JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Pungency in Paprika (*Capsicum annuum*). 1. Decrease of Capsaicinoid Content Following Cellular Disruption

PETRA KIRSCHBAUM-TITZE, CONSTANZE HIEPLER, ERIKA MUELLER-SEITZ, AND MICHAEL PETZ*

Department of Food Chemistry, University of Wuppertal, D-42097 Wuppertal, Germany

The capsaicinoid content in fruits of *Capsicum annuum* decreased within several days to a level of only 10% of the starting value when cells were disrupted by homogenization. This decrease was not observed in fruits that were carefully cut into halves. The analysis of one half made it possible to determine the reference content at time zero for the second half. A much lower decrease was observed when minced fruits were stored under nitrogen, whereas storage under oxygen resulted in considerable losses of capsaicinoids, indicating oxidative processes as a cause for the decrease of capsaicinoid content.

KEYWORDS: Paprika; capsaicinoids; oxidative degradation

INTRODUCTION

Capsicum fruits are valued as part of the diet for different reasons in many parts of the world. They are consumed as fresh fruits, canned as pickles, constituents in condiments and sauces, and dried as powders in spices and seasonings. Color, aroma, and pungency are important for quality classification. High levels of pungency as well as very low levels or even the absence of pungency may be desirable. Capsaicin, dihydrocapsaicin, and nordihydrocapsaicin are the most important capsaicinoids (**Figure 1**), which are responsible for pungency. Attempts to influence capsaicinoid contents have focused on genetic, agricultural, and technological aspects.

Within the genus *Capsicum* numerous species and varieties are cultivated, the highest level of pungency being associated with *C. frutescens*, whereas *C. annuum* comprises also rather mild varieties (*1*). The environment and specifically the climate, light, and temperature during growing of the plant and ripening of the fruit are thought to have an impact on capsaicinoid levels. Processing after harvesting also plays an important role, especially drying conditions, and the amount of seeds included has an influence on pungency, although the main concern is the stability of color and aroma (*2*).

Pungency first appears 20 days after flowering, increases in the next 10−20 days, and levels off ∼50 days after flowering (*3*). The same research group reports for the same cultivar *C. annuum* var. *annuum* cv. Karayatsubusa grown one year later that pungency was detected at day 15 with a maximum at day 25 after flowering (*4*). Another cultivar, *C. annuum* var. *annuum* cv. Padron, exhibits a similar time dependence (*5*−*7*) but shows the highest capsaicinoid content at day 42 (*8*). In *C. frutescens* an increasing capsaicinoid content was detected between days 20 and 40, and varying concentrations were detected until day 70 (*9*).

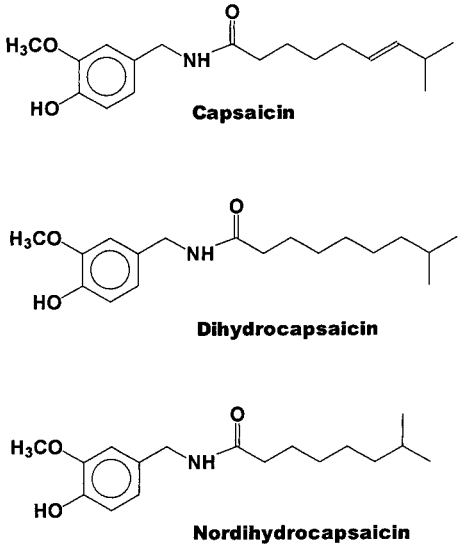

Figure 1. Chemical structures of the most important capsaicinoids.

In commercial fields of *C. annuum* cv. NuMex R Naky statistically significant differences in pungency among individual plants within a single plot were reported, indicating a greater influence of environment over genotype on pungency (*10*−*12*).

Capsaicinoid contents have been reported determined as milligrams per kilogram of fresh fruit, milligrams per kilogram of dried fruit or partly dried fruit, and milligrams per fruit with or without seeds or part of the fruit such as pericarp or placenta, making it difficult to compare various results. It was not stated clearly whether individual fruits were analyzed and mean values calculated or whether material of several fruits was combined before sample preparation took place (*3*, *5*−*8*), allowing no statement on the range of capsaicin content in several fruits at a well-defined stage after flowering. A range of 41−82 mg/

10.1021/jf010527a CCC: $22.00
Published on Web 02/02/2002

Table 1. Capsaicinoid Contents of Eight Individual Fresh Fruits (One Purchase from Spain)

color of fruit	mg/kg of fresh wt	color of fruit	mg/kg of fresh wt
orange	189	orange	259
orange-green	192	red	264
green	205	red	287
green	246	orange-green	373

100 g of fresh fruit was obtained by rinsing the interior of 10 individual fruits with methanol and 22−40 mg/100 g for the remaining rinsed tissues (*13*).

With respect to the lower pungency levels observed in late stages of maturity, possible mechanisms for chemical reactions of capsaicinoids within the fruit have recently been suggested (*9*, *14*−*16*). The basic peroxidase isoenzyme B6 located in vacuoles of placental epidermal cells (*15*, *17*) and the acidic peroxidase purified from pericarp (*18*) have been implicated in the conversion of capsaicin and dihydrocapsaicin into a lignin-like material with a dimerization as a first step (*19*). One such dimer has been characterized as an oxidation product in a cell-free system with riboflavin as photocatalyst without any enzyme involved (*20*).

These in vitro results were not yet corroborated by observations in vivo to explain intrinsic capsaicinoid levels and stability. In this paper we report the decrease of capsaicinoid contents in *Capsicum* fruits following the disruption of cell structure.

MATERIALS AND METHODS

Plant Material. Fresh green and red peppers (*C. annuum*) of different origins were purchased from a local market. Fruits of similar size were selected and processed within 1 day.

Sample Preparation. The individual fruits were cut into small pieces, combined as indicated, and then minced in a blender (Moulinex type 643 or hand-held Braun MR 505). The batch was carefully stirred in an ice bath to ensure homogeneity. Aliquots (10 g) were weighed into glass beakers for storage under conditions as indicated. Prior to analytical determination, the original weight was reconstituted with water, 30 mL of methanol was added, and this suspension was homogenized for 1 min (Ultra-Turrax) and left standing at room temperature for 30 min. The extract was filtered through a paper disk (Schleicher & Schuell, grade 595) into a Büchner funnel, the cell material was washed with 30 mL of methanol, and the combined filtrate filled up to 100 mL with methanol. Depending on capsaicin content and further dilution with methanol/water (70:30), 10−30 μL was analyzed by HPLC.

Chromatography. Liquid chromatography was performed using a Shimadzu HPLC system (autosampler SIL-6A, controlling unit SCL-6A, pump LC-6A) on an 250 × 4 mm LiChrospher RP-18e 5 μm cartridge with a corresponding guard column at room temperature. The eluant was acetonitrile/water/acetic acid (50:50:0.5 by volume) at a flow rate of 1 mL/min. Column effluent was monitored by fluorescence detection (Shimadzu RF-551, excitation at 280 nm, emission at 320 nm) connected to an APEX chromatography workstation (Autochrom version 2.06). Nonanoic acid vanillylamide (Serva) was used as external standard for quantification (picomoles per area of the peak). The capsaicinoid content was calculated as the sum of nordihydrocapsaicin (NDC), capsaicin (C), and dihydrocapsaicin (DC). Minor capsaicinoids were not considered in this study.

RESULTS AND DISCUSSION

Fresh pepper fruits purchased as one batch showed a wide spread of pungency level when analyzed as individual fruits (**Table 1**). Because the capsaicinoid content did not correlate with color, it was not feasible to select fruits with comparable contents as starting material for studies on the capsaicin metabolism. We therefore determined how many individual fruits would have to be combined in one sample preparation procedure to get an acceptable precision for the determination of the capsaicinoid content for the entire batch. To allow for a duplicate analysis, individual fruits were dissected from the tip to the peduncle as carefully as possible with respect to placenta and dissepiments into two halves, which were then allocated to group A and group B. Between 2 and 10 half fruits were combined and subjected to sample preparation and analysis as described.

Table 2. Capsaicinoid Contents (Milligrams per Kilogram of Fresh Weight) of Corresponding Groups of Combined Halves of Fruits, Precision of Determination as Indication of Homogeneity of the Batch

no. of fruits dissected in halves	value for fruit halves			
	group A	group B	SD	CV %
2	223.6	285.8	44.0	17.3
	328.6	283.8	31.7	10.3
	179.7	176.4	3.3	1.3
5	268.8	256.9	8.4	3.2
	322.9	297.5	18.0	5.8
	222.8	252.8	21.2	8.9
8	93.4	88.5	3.5	3.8
	116.8	129.6	9.1	7.8
	190.3	177.9	8.8	4.6
10	234.6	240.4	4.1	1.7
	212.5	219.5	4.9	2.3
	159.0	165.9	4.9	3.0

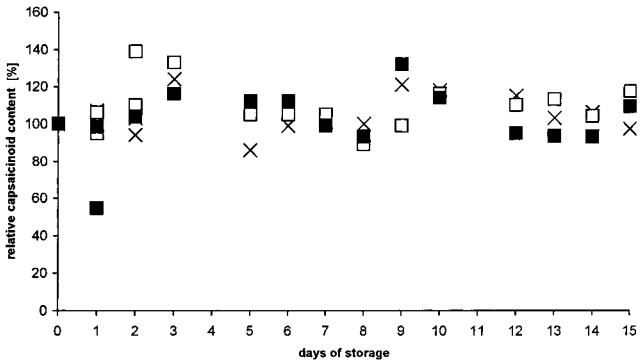

Figure 2. Storage of halved fruits (×) at 4 °C in an open dish, (■) at room temperature in a closed cabinet (slow reduction of water activity), and (□) at room temperature on an open shelf (faster reduction of water activity). Capsaicinoid content of batches of five fruit halves is shown as a percentage of the value obtained at time zero for the corresponding batch of halves.

Balancing the precision needed and the work involved in cutting many fruits, the following studies were made with batches of five fruits (**Table 2**).

Preliminary work suggested an enzymatic metabolism of capsaicinoids in minced fruits depending on temperature and activity of water (*21*). To check whether the loss of cellular compartmentalization is a prerequisite for such a metabolic process, 42 groups of five fruits each were cut in halves and allocated to groups A and B. Group A fruits were analyzed immediately. This capsaicinoid content provided the 100% value for the analysis of the corresponding halves of group B. Group B fruits were stored as indicated (different conditions) without further destruction. It was assumed that the one cut during the halving would not contribute substantially to cell disruption.

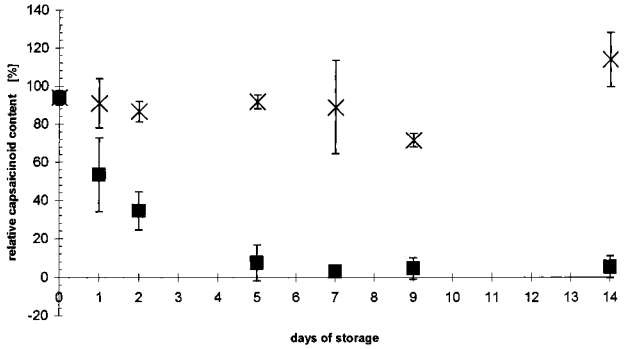

Figure 3. Parallel storage of minced fruits and halved fruits at (×) 4 °C and (■) at room temperature in a closed cabinet. Capsaicinoid content of batches of five fruit halves minced at time zero is shown as a percentage of the value obtained for the corresponding group of five fruit halves stored unminced [mean value and standard deviation (SD), $n = 2$].

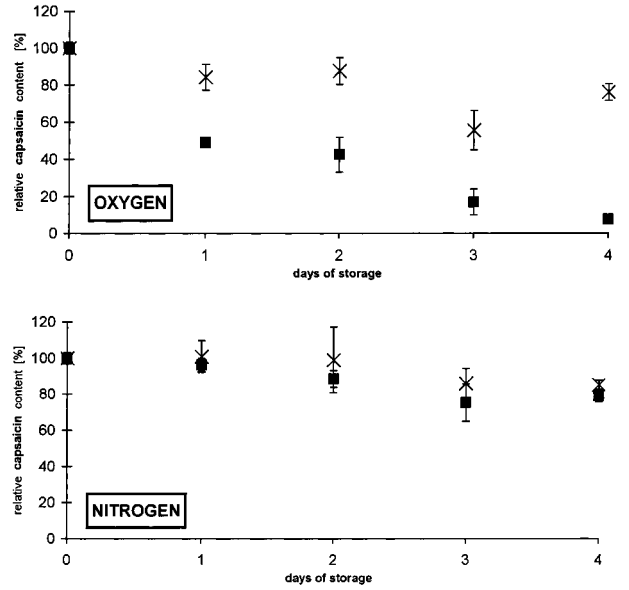

Figure 4. Storage of minced fruits (×) at 4 °C and (■) at room temperature under either pure oxygen or pure nitrogen. Capsaicin content is shown as a percentage of the value obtained at time zero (mean value and SD, $n = 3$).

On the basis of a coefficient of variation (CV) of 6% (**Table 2**) and a t factor of 12.7, a 95% confidence interval of 24−176% for group B values would result. No decrease of capsaicinoid content with time is observed under various storage conditions (**Figure 2**). The dispersion of values reflects the difficulties encountered during the dissection.

We then compared halved fruits (group A) with fruits minced at time zero (group B), both stored in an open dish (i) in the refrigerator at 4 °C and (ii) in a closed cabinet at room temperature. The capsaicinoid content of group A was assumed to have stayed constant, and the capsaicinoid content of the corresponding group B was expressed as a percentage of the value obtained for group A at the same time after storage. Results in **Figure 3** can be interpreted in accordance with a necessary destruction of cell compartments to result in capsaicinoid reduction. This reduction is not observed at low temperature.

To further elucidate the underlying mechanism, we used as storage conditions either pure nitrogen or pure oxygen in a desiccator instead of air. A batch of 500 g of minced fruits was prepared, and the capsaicinoid content at time zero was

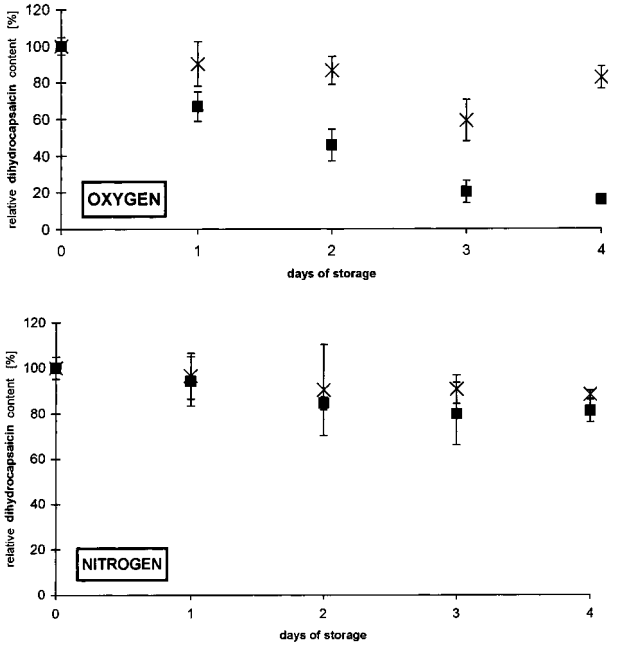

Figure 5. Storage of minced fruits (×) at 4 °C and (■) at room temperature under either pure oxygen or pure nitrogen. Dihydrocapsaicin content is shown as a percentage of the value obtained at time zero (mean value and SD, $n = 3$).

determined in triplicate. Aliquots (10 g) were stored in open glass beakers in the desiccators under conditions as indicated. Mean values from triplicate analysis were reported as a percentage of the value obtained at time zero. In some chromatograms of samples stored under oxygen we observed signals interfering with the determination of nordihydrocapsaicin. Therefore, only results for capsaicin and dihydrocapsaicin are reported. As can be seen from **Figures 4** and **5**, both compounds decrease to the same extent under oxygen but not under nitrogen. Again, temperature plays an important role.

It has already been shown that peroxidase B6 isolated from *Capsicum* fruits can oxidize dihydrocapsaicin as well as capsaicin with hydrogen peroxide as reaction partner (*14*, *17*).

Our results show a decrease due to chemical reactions under oxidative conditions of these intrinsic compounds in vivo after cell compartmentalization has been disrupted.

It remains to be seen if there are differences among various *Capsicum* species with respect to heterogeneity of capsaicinoid contents and metabolism in individual fruits using botanically well-defined plant material at different times after flowering.

LITERATURE CITED

(1) Jurenitsch, J.; Kubelka, W.; Jentzsch, K. Identification of cultivated taxa of *Capsicum*. Taxonomy, anatomy, and composition of pungent principle. *Planta Med.* **1979**, *35*, 174−183.

(2) Govindarajan, V. S. *Capsicum*—production, technology, chemistry, and quality. III. Chemistry of the color, aroma, and pungency stimuli. *Crit. Rev. Food Sci.* **1986**, 245−355.

(3) Iwai, K.; Suzuki, T.; Fujiwake, H. Formation and accumulation of pungent principles of hot pepper fruits, capsaicin and its analogues in *Capsicum annuum* var. *annuum* cv. Karayatsuba at different growth stages after flowwering. *Agric. Biol. Chem. Tokyo* **1979**, *43*, 2493−2498.

(4) Suzuki, T.; Kawada, T.; Iwai, K. The precursors affecting the composition of capsaicin and its analogues in the fruits of *Capsicum annuum* var. *annuum* cv. Karayatsubusa. *Agric. Biol. Chem. Tokyo* **1981**, *45*, 535−537.

(5) Estrada, B.; Pomar, F.; Díaz, J.; Merino, F.; Bernal, A. Evolution of capsaicinoids in *Capsicum annuum* L. var. *annuum* cv. Padrón at different growth stages after flowering. *Capsicum Eggplant Newsl.* **1997**, *16*, 60−64.

(6) Estrada, B.; Pomar, F.; Díaz, F.; Merino, F.; Bernal, A. Effects of mineral fertilizer supplementation on fruit development and pungency in 'Padrón' peppers. *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 493−497.

(7) Estrada, B.; Díaz, J.; Merino, F.; Bernal, M. A. The effect of seasonal changes on the pungency level of Padrón pepper fruits. *Capsicum Eggplant Newsl.* **1999**, *18*, 28−31.

(8) Estrada, B.; Bernal, M. A.; Díaz, J.; Pomar, F.; Merino, F. Fruit development in *Capsicum annuum*: Changes in capsaicin, lignin, free phenolics, and peroxidase patterns. *J. Agric. Food Chem.* **2000**, *48*, 6234−6239.

(9) Sukrasno, N.; Yeoman, M. M. Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. *Phytochemistry* **1993**, *32*, 839−844.

(10) Lindsey, K.; Bosland, P. W. A field study of environmental interaction on pungency. *Capsicum Eggplant Newsl.* **1995**, *14*, 36−38.

(11) Harvell, K. P.; Bosland, P. W. The environment produces a significant effect on pungency of chilis. *Hortic. Sci.* **1997**, *32*, 1292.

(12) Zewdie, Y.; Bosland, P. W. Evaluation of genotype, environment, and genotype-by-environment interaction for capsaicinoids in *Capsicum annuum* L. *Euphytica* **2000**, *111*, 185−190.

(13) Rymal, K. S.; Cosper, R. D.; Smith, D. A. Injection−extraction procedure for rapid determination of relative pungency in fresh Jalapeño peppers. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 658−659.

(14) Bernal, M. A.; Calderón, A. A.; Pedreño, M. A.; Muñoz, R.; Ros-Barceló, A.; Merino de Cáceres, F. Capsaicin oxidation by peroxidase from *Capsicum annuum* (var. *annuum*) fruits. *J. Agric. Food Chem.* **1993**, *41*, 1041−1044.

(15) Bernal, M. A.; Calderón, A. A.; Ferrer, M. A.; Merino de Cáceres, F.; Ros-Barceló, A. Oxidation of capsaicin and capsaicin phenolic precursors by the basic peroxidase isoenzyme B_6 from hot pepper. *J. Agric. Food Chem.* **1995**, *43*, 352−355.

(16) Contreras-Padilla, H.; Yahia, E. M. Changes in capsaicinoids during development, maturation and senescence of chile peppers and relation with peroxidase activity. *J. Agric. Food Chem.* **1998**, *46*, 2075−2079.

(17) Bernal, M. A.; Calderón, A. A.; Pedreño, M. A.; Ferrer, M. A.; Merino de Cáceres, F.; Ros-Barceló, A. Purification of a basic peroxidase isoenzyme from *Capsicum* fruits and the immunoinhibition of its capsaicin oxidation capacity by antibodies raised against horseradish peroxidase. *Z. Lebensm. Unters. Forsch.* **1994**, *199*, 240−242.

(18) Pomar, F.; Bernal, M. A.; Díaz, J.; Merino, F. Purification, characterization and kinetic properties of pepper fruit acidic peroxidase. *Phytochemistry* **1997**, *46*, 1313−1317.

(19) Bernal, A. M.; Ros-Barceló, A. 5,5′-Dicapsaicin, 4′-*O*-5-dicapsaicin ether, and dehydrogenation polymers with high molecular weights are the main products of the oxidation of capsaicin by peroxidase from hot pepper. *J. Agric. Food Chem.* **1996**, *44*, 3085−3089.

(20) Tateba, H.; Mihara, S. Photochemical oxidative dimerization of capsaicin in an aqueous solution. *Agric. Biol. Chem. Tokyo* **1991**, *55*, 873−874.

(21) Titze, P.; Petz, M. Untersuchungen zum Capsaicinoidgehalt in frischen Gewürzpaprika (Studies on the capsaicinoid content in fresh paprika). *Lebensmittelchemie* **1999**, *53*, 68.

Received for review April 23, 2001. Revised manuscript received December 3, 2001. Accepted December 5, 2001. We thank the German Research Foundation (DFG) and the Fonds der Chemischen Industrie for financial support.

JF010527A