielded a few tens of interesting genotypes. In a view of reciprocal

similarity in some of them, they were divided into three groups. Every group contained a few genotypes with similar levels of morphological traits and a similar content of capsaicinoids. They were marked by the symbols of SF1 (90 g), SF2 (10 g), SF3 (13 g). In the parentheses the average weight of the fruit was noted.

For every line, the yield of plants selected at random was used to prepare samples of fully ripened fruits of approximately 1 kg in weight, free of peduncles and sepals of calyces. After washing, a few fruits were taken at random from every sample. The placenta with seeds and septa were removed from them. Then, the pericarp was dried at the temperature of 60°C for 3 days. In every sample the remaining fruits were broken up and rubbed through a sieve. In this way an uniform puree was obtained. The pomace remaining on the sieves included tissues of placenta, septa, skin, seeds and certain parts of the pericarp tissue. The puree in thin layers was dried in Petri dishes in conditions identical to those in which the above-mentioned samples were dried.

In the studies, the contents of capsaicin and dihydrocapsaicin were determined in the pericarp and in the puree. For this aim, the HPLC technique was employed, as it was presented by Collins et al. [8], with a modification involving the preparation of the samples. Every sample was subjected to three HPLC analyses. In order to extract capsaicinoids, ground samples of 1.5 g poured over with 15 ml of acetonitrile were placed in 50-ml glass bottles with teflon-lined lids. The bottles were capped and placed in an 80°C water bath for 4 h and stirred manually every hour. Then samples were cooled to room temperature. About 3 ml of supernatant were extracted and filtered (0.45 μm Waters Millex – HN filter unit on a 5-ml disposable syringe) into a glass vial, capped and stored at 5°C until analysed. A 10 μl aliquot was used for each HPLC injection.

Determinations were made with the use of Perkin Elmer, Series 200 HPLC device equipped with an autosampler system and PE Nelson Network Chromatography Interface NC 1900. The amount of capsaicin and dihydrocapsaicin was determined by isocratic flow of the analysed solution through the column (Waters S50DS2 4.6´100 mm column) at the rate of 1 ml·min^{-1} for the period of 7 min using a detector set with excitation at 280 nm. The mobile phase was isocratic, with 70% solvent A (100% methanol) and 30% solvent B (10% methanol in water, by volume). Standards of 8-methyl-N-vanillyl-6-nonenamide (capsaicin) and N-vanillylnonanamide (dihydrocapsaicin) were obtained from Sigma-Aldrich. Standard solutions of 1000, 500, 100, 50, 25, 10, 5 and 1 ppm were prepared in 100% methanol by dilution of a 2000 ppm stock solution. Results obtained during the experiments were subjected to statistical analysis and the data of standard deviation (±SD) were included.

RESULTS AND DISCUSSION

The selection of hybrid materials aimed to provide genetically stable soft-flesh lines which were studied in these investigations. It was conducted to obtain opti-

mum levels of agrotechnical and technological traits and a spicy taste, reflecting the concentration of capsaicinoids. If the product resulting from the mechanical separation of pericarp soft tissues from inedible remains is supposed to serve as a nutraceutic agent, the levels of capsaicinoids in the puree cannot be exceedingly high. The pungency of juices or concentrates prepared from the soft-flesh raw material must be acceptable for the potential consumer. On the other hand, it should be sufficiently high to retain nutraceutic properties of capsaicinoids. Since this represents a difficult task, it is indispensable to recognise the variability range of capsaicin and dihydrocapsaicin levels as well as the reciprocal relationships between the two compounds and, above all, their content in the puree and the pericarp.

The data presented in table 1 demonstrate the contents of capsaicin and dihydrocapsaicin in the walls of the fruit, i.e. in the pericarp and in the puree, obtained by the separation of pericarp soft tissues from the inedible and ballast parts, such as the placenta, seeds, septa and the skin. As mentioned in the previous chapter, the puree was obtained by mixing the fruits and rubbing them through a sieve. The obtained products were homogenous, took the form of a very dense juice and they retained the intense red colour of the fruits, from which the puree was prepared. Within every genotype group, the content of capsaicin was always higher than the content of dihydrocapsaicin. The levels of each studied capsaicinoid were higher in the puree than in the pericarp. The differences were particularly evident upon comparing the sums of the two compounds in the pericarp and in the puree.

Table 1.

Capsaicinoids content in pericarp and puree in mg·kg-1

group of genotypes	CAP (1)[a]	CAP(2)[b]	DHC(1)[c]	DHC(2)[d]	CAP + DHC (1)	CAP + DHC (2)
SF 1	13.0±4.1	32.5±11.3	6.8±4.7	14.3±8.1	19.8±8.8	46.8±18.6
range	9–21	20–49	3–16	6–28	12–37	26–77
SF 2	27.7±9.5	210.5±55.3	11.0±4.3	60.7±10.3	38.5±13.6	271.3±61.6
range	19–46	146–292	5–18	45–75	24–64	192–353
SF 3	43.2±17.4	359.0±21.5	15.2±5.6	144.2±7.3	58.3±23.3	503.2±28.7
range	32–77	322–387	10–26	133–155	42–103	455–542

[a]CAP (1) – capsaicin in pericarp, [b]CAP(2) – capsaicin in puree, [c]DHC(1) – dihydrocapsaicin in pericarp, dDHC(2) – dihydrocapsaicin in puree (±SD included)

Earlier studies [9] showed that capsaicinoids, compounds typical for the *Capsicum* genus, are synthesised mainly in placenta tissue. In the course of fruit maturation and ripening, their amount increases. The process of decreasing the concentration in the mature, senescent fruits was also recognised [10]. The labile character of the compounds is manifested also in the other parts of fruits to which they diffuse. Their high amounts are noted in seeds and, naturally, in the pericarp. The interesting studies of Estrada et al. [6] unequivocally indicate that they may be also transported to vegetative parts of the plants.

The described possibilities for transporting the compounds both within the fruits and in tissues of vegetative organs results from natural physiological processes in the plants. The differences that we observed in capsaicinoid levels between pericarp and the puree obtained from pericarp tissues after mixing the whole fruits resulted from their free penetration into the obtained product as a sequel of the breaking up and decomposition of the placental tissue. Among others, the range of the capsaicinoid level variability within genotype groups presented in table 1 confirms the complex type of inheritance of the trait. Moreover, it is known [11, 12], that the intensity of the synthesis and the accumulation of capsaicinoids depend on the conditions of plant vegetation.

Taking into account the variable level of capsaicinoids and their evidently higher content in the puree we have undertaken an attempt to evaluate the range of detected differences. The share of capsaicin in the puree in genotypes of low content of the substance (SF1) was 2.5-fold higher than in the pericarp (fig. 1). Even more extensive differences were noted in the other group (SF2). The most extensive, up to ten-fold differences in the share between the puree and the pericarp characterised the latter of the evaluated groups (SF3) which manifested the highest content of capsaicin. The same relationships were disclosed in the case of dihydrocapsaicin. Thus, the applied way of separating pericarp tissues from the inedible parts allowed for an uniform penetration of both capsaicinoids into the product, i.e. into the puree. The other conclusion may prove important for the standardisation of the raw material. It might be formulated as follows: the increasing content of capsaicinoids was paralleled by an even more accentuated increase in their content in the puree. The reciprocal relationships of capsaicin and dihydrocapsaicin in the puree and the pericarp, separately for each of the compounds, resulted in a very similar share of the sum of the two capsaicinoids in materials subjected to HPLC analysis.

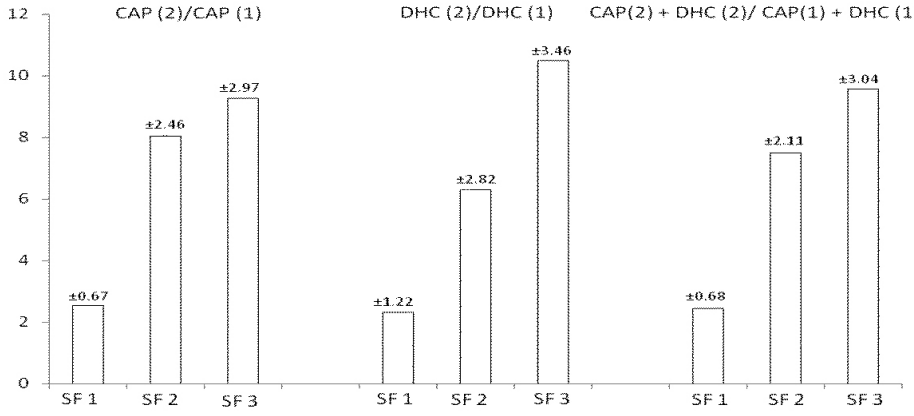

Figure 1. Ratio of capsaicinoids in puree (2) and pericarp (1) for capsaicin (CAP), dihydrocapsaicin (DHC) and sum of capsaicinoids respectively, in the group of genotypes. (±SD included)

The reciprocal relationship are presented in figure 2. In the studied genotypes, the amount of capsaicin, as related to that of dihydrocapsaicin, was similar in the fruits and the puree: the content of the former was approximately 2.5-fold higher than that in the latter. Slightly more extensive differences were noted in the puree of fruits of the SF2 group. Similar relationships could be observed in the set of results obtained by Kurian and Starks [7]. The authors studied the high-capsaicinoid standard, i.e. hard-flesh cultivar 'Orange Habanero'. The contents were, approximately, 20-fold higher as compared to those noted in our research material. However, the quantitative ratio of the two capsaicinoids closely resembled that which was noted by us. In the same cultivar [13] content of capsaicin in the placenta was over thirty times higher than dihydrocapsaicin. The less pronounced differences in capsaicin and dihydrocapsaicin ratio, at the level of 1.71, were noted by Zewdie and Bosland [4] in the cultivar 'Habanero'. Similar values of the ratio (1.54) for the same cultivar were observed by Kozukue et al. [14] In the list of data originating from the article, pertaining the placenta, seeds and pericarp in the spicy of cultivar 'Seongrok', the capsaicin content was found to be, respectively, 3.46-; 2.98-; and 2.11-fold higher than that of dihydrocapsaicin. In the fruit of 13 cultivars and F_1 hybrids [15], ratio of capsaicin and dihydrocapsaicin content ranged between 0,87 – 1,63, and in a few Turkish cultivars of paprika [16] between 1.25 and 1.97 and between 1.48 and 1.94, in the pericarp and seeds, respectively. Similar values (1.43–1.69) can be obtained upon the conversion of the data for cv. Padron [6]. The same paper contained data on the vegetative organs of plants which set fruits. The relationships between capsaicinoid contents were completely distinct from those presented above: the capsaicin content in the lower and middle fragments of the plants (the leaves, the stalk) was several-fold lower than the content of dihydrocapsaicin. Only in the apical parts of the plants the amounts of capsaicinoids were similar. The authors interpreted the differences as a result of the metabolic transformation of the two compounds and their divergent distribution to the vegetative organs.

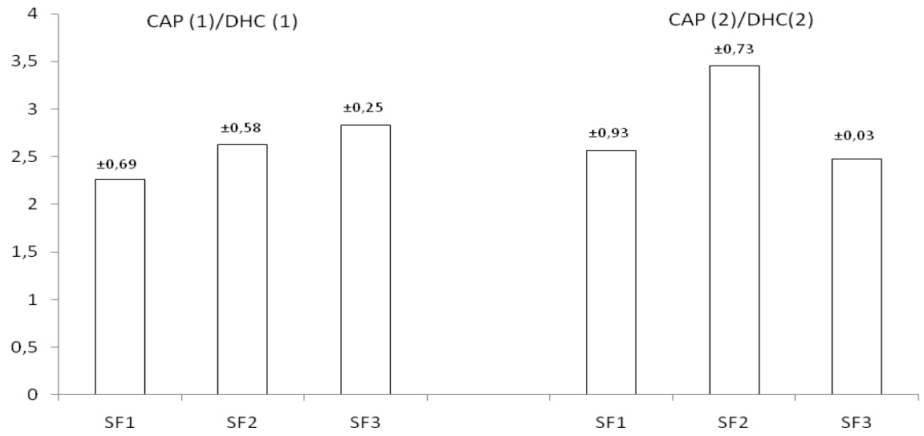

Figure 2. Ratio of capsaicin (CAP) to dihydrocapsaicin (DHC) in pericarp (1) and puree (2) of the genotype groups. (±SD included)

No unequivocal effect was detected of the applied technique of puree production on changes in capsaicin share as related to the pericarp and the puree. Nevertheless, the observed differences have proven less pronounced than those described by other authors. Therefore, a careful suggestion can be advanced that the standardisation of the soft-flesh raw material and products from the raw material will be possible as related to the content and relationship of capsaicinoids.

CONCLUSION

The applied way of separating the soft-flesh pericarp tissues from the inedible parts allowed for a uniform penetration of both capsaicinoids into the product, i.e. into the puree. The increasing content of capsaicinoids was paralleled by an even more accentuated increase in their content in the puree. The reciprocal relationships of capsaicin and dihydrocapsaicin in the puree and the pericarp, separately for each of the compounds, resulted in a very similar share of the sum of the two capsaicinoids in materials subjected to HPLC analysis.

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ZALEŻNOŚC MIĘDZY ZAWARTOŚCIĄ KAPSAICYNY I DIHYDROKAPSAICYNY W OWOCACH GENOTYPÓW SOFT-FLESH *CAPSICUM* SPP.

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

Celem podjętych badań było określenie poziomu i wzajemnych proporcji kapsaicynoidów (kapsaicyna i dihydrokapsaicyna) w perykarpie i przecierze uzyskanym przez mechaniczne oddzielenie tkanki miękiszowej od niejadalnych części owoców. Materiałem badawczym było kilka linii wyselekcjonowanych z mieszańca międzygatunkowego *Capsicum frutescens* L. X *C. annuum* L. charakteryzujących się miękką tkanką perykarpu (soft-flesh) dojrzałych owoców. Oznaczenia ilościowe kapsaicynoidów przeprowadzono metodą HPLC. Zawartość kapsaicyny w badanych liniach wahała się w granicach 9–27 i 20–387 $mg \cdot kg^{-1}$ odpowiednio w perykarpie i przecierze. Dla dihydrokapsaicyny wartości te wynosiły 3–26 i 6–155 $mg \cdot kg^{-1}$. W zależności od grupy genotypów, zawartość każdego ze związków w przecierze była od 2,5 do około 10 razy większa niż w tkankach perykarpu. Poziom kapsaicyny był około 2,5 razy wyższy niż dihydrokapsaicyny zarówno w perykarpie jak i w przecierze, a udział kapsaicyny w sumie obydwu związków wahał się od 67 do 77% w zależności od genotypu i analizowanego materiału.

Słowa kluczowe: *C. annuum L., C. frutescens L., HPLC, kapsaicynoidy, perykarp, przecier*