JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Pungency in Paprika (*Capsicum annuum*). 2. Heterogeneity of Capsaicinoid Content in Individual Fruits from One Plant

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The capsaicinoid content of individual fruits from a single plant harvested at the same time after flowering exhibits a wide range of values with a rather uniform pattern for the ratio of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. This observation is confirmed by the analysis of fruits from a second and third plant and for several harvest times at different stages of maturity. Competition with lignin-like material, environmental influences, and subcellular distribution may play a role in the synthesis and transformation of capsaicinoids.

KEYWORDS: Paprika; individual fruits; capsaicinoids; heterogeneity

INTRODUCTION

Capsaicinoids are found only in the fruits of *Capsicum* plants. Extensive work (*1*−*3*) on their biosynthesis has shown that the vanillylamine part of the molecule is synthesized via the phenylpropanoid pathway and the fatty acid part originates from valine, leucine, or isoleucine. The final step combining both parts is thought to occur on the membrane of vacuoles in the placenta with subsequent accumulation of the capsaicinoids in these vacuoles and extracellular secretion (*3*, *4*). A high amount of capsaicin could be obtained by rinsing the interior of intact *Capsicum* fruits with methanol, and only smaller amounts were obtained with the acetone extract of the remaining minced tissue (*5*). In cell cultures derived from *Capsicum annuum* (*6*) or *Capsicum frutescens* (*7*, *8*) a major part of capsaicin was excreted in the medium.

The individual capsaicinoids are not converted into one another (*2*). The different ratios in *Capsicum* cultivars seem to be controlled by differences in the pool of CoA derivatives of fatty acids; they do not reflect the specific activity of the capsaicinoid-synthetase (*3*, *9*).

Although results from fruits of *C. annuum* var. *grossum*, a mild variety, indicate a changing pattern of capsaicinoids if harvested at different times (*10*), no change was observed in fruits from a hot hybrid species, *C. annuum* var. *annuum* cv. Karayatsubusa (*11*). The authors report some change when cultivating the same species one year later (*12*). Data for the mild variety *C. annuum* var. *annuum* cv. Padron grown in 1996 in a greenhouse show changing ratios with time (*13*), but other values were reported later in a study exploring the influence of fertilizer supplementation (*14*) or can be calculated from capsaicin and dihydrocapsaicin contents in fruits grown 1997 in a greenhouse with different water supplies (*15*).

Recent studies with cell cultures of *C. frutescens* show different ratios for capsaicin and dihydrocapsaicin within immobilized callus cells, placenta cells, and culture medium when compared to the corresponding part of the fruit grown on the related plant (*7*). It is not clear how feeding precursors to the cell culture has an influence on this capsaicinoid ratio (*8*).

Interaction effects of several genes and the environment have an influence on capsaicinoid levels (*16*, *17*). The capsaicinoid profile cannot be used for taxonomic characterization (*18*).

In all of these studies fruits from several plants were analyzed and compared, grown either in greenhouses or in the field. We report in this paper results obtained from individual fruits from three individual plants grown in a greenhouse.

MATERIALS AND METHODS

Plant Material. Seeds of *Capsicum annuum* (Pfeffer de cayenne G974, lot 18G85St) were obtained from EGESA (Giessen, Germany). Plants were grown in a greenhouse from April to September 1999. Fruits of similar size (length = 12 cm, width = 1 cm, weight = 3−5.5 g) were harvested from 14 to 66 days after flowering. Individual fruits were kept wrapped in plastic foil at 1 °C for up to 4 days before analysis.

Sample Preparation and Chromatography. Each fruit was cut into small pieces and minced in a small-volume blender (Krups 708). The purée of one individual fruit was weighed and used for capsaicinoid determination as described in the preceding paper (*19*).

RESULTS AND DISCUSSION

In our work on the stability or conversion of capsaicinoids we observed considerable differences in the capsaicinoid content among individual fruits of one purchase (*19*). The origin, age, and transport conditions of these fruits were not known and could not be controlled. Therefore, *C. annuum* plants were cultivated in a greenhouse. At day 14 after flowering 16 green fruits were harvested from the exterior of one individual bush (bush 1). During storage at 1 °C in the dark, wrapped in plastic foil, five fruits had turned red 2 days later. Six fruits were selected randomly for analysis. Their capsaicinoid content comprises a wide range between 217 and 632 mg/kg of fresh weight with small variations in the profile of the three main

10.1021/jf0105283 CCC: $22.00
Published on Web 02/02/2002

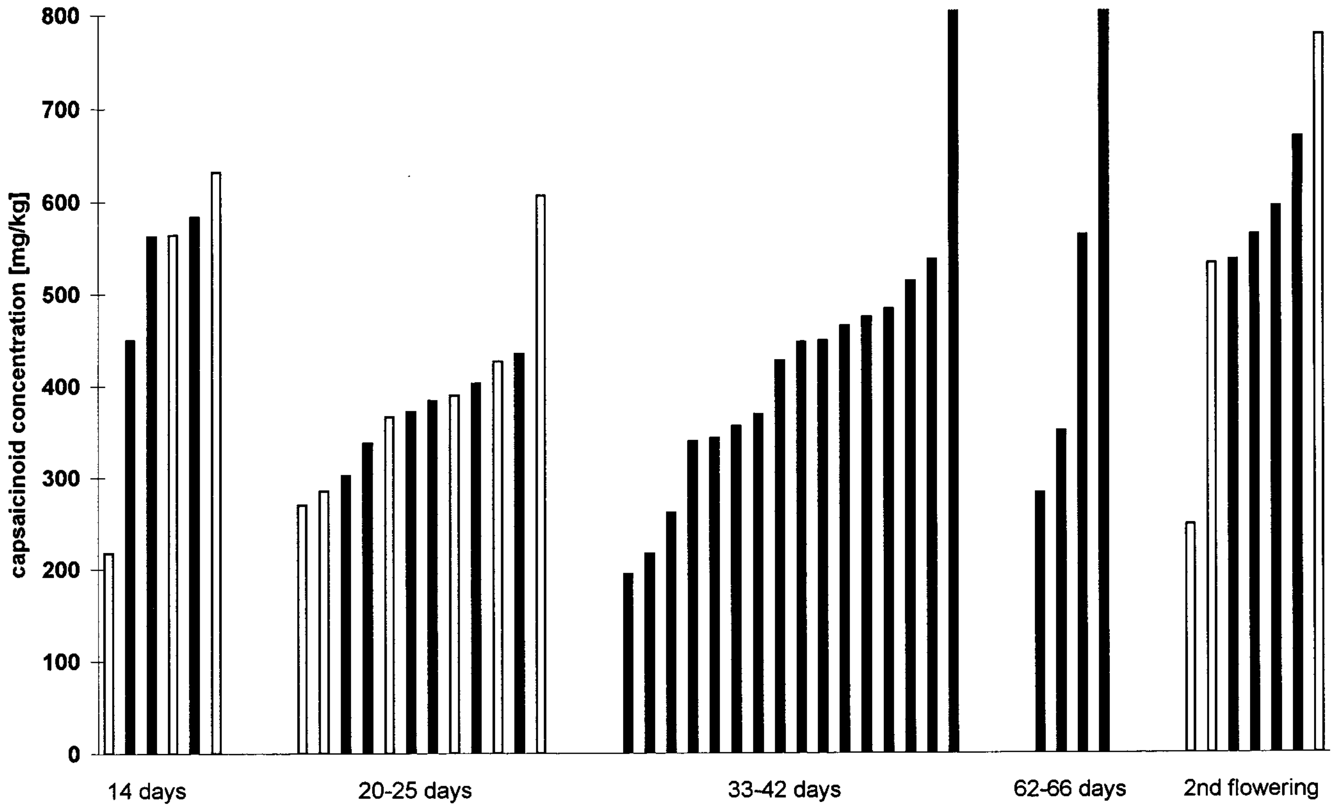

Figure 1. Capsaicinoid content of individual fruits harvested at different times after flowering: (black bars) red fruits; (white bars) green fruits.

Table 1. Capsaicinoid Content and Profile of Six Individual Fruits from Bush 1, Harvested 14 Days after Flowering[a]

color of fruits	capsaicinoid content (mg/kg)	C %	DC %	NDC %
green	217	59	31	10
red	450	48	37	15
red	562	55	33	12
green	564	59	31	10
red	583	48	35	17
green	632	54	34	12

[a] C, capsaicin; DC, dihydrocapsaicin; NDC, nordihydrocapsaicin.

components, capsaicin (C), dihydrocapsaicin (DC), and nordihydrocapsaicin (NDC). There is no correlation with color as an indication for a different stage in maturity (**Table 1**). Fruits harvested one week later (days 20−25) from the three bushes exhibited the same range of capsaicinoid content and profile, except for one fruit from bush 3 with less capsaicin (**Table 2**). Between days 33 and 42 fruits were harvested from the three bushes, and 16 individual red fruits were analyzed. A similar variation in capsaicinoid content was obtained, as was the case with four fruits harvested between days 62 and 66 (**Figure 1**).

The three bushes in the greenhouse blossomed a second time in September. The resulting fruits showed various capsaicinoid contents within the same range (**Table 3**). The profile again was quite uniform with slightly less capsaicin compared to the fruits of the spring series (**Table 1**).

Seasonal changes, for example, higher capsaicinoid accumulation, were observed in fruits of *C. annuum* var. *annuum* cv. Padron ripened in June to September compared to those in October (*20*).

The heterogeneity of capsaicinoid contents among fruits from plants grown under identical conditions, and even among individual fruits harvested from the same plant at the same time,

Table 2. Capsaicinoid Content and Profile of 12 Individual Fruits from Three Bushes, Harvested 20–25 Days after Flowering[a]

bush	color of fruits	capsaicinoid content (mg/kg)	C %	DC %	NDC %
1	green	285	48	37	15
	red	302	53	33	14
	red	383	51	36	13
	green	427	53	36	11
2	red	372	49	36	15
	green	389	51	37	12
	red	402	53	34	13
	green	606	54	34	12
3	green	269	37	41	22
	red	337	43	38	19
	green	365	47	38	15
	red	435	48	35	17

[a] C, capsaicin; DC, dihydrocapsaicin; NDC, nordihydrocapsaicin.

Table 3. Capsaicinoid Content of Seven Individual Fruits from Three Bushes, Harvested after Second Flowering within the Same Year[a]

color of fruits	capsaicinoid content (mg/kg)	C %	DC %	NDC %
green	248	45	40	15
green	532	50	40	10
red	536	48	40	12
red	563	49	38	13
red	593	52	36	12
red	669	50	37	13
green	778	53	37	10

[a] C, capsaicin; DC, dihydrocapsaicin; NDC, nordihydrocapsaicin.

raises the question of whether other factors apart from the genes involved in biosynthesis and environmental conditions influence pungency. The level of capsaicinoids at a certain time after flowering may result from biosynthesis, subcellular distribution, and chemical conversion.

In biosynthesis, lignin-like material mainly in developing seeds shares phenylpropanoid precursors with capsaicinoids and is synthesized within a comparable time schedule. Compartmentalization and competition for common precursors in different pathways of plant metabolism are discussed with respect to the synthesis and accumulation of capsaicinoids (*14*, *15*, *21*, *22*). Stress is known to influence the phenylpropanoid metabolism (*23*) and may indirectly affect capsaicinoid synthesis. Because we observed a decrease of capsaicinoid content only after cell disruption (*19*), those capsaicinoids that are still in the same cell compartments as the peroxidases (*24*, *25*) can undergo an oxidative conversion. Extracellularly excreted capsaicinoids (*3*) may not be amenable to these enzymes anymore. Nothing is known yet about these transport mechanisms and what factors may influence them.

ACKNOWLEDGMENT

We thank C. Hiepler and R. Kreuer for technical assistance.

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

JF0105283