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Short Communication

Nitrogen Fertilization Effect on Antioxidants Compounds in Fruits of Habanero Chili Pepper (*Capsicum chinense*)

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ABSTRACT

Habanero chili pepper (*Capsicum chinense* Jacq.) was cropped in an open field. Fruit quality was evaluated in relation to four nitrogen fertilization doses (32, 80, 160 & 320 kg N ha^{-1}), with the objective to identify their effect on several chemical and biochemical compounds. Total soluble solids (TTS), titratable acidity (TA), pH, antioxidant activity, and total phenols were determined. TA increase twice as high nitrogen dose was applied, but bioactive compounds as antioxidant activity and total phenols were not affected significantly with any nitrogen dose applied. In conclusion it is possible to obtain appreciable nutritional quality on habanero chili pepper fruit, independently of nitrogen management fertilization. © 2011 Friends Science Publishers

Key Word: Capsicum chinense; Nutrients; Phenolics compounds; Nitrogen; Antioxidants

INTRODUCCION

Pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional values of its fruits, mainly due to the fact that they are an excellent source of natural colors and antioxidant compounds (Howard *et al*., 2000). Ascorbic acid, carotenoids and phenolic compounds are its main antioxidants constituents (Marin *et al*., 2004; Gil-Guerrero *et al*., 2006). The intake of these compounds in foods is an important health protecting factor. They have been recognized as being beneficial for prevention of widespread human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts (Kaur & Kapoor, 2001; Sardas, 2003). It is generally assumed that environmental factors and agricultural techniques have an effect on vegetables and fruits quality (Wang, 2006; Bafeel & Ibrahim, 2008). In particular, mineral fertilization influences antioxidant composition in some fruit and vegetables (Jeppsson, 2000; Kaur & Kapoor, 2001; Kopsell & Kopsell, 2006; Kemal *et al*., 2007).

Some studies are available on the identification of antioxidant properties according ripening (Howard *et al*., 2000; Menichini *et al*., 2009; Navarro *et al*., 2006), genetic-environmental conditions (Lee *et al*., 2005) and postharvest life (González *et al*., 2005). However, information on the effect of mineral fertilization on nutritional quality of this kind of chili pepper is scarce. The aim of the present work was to evaluate the effect of four nitrogen doses of fertilization in habanero chili pepper (*C. chinense*) on their bio-compounds antioxidant content.

Additionally, any information on the physicochemical properties and bioactive compounds of fruit of this cultivar will provide a knowledge base that may be of some benefit to the developing fruit processing industry in Mexico and Latin America.

MATERIALS AND METHODS

The experiment was conducted at the Forestry Research Institute of Agricultural and Livestock, research field center located at Caborca, Sonora, Mexico (30°42'55" N, 112°21'18" W), during period of July-November 2009. Seedlings of habanero chili pepper (*Capsicum chinense* Jacq.) "Naranja" were transplanted in randomized complete block design with three replications per treatment. Phosphorus and potassium were not applied because soil test indicated sufficient quantities (53 & 474 mg kg^{-1}; Castellanos *et al.,* 2000). Nitrogen treatments applied in this trial, were 32, 80, 160 and 320 kg ha^{-1} in form of Urea-Nitrate-Ammonium Solution (8-8-16) applied in split doses during complete season following guidelines of previous trial developed under similar soil and environment conditions. Irrigation was done when soil reached a tension of 30 kPa, according to tensiometer inserted 30 cm of profundity. Harvest was done when fruits were fully matured (50 days of age). The fruits were transported at laboratory and frozen at -80°C for analysis.

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Physiochemical measurements: For determination of titratable acidity (TA), 30 grams of pulp-skin was macerated in mortar with 10 mL distilled water. A solution with NaOH (0.1 N) was used as a standardized titratation solution. Results were expressed in percent acidity of citric acid; On the other hand, pH determination was made using a digital pH-meter (Hanna HI 9813) with the application of the electrode directly in the juice. Total soluble solids (TSS) were measured with a manual refractometer placing a small drop of juice on the reading prism. Results were expressed as °Brix and additional TSS/TA ratio was determined.

Total antioxidant activity: This assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acid pH (Prieto *et al*., 1999). A sample of fruit (0.3 g) was mixed with 1.5 mL of ethanol (80%) and macerated in mortar at dark. After centrifugation (14000 rpm x 15 min), an aliquot (0.1 mL) of fruit extract was combined to 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate & 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 min. After, the mixture had cooled to room temperature, this was diluted in distilled water (1:9) and the absorbance of each solution was measured at 695 nm (Thermo Scientific Genesys 20) against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0–500 µg/mL.

Total polyphenols: Total phenolic content in habanero chili fruit was determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) and modified by Khandaker *et al.* (2008), using Gallic Acid as a standard phenolic compound. Briefly, 50 µL of habanero fruit extract solution was placed in a test tube, then 1 mL of Folin-Ciocalteu reagent (previously diluted by distilled water; Reagent: Water = 1:4) was added and the content was mixed thoroughly. After 3 min, 1 mL of Na_2CO_3 (10%) was added, the mixture was allowed to stand for 1 h in the dark. Absorbance was measured at 760 nm using a (Thermo Scientific Genesys 20) spectrophotometer. Total phenolic content of tissue was expressed as mg gallic acid equivalents per gram of fresh weight (mg GAE/g DW) through the calibration curve with Gallic Acid, ranging from 0 to 300 µg/mL.

Statistical analysis: All the experiments were conducted in triplicates and the results were calculated as mean±standard deviation (SD) in this study. Analysis of variance (ANOVA) was carried out using Statgraphics® Centurion XV (StatPoint Inc, USA).

RESULTS AND DISCUSSION

Chemical analysis: Concentrations of free sugars and organic acids in pepper fruit affect fruit flavor (Pieternel *et al*., 1994), and analysis of pH, TSS and AT is a friendly and quick way to know quality of fruit. Actually, there are research concerning to effect of ripening state on physical-chemical quality for other kind as yellow bell peppers (Antoniali *et al*., 2007), but for habanero pepper is scarce. In this nitrogen fertilization study, results showed that increasing nitrogen doses had no significant effects on quality determination relative to pH and TSS (Table I); values pH fluctuation was low (5.72-6.41), whereas for TSS, the values were 8.73 and 10.16 °Brix. In *Capsicum spp*, citric acid is reported as major acid in relation with others (malic, fumaric & succinic acid). Citric acid is present in *Capsicum chinense* fruit within a range of 0 to 0.2443 g 100 g^{-1} fresh of weight (Jarret *et al*., 2009). In this study citric acid presented as TA, showed significant increase with applied nitrogen doses, beginning with 0.1170 g 100 g^{-1} at 32 kg N, and ending a value of 0.2285 g 100 g^{-1}, with 320 kg N ha^{-1}, similar tendency with management of nitrogen applications has been reported in tomato (Bénard *et al*., 2009). Consequently, this situation decrease the TSS/TA ratio whit increase nitrogen dose (Table I). Recently, Jarret *et al.* (2009) studied various landraces of habanero chili pepper acquired from diversity origins as North, Central and South America; in this research they reported high variability for free sugars and organic acids in *C. chinense* and suggest that moderate level of sugars and high levels of organic acids contribute to the typically non-sweet taste of this kind of fruit.

Antioxidant activity: Antioxidant activity is the capacity to prevent auto-oxidation of free-radical-mediated oxidation of the substrate when present in low concentration (Halliwell, 1992). In the present study, average antioxidant activity for habanero pepper as a function of nitrogen fertilization ranged from 6.9 mg of GAE/g FW at intermediate nitrogen dose (80 kg ha^{-1}) until 8.1 mg of GAE/g FW at high nitrogen dose (320 kg N ha^{-1}), with no difference found among treatments (Fig. 1). Actually, researchers reports the relationship between antioxidant activity and ripening stage in diverse kind of pepper (Conforti *et al*., 2007) and according different color type (Zhang & Hamauzu, 2003). However, probably, this is the first report mentioning the effect of nitrogen fertilization on antioxidant activity on habanero chili pepper. Other has studied the effect on nitrogen fertilization on quality parameters **as** melons (Ferrante *et al*., 2008) and leafy vegetables (Kopsell *et al*., 2007). In both studies, have been found no difference in antioxidant activity.

Total polyphenols: Pepper fruit is considered an excellent source of bioactive nutrients such as carotenoids, vitamin C and phenolics compounds (Navarro *et al*., 2006). In this research, values of total polyphenols range between 798 and 650 mg/100 g GAE, with no response found among nitrogen treatment in this quality parameter of habanero chili fruit (Fig. 2). In current studies of crop mineral system on red pepper grown under soilless cultivation versus to conventional and organic systems, Flores *et al.* (2009) found differences among treatments, being

Table I: Nitrogen fertilization effect on chemical properties in habanero pepper fruit

Nitrogen (kg ha^{-1})	pH	TSS (Brix)	TA (g 100g^{-1})	TSS/TA (g 100g^{-1})
32	5.76 ± 0.099 a [z]	10.03 ± 0.636 a	0.1170 ± 0.020 a	88.555 ± 23.55 b
80	6.41 ± 0.184 a	8.73 ± 0.707 a	0.1436 ± 0.042 a	65.823 ± 21.24 ab
160	6.15 ± 0.064 a	9.92 ± 0.354 a	0.1877 ± 0.040 ab	53.812 ± 9.516 a
320	5.72 ± 0.092 a	10.16 ± 0.141 a	0.2285 ± 0.020 b	45.222 ± 7.829 a

[z]Standard deviation; TSS: Total Soluble Solids; AT: Titratable acidity; Means followed by the same letter are not statistically different at $P=0.05$; Values are expressed as means ± SD for n=3

Fig. 1: Nitrogen fertilization on antioxidant activity on habanero pepper fruits

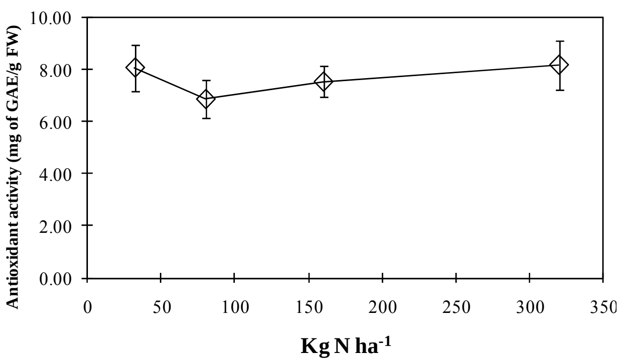
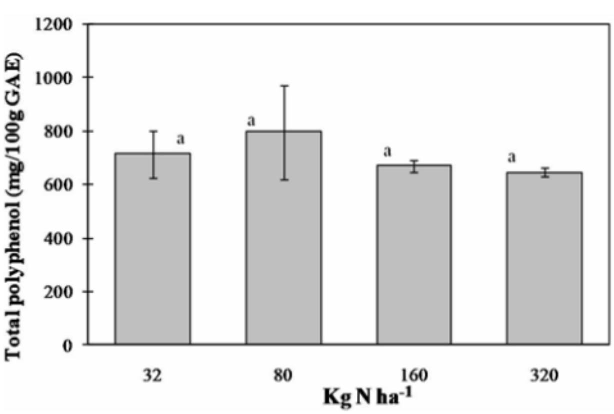


Fig. 2: Nitrogen fertilization effect on total polyphenols in habanero pepper fruits



soilless culture the most suitable for to obtain high phenols concentrations in fruit. On the other hand, recent studies on limitation of nitrogen supply a tomato crop cause increments in polyphenols in fruit (Bernard *et al*., 2009).

CONCLUSION

Nitrogen fertilization on habanero pepper caused effect on fruit flavor increasing the parameter measured as TA and decreasing the relation TSS/TA. On the other hand, the antioxidant activity and total polyphenols were not affected by any nitrogen doses applied. Therefore, it is advisable to reduce nitrogen input for cultivation on habanero pepper, without compromising phytochemical quality.

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