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Effects of organic fertilizer in the capsaicinoids of red pepper (*Capsicum baccatum* L.)

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This study aimed to quantify the contents of capsaicinoids (capsaicin, dihydrocapsaicin and nordihydrocapsaicin) in the *Capsicum baccatum* L. species grown in soil with different concentrations of organic fertilizer (0, 1, 2 and 4%). The quantification of capsaicinoids was made by reverse-phase chromatography using the high-performance liquid technique (HPLC) with ultraviolet (UV) detection. The peppers were harvested from November 2009 to March 2010. The capsaicin content of the fruits of *C. baccatum* L. was higher for plants grown with organic fertilizer. The highest dihydrocapsaicin contents (10.49 ± 0.38 mg/100 g dry fruits) were obtained from the first harvest of pepper cultivated in soil at 2% organic fertilizer. The content of nordihydrocapsaicin did not change (p≥0.05) with different concentrations of organic fertilizer. The contents of capsaicinoids in pepper fruits varied with the different organic fertilizer concentrations used to grow the crop. Plants grown at 2% organic fertilizer presented the highest capsaicin content, with values of 44.70 ± 3.6 and 50.42 ± 4.80 mg/100 g dry fruits, in the first and second harvest, respectively.

Key words: Red pepper, capsaicinoids, capsaicin, organic fertilizer, high performance liquid chromatography (HPLC).

INTRODUCTION

In Brazil, *Capsicum* pepper crop is of major prominence in the economic, social and nutritional areas (Moreira et al., 2006; Sudré et al., 2010). Popularly known as red pepper, *Capsicum baccatum* L. is economically the most demanded pepper species of all cultivated in Brazil. The crop is present especially in the States of Minas Gerais,

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São Paulo, Goiás, Ceará and Rio Grande do Sul (Reischeneider and Ribeiro, 2004). Pungency is the key feature of pepper fruits. It is scaled according to the contents of capsaicinoids that are alkaloids accumulated only in the fruits of the *Capsicum* genus (Ishikawa et al., 1998; Carvalho and Bianchetti, 2004).

The production of capsaicinoids in red pepper depends on the Pun 1 gene (formerly known as C), which is dominant for pungency. However, the amount, quality and diversity of capsaicinoids in fruits depend on the soil, climate and growing conditions (Dias et al., 2008; Kappel et al., 2008; Domenico et al., 2012). The water regime, position of the fruits on the plant as well as the farming practices (that is, fertilization) are some important factors affecting the production and accumulation of capsaicins in peeper fruits (Carvalho et al., 2009).

Therefore, knowing the growing conditions of *Capsicum* plants that suit the production of alkaloids is very important since these alkaloids are valued both in the food and in the pharmaceutical industries (Domenico et al., 2012). So, this study aim to quantify the capsinoids content (capsaicin, dihydrocapsaicin and noridihydrocapsaicin) by the HPLC method in the fruits of red pepper (*C. baccatum* L.) grown at different concentrations of organic fertilizer. Scientifically, it implies on the identification of effects of the organic fertilizer on the concentration and diversity of capsaicinoids produced by pepper plants. In practical terms, the increased production of active ingredients may add value to this pepper species for being used as seasoning or medicinal plant and significantly contributing to the field of health and agribusiness.

MATERIALS AND METHODS

The cultivation of *C. baccatum* L. var. *baccatum*

The experiment was conducted in the Medicinal Plant Garden of the University of Maringá (UEM, Maringa-PR, Brazil) from July 2009 to March, 2010. Maringá is located at 23° 25' 31" S and 51° 56' 19" W, 500 to 600 m height. The soil type is Oxisol (Embrapa, 2006).

The experiment was designed in a 2×4 factorial completely randomized to two harvest periods (November 2009 and March 2010) and four levels of organic fertilizer (0, 1, 2 and 4%), with three repetitions. Irrigation was carried out by a drip system with 0.20 m drip spacing (Melo and Nagai, 1998). The seeds of *C. baccatum* were placed to germinate into 128-cell polystyrene trays, 3 seeds per cell, in July 2009. Seedlings were grown in the nursery. Pepper beds were prepared five days before planting with increasing concentrations of plant organic fertilizer (0, 1, 2 and 4% of the soil volume). The organic fertilizer in all mentioned concentrations was incorporated at 0 to 20 cm depth. The transplanting of seedlings to the beds (4.5 m^2, 0.20 m height and 0.50×0.50 m each) was made in August 2009 (Embrapa, 2004). Soil samples were collected at 20 cm deep in the Garden of Medicinal Plants of the UNI-Cesumar Maringa-PR. The samples were homogenized and sent for analysis to the Soil Fertile Laboratory (CRQ-03 751 and CRF-PR 015963/0) in Maringá-PR (Table 1). The fruits of *C. baccatum* L. were harvested in the early maturation stage. The first harvest was made in November 30, 2009 (three months after seedling transplanting) and the second in February 28, 2010; both in the morning (between 8:00 and 9:00 am).

Plant

The voucher specimens of *C. baccatum* L. var. *baccatum* have been deposited in the BHCB Herbarium at the Department of Botany, Federal University of Minas Gerais (UFMG, BHCB 139438).

Plant extracts

Fruits with seeds were washed under running water, chopped into small pieces, dried at 45°C and then milled. The fruit powder (10 g) was added to ethyl alcohol (96%) at 1:10 and subjected to hydrodynamic maceration (Brazilian Pharmacopeia, 2010). The organic solvent was removed using a rotary evaporator at 45°C and then lyophilized to yield 635 mg of the crude extract, which was stocked in a freezer at -15°C.

HPLC method

The capsaicinoids were analyzed using 20 mg of the lyophilized sample of *C. baccatum* L., which was subjected to extraction with 1 ml acetonitrile in an ultrasound bath (Unique, Ultracleaner 1400 A) for 10 min, twice consecutively. The extracts were combined and centrifuged at 13,000 rpm for 5 min at room temperature and then pellets were suspended in 1 ml of acetonitrile and filtered through a membrane filter cellulose acetate 0.45 µm pore size. Samples were then stored at -20°C until analysis. The contents of capsaicinoids in the extracts of red pepper fruits grown with different concentrations of organic fertilizer (0, 1, 2 and 4%) were quantified by the HPLC, according to the technical standards of the Association of Official Analytical Chemists (AOAC, 2000). The HPLC data handling was made by the Gilson system controller software equipped with the Rheodyne manual injector for 20 µl injection volume (pump model 307) and UV detector (model 151) at 280 nm. The mobile phase was acetonitrile/water/acetic acid (39.9/60/0.1 v/v) and Microsorb-MV column (4.6 mm × 250 mm, I.D., 5 µm) with a flow rate of 0.8 ml/min at 25°C. The calibration curves were obtained with standard solutions from 20 to 200 µg ml^{-1} capsaicin (Sigma Aldrich), 5 to 200 µg ml^{-1} dihydrocapsaicin (Sigma Aldrich) or nordihydrocapsaicin (Crescent Chemical Company). All analyzes were performed in triplicate. Statistical analysis was performed by SISVAR (a computer statistical ANOVA system) and complemented by the Tukey test using the 5% probability level.

RESULTS

This study quantified the amount (%) of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin of the *C. baccatum* L. species that were cultivated in North Parana State, Southern Brazil. The Reversed-Phase Chromatography was the HPLC technique applied to quantify the contents of capsaicinoids in dry fruits of *C. baccatum* L. that were obtained from different concentrations of organic fertilizer. The capsaicin contents of the fruits harvested in November (First Harvest) were 12.00 ± 0.54, 19.40±0.80*, 44.70 ± 3.6* and 40.39 ± 0.77 mg/100 g dry fruits for 0, 1, 2 and 4% organic fertilizer, respectively (Table 1). The capsaicin

Table 1. Chemical analysis of the soil and organic fertilizer.

Soil analysis							
$cmol_c\ dm^{-3}$					$mg\ dm^{-3}$	$g\ dm^{-3}$	pH
H^+Al^{3+}	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	P	C	$CaCl_2$
4.61	0.20	2.44	1.27	0.09	1.66	8.96	4.98

Organic fertilizer										
Humidity (%)		%								pH
65ºC	110ºC	C	MO	N total	CaO	MgO	K_2O	P_2O_5	Rel. C/N	H_2O
4.61	6.80	16.90	30.76	1.10	1.4	0.47	0.70	0.01	15:1	7.23

content of the fruits harvested in March (Second Harvest) were higher, reaching 26.15 ± 4.17, 37.73 ± 3.55, 50.42 ± 4.80* and 44.32 ± 1.22 mg/100 g dry fruits for 0, 1, 2 and 4% organic fertilizer, respectively (Table 2). The plants grown at 2% organic fertilizer presented the highest capsaicin contents, with values of 50.42 ± 4.80 mg/100 g dry fruits from the second harvest. The retention times for fruit extracts obtained from 2% organic fertilizer are 29 min for capsaicin, 26 min for nordihydrocapsaicin, and 46 min for dihydrocapsaicin (Figure 1). In this work, treatments up to 2% organic fertilizer favored the accumulation of capsaicin in the *C. baccatum* fruits. When compared to treatments with 4% organic fertilizer, the capsaicin contents declined to 40.39 ± 0.77 and 44.32 ± 1.22 mg/100 g dry fruits in the first and second harvest, respectively.

DISCUSSION

The pungency of red peppers is a distinguished quality parameter. About 20 capsaicinoids have already been identified in red peppers. The capsaicin, dihydrocapsaicin, and nordihydrocapsaicin are responsible for the pungent taste. The capsaicin and dihydrocapsaicin together represent 90% of the capsaicinoids (Nwokem et al., 2010). The *C. baccatum* L. spp. is very similar to the *Capsicum annuum* L. species that is one of *Capsicum* spp. most commercialized worldwide. Contents of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin were determined by the HPLC technique in the dry fruits of *C. baccatum* L. grown at different concentrations of organic fertilizer. Capsaicin was the major compound of the capsaicinoids. Giuffrida et al. (2013) also found capsaicin as the major compound of all capsaicinoids obtained from 12 different varieties of *Capsicum* by the HPLC technique. The highest capsaicin contents were found in soils that received 2% organic fertilizer: 44.70 ± 3.6 and 50.42 ± 4.80 mg/100 g dry fruits in the first and second harvest, respectively.

These results agree with the findings of Pino et al. (2007) for the *Capsicum chinense* species with total capsaicinoids of 41.8 to 65.9 mg/100 g dry fruit. Materska and Perucka (2005) quantified the capsaicin contents in different varieties of *C. annuum* L. by reversed-phase chromatography using the HPLC technique. The authors obtained 30 to 50 mg/100 g dry fruits, similarly to the values found in this present work for the *C. baccatum* L. spp. grown at concentration of 2% organic fertilizer. *C. baccatum* L. is similar to the pepper species most commonly used in the world and might therefore serve as a substitute in the manufacture of sauces and use by the food industry. Higher capsaicin contents were found in the second harvest of fruits grown with 2% organic fertilizer, in the months from January to March, which present high temperature and high rainfall. Pepper crops require high temperatures (25 to 30°C) for their development (Mercado et al., 1997).

Therefore, the high temperature observed in March in the second harvest perhaps increased the plant metabolism (Yaldiz et al., 2010) and consequently the contents of capsaicin in fruits. In addition, the hot and rainy weather generally observed in the months of January and March (second harvest) suits the development of pests and pathogens (Torquato, 2010) and these can induce the plant to produce capsaicinoids (Carvalho et al., 2009). Rodrigues et al. (2013) reported the effect of an organic fertilizer (concentrations of 0, 1, 2 and 4%) on the *C. baccatum* biomass development. The authors observed a decline in the pepper biomass performance for 4% organic fertilizer, suggesting a lower content of capsaicin. In pepper, the capsaicin production results in pungency and this is expressed by a dominant gene (Gene C). However, the accumulated capsaicin is as a result of the crop, soil and climate conditions (Dias et al., 2008; Kappel, 2008; Domenico et al., 2012).

In this work, the increased concentrations of organic fertilizer favored the accumulation of capsaicin in the *C. baccatum* fruits. It can be explained by the factors stimulating the primary metabolism and plant growth. Fertilization favors the availability of nutrients, especially N in soil, and can induce the production of secondary metabolites such as alkaloids, in this case, capsaicin (Costa et al., 2000). Other authors also verified the benefits of organic fertilization in crops, such as increased biomass production, improved productivity and

Table 2. Capsaicinoids contents (mg/100 g dry fruits) of *Capsicum baccatum* obtained to 4 different soil fertility concentrations and 2 harvest periods (November 2009 and March 2010).

	November 2009 (Soil fertility levels)				March 2010 (Soil fertility levels)			
Capsaicinoids	0%	1%	2%	4%	0%	1%	2%	4%
Nordihydrocapsaicin	2.46 ± 0.006	3.49 ± 0.10	3.52 ± 0.09	5.08 ± 0.29	2.64 ± 0.44	3.49 ± 0.015	4.74 ± 0,17	7.56 ± 0.17
Capsaicin	12.00 ± 0.54	19.40 ± 0.80*	44.70 ± 3.6*	40.39 ± 0.77	26.15 ± 4.17	37.73 ± 3.55*	50.42 ± 4.80*	44.32 ± 1.22
Dihydrocapsaicin	3.13 ± 0.14	3.70 ± 3.25	10.49 ± 0.38*	14.69 ± 0.18	3.29 ± 2.89	7.21 ± 0.34	7.70 ± 6.65	10.54 ± 0.66

The values correspond to the mean and standard deviation of triplicate extraction and quantification by HPLC. *The statistical significance ($p \leq 0.05$) was calculated between the harvest periods and among the fertility concentrations (ANOVA).

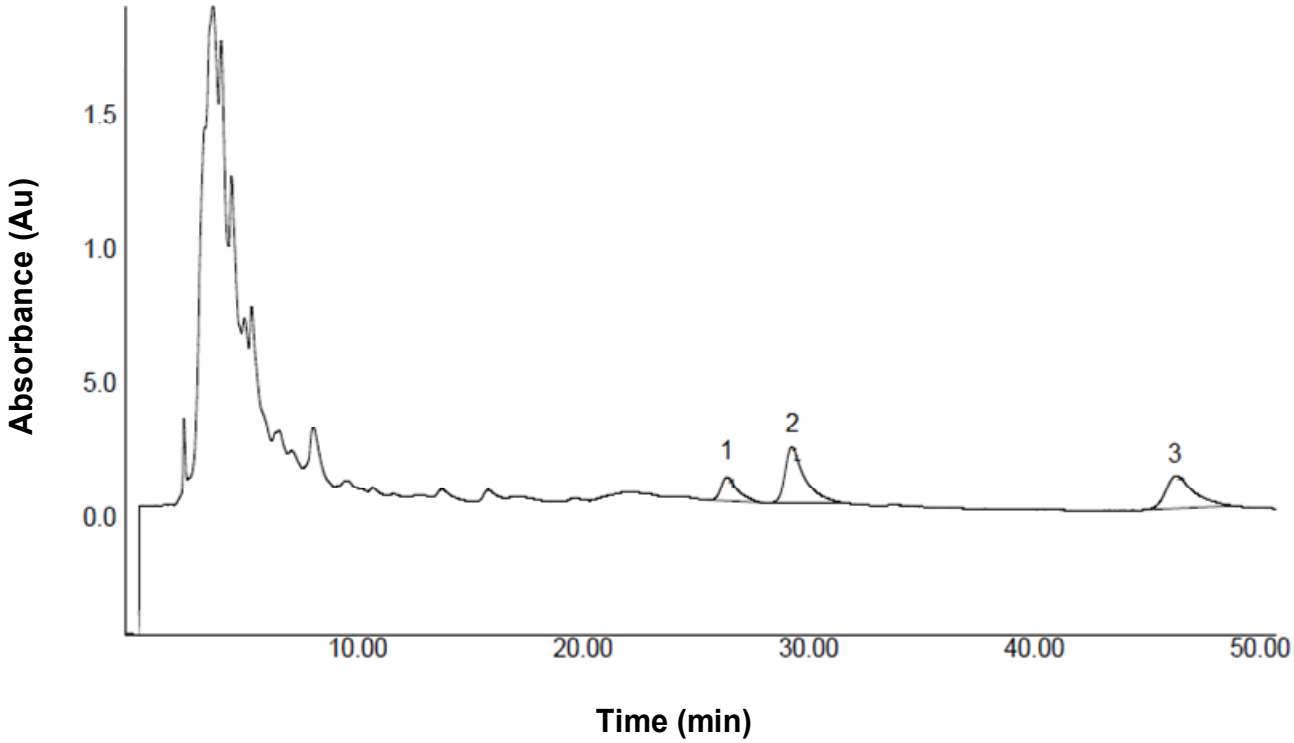

Figure 1. HPLC chromatogram of the extract of pepper fruits of *Capsicum bacattum* L obtained in the second harvest (March, 2010) and grown to 2% organic fertilizer: (1) Nordihydrocapsaicin, (2) Capsaicin and (3) Dihydrocapsaicin. Column: Microsorb-MV (4.6 mm × 250 mm, I.D., 5 µm). The mobile phase was an acetonitrile/water/acetic acid (39.9/60/0.1 v/v). Other parameters: flow rate, 0.8 ml/min; wavelength, 280 nm; column temperature, 25°C.

higher yield of active ingredients (Demeyer and Dejaegere, 1992; Becker et al., 2000; Chaves et al., 2006; Gómez-López and Del Amor, 2013).

Conclusion

The capsaicinoids content of red pepper fruits (*C. baccatum*) may vary according to different organic fertilizer concentrations used to grow the crop. The organic fertilizer at 2% concentration promoted the highest increase in the capsaicin content in pepper fruits, regardless of the harvest period.

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Conflicts of interest

The authors have not declare any conflict of interest.

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