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CAPSAICINOID CONTENT OF SOFT-FLESH FRUITS OF PEPPER *CAPSICUM FRUTESCENS* L. X C. *ANNUUM* L. INTERSPECIFIC HYBRIDS – A SHORT REPORT

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Soft-flesh fruits of *Capsicum* spp. may constitute a raw material for the production of biologically active foodstuffs. Separating the soft pericarp from ballast parts such as placenta with seeds, cross walls and skin is a mechanical process making it possible to preserve full flavour and nutritional qualities. The research material consisted of fruits of two lines selected from *Capsicum frutescens* L. × *C. annuum* L. interspecific hybrids, which differed from one another in the level of capsaicinoids. The content of these compounds was analysed by means of high performance liquid chromatography (HPLC) by determining the level of capsaicin and dihydrocapsaicin in the fruits and paste obtained by the separation of the soft pericarp of the fruits. In the high-capsaicinoid line, the level of capsaicin and dihydrocapsaicin in the pulp was several times higher than in the pericarp. Paste obtained from low-capsaicinoid fruits contained about 50% more capsaicinoids in comparison with the pericarp. Taste pungency was determined with the use of Scoville Units (1000) and reached 22.8 and 103.7 for the fruits and pulp of 34/2 line and 7.9 and 12.5 for 34/4 line, respectively. Biotechnological performance of the fruits, understood as the percentage of paste in raw material weight, oscillated between 31% and 73%.

INTRODUCTION

Soft-flesh fruits of pepper may constitute an interesting raw material for the food processing industry as regards the production of biologically active foodstuffs or nutraceuticals. Mature fruits are characterised by a very soft pericarp, which enables easier separation of inedible parts of fruits, *i.e.* placenta with seeds, cross walls and skin. The separation consists of passing mixed fruits through a sieve with adequate mesh sizes. Thus it is a process enabling the preservation of the full nutritional qualities and flavour of the pulp obtained.

Capsaicinoids are the most characteristic biological elements of capsicum fruits. Their antioxidant activity [Perucka & Materska, 2003] has a particular significance. The level of capsaicinoids determines the pungency of taste of fruits or their products. The concentration of capsaicinoids varies depending on particular parts of fruits. According to Huffmann *et al.* [1978], the highest concentration of capsaicinoids occurs in cross wall and placenta. The most significant capsaicinoids, with regard to the content, are capsaicin and dihydrocapsaicin. They constitute over 90% of all capsaicinoid compounds. In *Capsicum pubescens* itself, which has no practical importance, nordihydrocapsaicin has been confirmed to constitute 20%.

According to Collins *et al.* [1995], high performance liquid chromatography (HPLC) is a very exact and effective method for determining the level of capsaicinoids. This method makes it possible to separate natural capsaicinoids and determine their content. A comparative evaluation carried out by Wall & Bosland [1998] indicated explicitly that the HLPC method is the most reliable one for analysing both carotenoids and capsaicinoids.

One of the methods for genetic improvement of plants being a source of raw material for the food processing industry or the production of nutraceuticals, is the formation of interspecific hybrids. *S* gene, being responsible for soft flesh feature, discovered by us in *Capsicum frutescens* L., was transferred to *C. annuum* L., being the most significant of the cultivated species, by crossing. This study presents the evaluation of hybrid materials obtained in respect of their general practicability in horticulture production as well as more detailed practicability of raw material in the processing technology.

MATERIAL AND METHODS

The research material consisted of fruits of ten plants belonging to two lines of F_5 *Capsicum frutescens* L. × *C. annuum* L generation denoted with 34/2 and 34/4 symbols, respectively. Each of the lines was represented by 50 plants and the individuals were randomly chosen for analyses. The

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number of ripe fruits as well as their weight were determined. The contents of soluble solids were determined with the use of a refractometer. Biotechnological performance is understood as the percentage of soft pericarp obtained from mixed fruits in the weight of the fruit deprived of calyx.

The contents of capsaicinoids were determined for two kinds of raw material. One of them was a pericarp of each of the selected plants, which was separated from the placenta with seeds. The other was a pulp made of collective samples within the line. The shares of fruits form each of the plants in the paste were identical. The separation of the pericarp from the other inedible parts of fruits consisted of passing mixed fruits through a sieve with mesh diameters of 1 mm. The prepared material, pericarp and pulp, were dried for 5 days at a temperature from 58°C to 60°C. The dried samples were stored in tightly closed containers away from any light. Grinding was performed right before the determination of capsaicinoid contents.

An analysis of the contents of capsaicinoids represented by capsaicin and dihydrocapsaicin was carried out according to the method described and proved by Collins *et al.* [1995]. 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. 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. Each of the samples was analysed in four replications. 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 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. Making use of the formula given in the work referred to above, the level of taste pungency was also determined with the use of Scoville Units (SU). Results obtained during the experiments were subjected to statistical analysis. The volume of the least significant difference was determined by means of Tukey's test at p=95%. In tables, statistically different data were denoted with different letters. The data of standard deviation (± SD) were included as well.

RESULTS AND DISCUSSION

Soft-flesh feature conditioned by *S* gene was found in *Capsicum frutescens* L plants. Crossing *Capsicum frutescens* L with cultivated forms of *Capsicum annuum* L. resulted in obtaining hybrids that manifested a considerable morphological diversification. The selection, which was carried out for a few years, made it possible to obtain a few interesting lines which manifested uniformity of morphological features. Two of these lines and each of them represented by 5 plants constituting the subject of this study.

According to data of Table 1, the fruits were characterised by little average weight reaching several grams. However, their large number resulted in obtaining raw material yield exceeding the weight of 0.5 kg. Besides, soft-flesh type tiny fruits tolerate transport better as they are not crushed in the mass.

One of the essential features as regards the evaluation of the standard, *i.e.* hard-fruit forms of capsicum, is a level of soluble solids [Kmiecik & Lisewska, 1994]. It is very easy to determine it with the use of a refractometer, which shows approximate data at the level of sugars. The fruits of 34/2 line were characterised by a higher level of soluble substances.

Pericarp of soft-flesh fruits is so soft that it can easily be squeezed out of the fruit. As part of the technological process, the separation may be carried out by passing the fruit through a sieve. The efficiency of such activities may be increased by partial mixing the fruit before the separation. Passing the fruit through a sieve results in obtaining a uniform product with its consistency depending, among others, on the structure of pericarp and fruit sizes. Placenta with seeds, cross walls and skin all constitute waste products. According to the data shown in Table 1, biotechnological performance, meaning the percentage of the product in the raw material weight, was very diversified and oscillated between 31% and 73%. It is also worth mentioning that, although there are no experimental data, the product obtained in the above manner retains its original and natural taste. It also manifests aesthetic qualities connected with its lively and beautiful colour, which are the qualities difficult to assess, but which exist from an objective point of view.

As it was indicated before, the fruits from each of the lines were characterised by a high level of morphological uniformity. The contents of soluble solids as well as capsaicinoid analyses (Table 2) explicitly point to diversification of

TABLE 1. Technological characteristics of the raw material.

Symbols of individuals	Number of mature fruits	Mean fruit weight (g)	Soluble solids (%)	Biotechnological performance (%)
34/2/1	38	14.4	8.83	53
34/2/2	38	18.4	7.37	73
34/2/7	40	13.0	10.20	65
34/2/9	55	9.6	9.23	54
34/2/20	46	12.8	9.07	59
Mean	43	13.6	8.95	62
34/4/6	53	16.2	6.47	60
34/4/12	36	19.7	6.13	52
34/4/22	50	17.2	9.00	31
34/4/26	39	14.5	7.27	69
34/4/50	39	18.0	6.73	62
Mean	43	17.1	7.12	55

TABLE 2. The content of capsaicinoids in ppm and pungency in Scoville Units (SU).

Symbols of individuals	Capsaicin	Dihydro-capsaicin	Total	SU (1000)
34/2/1	$58.8^{d}\pm5.4$	$19.7^{bc}\pm0.4$	$78.5^{c}\pm5.6$	17.5
34/2/2	$58.2^{d}\pm5.1$	$24.2^{c}\pm2.3$	$82.4^{c}\pm3.8$	18.1
34/2/7	$52.9^{cd}\pm7.7$	$17.4^{bc}\pm1.0$	$70.3^{c}\pm7.8$	15.7
34/2/9	$158.2^{e}\pm7.7$	$47.4^{d}\pm0.9$	$205.6^{d}\pm7.7$	45.9
34/2/20	$56.0^{d}\pm4.4$	$19.9^{bc}\pm0.2$	$75.9^{c}\pm4.6$	16.9
Mean	76.8	25.7	102.5	22.8
34/2 pulp	$322.7^{f}\pm21.8$	$132.6^{e}\pm10.5$	$465.3^{e}\pm32.2$	103.7
34/4/6	$18.1^{a}\pm0.6$	$7.0^{ab}\pm1.2$	$25.1^{a}\pm1.5$	5.5
34/4/12	$22.8^{ab}\pm0.7$	$11.8^{ab}\pm2.4$	$34.6^{ab}\pm1.8$	7.6
34/4/22	$56.6^{d}\pm 0.4$	$20.7^{bc}\pm0.7$	$77.3^{c}\pm1.0$	17.2
34/4/26	$13.2^{a}\pm0.3$	$8.2^{ab}\pm1.9$	$21.4^{a}\pm1.8$	4.7
34/4/50	$15.0^{a}\pm2.9$	$5.1^{a}\pm1.1$	$20.1^{a}\pm3.6$	4.4
Mean	25.1	10.6	35.7	7.9
34/4 pulp	$42.7^{bc}\pm 2.6$	$13.2^{ab}\pm1.1$	$55.9^{bc}\pm3.6$	12.5

the raw material in respect of physiology. The fruits of 34/2/9 and 34/4/22 plants respectively were distinctive as regards the considerably higher level of capsaicinoids than that of all the other fruits within the two lines. According to results of Zewdie & Bosland [2000a, b; 2001], the content of capsaicin was several times higher than that of dihydrocapsaicin. The above conclusion also refers to 34/2 high-capsaicinoid lines as well as 34/4 low-capsaicinoid lines. The difference in the mean values of the lines reached about 300%.

The use of soft-flesh hybrid forms as a potential raw material for obtaining new foodstuffs can be regarded as an original look at raw material resources within *Capsicum* spp.. The results of the evaluation of the product obtained by mechanical separation of the raw material from ballast parts constitute the most essential part of the study. Analyses of capsaicinoids were made on a dried pulp composed of pericarp of the fruits. The raw material was mixed prior to passing it through a sieve. There are no doubts that it was then that capsaicinoids were released from placenta tissues and cross walls. It is only on the basis of the above that we can explain the considerably higher level of capsaicinoids in the pulp than in the raw material made from dried pericarp. For the 34/2 high-capsaicinoid line the level of capsaicinoids raised 4.5 times, whereas for 34/4 low-capsaicinoid line the difference was about 50%.

In order to compare the analysed results for practical purposes, the level of capsaicinoids was converted to Scoville Units. Having been used for a very long time, they are commonly understood. At the same time [Wall & Bosland, 1998] a high interrelationship (r=0.90) was proved to exist between sensory and instrumental methods for the determination of the level of capsaicinoids. A comparative analysis of our own results (Table 2) expressed in SU suggests that the pulp obtained from 34/2 line can be regarded as very hot; whereas the paste obtained from 34/4 line can be regarded as gently piquant.

CONCLUSIONS

1. The pulp made of soft-flesh fruits was characterised by a higher level of capsaicinoids than it would follow from the analyses of raw material in the form of dried fruits (pericarp with skin and without placenta).

2. Standardization of the product becomes easier and more reliable if the pulp constitutes the subject of analyses. As regards the reference to the general practicability of the raw material, there are no doubts that the biotechnological performance of the raw material has the greatest significance for industry.

3. The research results proved that it is possible to obtain materials with an end product constituting 75% of the weight of the raw material. If juice is to be the end product, then water added in the course of separation of soft flesh from the ballast parts may increase the efficiency of the process.

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ZAWARTOŚĆ KAPSAICYNOIDÓW W OWOCACH TYPU SOFT-FLESH MIĘDZYGATUNKOWYCH MIESZAŃCÓW PAPRYKI *CAPSICUM FRUTESCENS* L. X *C. ANNUUM* L. – KRÓTKI KOMUNIKAT

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Owoce typu soft-flesh u roślin z rodzaju *Capsicum* mogą stanowić surowiec do produkcji artykułów spożywczych bogatych w związki biologicznie aktywne. Oddzielanie miękkiej tkanki perykarpu od części balastowych takich jak łożysko z nasionami, przegrody wewnętrzne i skórka, jest procesem mechanicznym, pozwalającym na zachowanie pełni walorów smakowych i odżywczych. Materiałem badawczym były owoce dwóch linii wyselekcjonowanych z mieszańców międzygatunkowych *C. frutescens* L. x *C. annuum* L., różniących się zawartością kapsaicynoidów. Analizy ich zawartości dokonano przy pomocy wysokosprawnej chromatografii cieczowej (HPLC), określając poziom kapsaicyny i dihydrokapsaicyny w owocach oraz w przecierze uzyskanym przez oddzielenie miękkiej tkanki perykarpu owoców. U linii wysokokapsaicynoidowych zawartość kapsaicyny i dihydrokapsaicyny w przecierze była kilkakrotnie wyższa niż w perykarpie. Przecier z owoców niskokapsaicydowych zawierał około 50% więcej tych związków (tab. 1). Ostrość smaku określona przy pomocy jednostek Scoville'a (SU 1000) wynosiła odpowiednio 22,8 i 103,7 dla owoców i przecieru u linii 34/2 oraz 7,9 i 12,5 u linii 34/4 (tab. 2). Wydajność biotechnologiczna owoców, rozumiana jako udział przecieru w masie surowca, to jest owoców bez szypułek i działek kielicha, wahała się w granicach 31–73%.