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## Vitamin C content in Habanero pepper accessions (*Capsicum chinense*)

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### ABSTRACT

Fruits of *Capsicum* species (peppers) accumulate high amounts of ascorbic acid or vitamin C. *C. chinense* occurs in the Midwest and Northeast regions and the Amazon Basin (where its greatest genetic diversity is found). The objective of the present work was to quantify the vitamin C content in peppers of 22 accessions of *C. chinense* 'Habanero' from the Breeding Program of Embrapa Vegetable Crops. Vitamin C was extracted from mature fruits with TCEP-HCl (tris 2-carboxyethyl-phosphine hydrochloride) and its content determined by high performance liquid chromatography (HPLC). Vitamin C content ranged from 54.1 to 129.8 mg/100 g. Accessions were divided into four heterogeneous groups of diversity. Vitamin C content of the first group varied between 116.2 and 129.8 mg/100 g; the second group ranged from 94.0 to 104.6 mg/100 g; the third group ranged from 76.7 to 87.5 mg/100 g; and the fourth group ranged from 54.1 to 66.6 mg/100 g. These results highlight the diversity of *C. chinense* collection in terms of vitamin C content.

**Keywords:** Pepper, ascorbic acid, genetic resource, high performance liquid chromatography (HPLC).

### RESUMO

#### Teor de vitamina C em acessos de pimenta (*Capsicum chinense*) do grupo varietal Habanero

Os frutos de *Capsicum* possuem elevados teores de ácido ascórbico ou vitamina C. A pimenta (*C. chinense*) ocorre nas regiões Centro-Oeste e Nordeste e na Bacia Amazônica (onde está localizada a sua maior diversidade genética). O objetivo deste trabalho foi quantificar o teor de vitamina C em 22 acessos de *C. chinense* do grupo varietal 'Habanero', procedentes do programa de melhoramento genético da Embrapa Hortaliças. A vitamina C foi extraída de frutos maduros com TCEP-HCl (tris 2-carboxyethyl-phosphine hydrochloride) e os teores foram determinados por cromatografia líquida de alta eficiência (CLAE). Os teores de vitamina C variaram entre 54,1-129,8 mg/100g. Foram formados, com base no teor de vitamina C, quatro grupos heterogêneos de diversidade. Os teores do primeiro grupo variaram entre 116,2-129,8 mg/100 g; o segundo variou entre 94,0-104,6 mg/100 g; o terceiro entre 76,7-87,5 mg/100 g; e o quarto entre 54,1-66,6 mg/100 g. Esses resultados evidenciam a diversidade dessa coleção de *C. chinense* para os teores de vitamina C.

**Palavras-chave:** Ácido ascórbico, recurso genético, cromatografia líquida de alta eficiência (CLAE).

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Peppers of the genus *Capsicum* were already used in the diet of pre-Colombian American peoples in many recipes, generally as part of sauces or as spice (Reifschneider & Ribeiro, 2008). *Capsicum chinense* occurs in the Brazilian Midwest and Northeast regions and in the Amazon Basin (where its greatest genetic diversity is found).

Several bioactive substances are present in fruits of hot and sweet pepper plants, especially the alkaloid capsaicin (that gives pungency), vitamin E, pro-vitamin A carotenoids, flavonoids and other secondary metabolites with antioxidant properties (Perucka & Materska, 2003; Matsufuji *et al.*, 2007; Oboh & Rocha, 2007; Sun *et al.*, 2007; Topuz & Ozdemir, 2007; Menichini

*et al.*, 2009). Fresh peppers are also excellent sources of ascorbic acid (vitamin C), which participates in several antioxidant processes in plants besides acting in the prevention of human chronic diseases, including certain types of cancer, coronary disorder, arteriosclerosis and cataracts (Howard *et al.*, 2000). The hot peppers (including several representatives of *C. chinense*) have great importance in the American continent and in tropical regions of the world. These products are essential in Mexican, African and Asian cuisines. In Brazil, *C. frutescens* (malagueta pepper) and *C. chinense* (pimenta de cheiro), are irreplaceable ingredients in several regional dishes. The acarajé and vatapá in Bahia state,

the chicken with okra in Minas Gerais state, the duck with tucupi in Pará state and the fish stew and chowder in several coastal cities of the Northeast and Southeast are some examples (Ribeiro & Henz, 2008).

Other lesser known varieties, like "Habanero", have begun to gain a greater importance in Brazil. The varietal group of hot peppers like "Habanero" presents, nowadays, a growing demand in North America and European Economic Community. The high value of this product in the international market is a result of an offer historically lower to the demand and, for this reason, has aroused the interest of national private groups in the development of processed products in this segment.

The Brazilian germplasm collection of peppers like “Habanero” is very diverse (Carvalho *et al.*, 1999). From the perspective of plant breeding, the characterization of the accessions with higher vitamin C content is interesting. Because of the red pigmentation of several samples of pepper, vitamin C content determined by classical method of dichlorophenolindophenol (DCFI) may suffer interference at the turning point. In this context, the aim of this work was to evaluate the methodology by high performance liquid chromatography (HPLC) (Fontanaz *et al.*, 2006) and quantify vitamin C content in 22 accessions of Habanero peppers (*C. chinense*) from the Breeding Program of Embrapa Vegetable Crops.

## MATERIAL AND METHODS

Twenty two accessions of *C. chinense* “Habanero” from the Breeding Program of Embrapa Vegetable Crops were evaluated (Table 1). Planting was done in the field, in April 2009, at Embrapa Vegetable Crops, Brasília, Federal District. The fertilization was carried out according to the recommendations for the pepper crop and the plot spacing in the field was 0.4 m between plants and 0.8 m between rows. Five plants were grown per accession. In September, about 30 to 40 mature fruits were harvested per accession. In the Chemistry of Natural Products Laboratory at Embrapa Genetic Resources and Biotechnology, the fruits were selected (about 20 fruits per accession), washed, dried with paper towels, wrapped in *kraft* paper bags and plastic and stored at 4°C until the time of extraction.

Each sample (about 20 fruits per accession) was homogenized with skin and seed in hammer mill IKA®. About 10 g of pepper pulp (three replications per sample) were weighed directly in a 100-mL volumetric flask. The vitamin C was extracted and analyzed using high performance liquid chromatography (HPLC), as described by Fontanaz *et al.* (2006). Briefly, the extraction was done with 40 mL of TCEP-HCl (tris 2-carboxyethyl-phosphine hydrochloride), adding about 30 mg of takadiastase with incubation at 30°C for

30 min; the volume was completed up to 100 mL with 1% trichloroacetic acid (TCA). The extract was filtered through paper and filter Millex LCR 13 mm with a modified PTFE membrane and it was analyzed using HPLC.

The equipment used was a chromatograph (HPLC) Varian® equipped with a quaternary pump, automatic injector and photodiode array detector controlled by software Galaxie®, using LiChospher column RP-18, 5 µm, 4.6x250 mm; isocratic elution 1 mL/min; detection at 265 nm; injection volume of 20 µL. Mobile phase: 1.6 g of decylamine: 80 mL of acetonitrile: 100 mL of 0.25 M sodium acetate pH 5.4: water up to 1000 mL; 50 mg of TCEP-HCl in the final solution. The ascorbic acid identification was made by co-chromatography with

standard and through comparison of the spectral profile provided by a diode array detector.

The quantification of the vitamin C content in the different accessions of *C. chinense* was carried out using external calibration curve, which was linear using six concentrations of ascorbic acid (Sigma®) ranging from 0.3 to 1.5 mg mL<sup>-1</sup> (r= 0.999).

For the statistical analysis, Euclidian distant matrices between the accessions were determined, used as a measure of dissimilarity for the cluster analysis of genotypes by the average distance method (UPGMA, Unweighted Pair-Group Method using an Arithmetic Average). The analysis of variance (ANOVA) at 5% significance was used to test the hypothesis of the difference between the mean vectors of each group.

**Table 1.** Vitamin C content in 22 genotypes of *Capsicum chinense* (Habanero) [teor de vitamina C em 22 genótipos de *Capsicum chinense* (Habanero)]. Brasília, Embrapa Recursos Genéticos e Biotecnologia, 2009.

Access CNPH	Color	Vitamin C* (mg/100 g)	DP	CV (%)
15035	light red	129.8	5.5	4.2
15027	light red	128.1	1.5	1.3
15041	light red	124.9	1.9	1.6
15045	red	124.0	0.6	0.5
15030	light orange	123.7	4.3	3.5
15033	dark red	123.4	1.8	1.6
15044	orange	121.7	5.6	4.8
15032	light orange	116.2	3.5	3.0
15042	light orange	104.6	2.9	2.8
15039	orange	103.8	1.7	1.6
15028	orange	98.2	3.6	3.7
15038	orange	98.1	0.8	0.8
15029	light red	94.0	1.5	1.6
15025	dark red	90.2	6.0	6.4
15036	light orange	87.5	2.8	3.2
15043	red	85.0	0.3	0.4
15040	orange	82.2	1.8	2.2
15037	red	76.7	2.0	2.6
15024	dark red	66.6	4.2	6.3
15026	dark red	64.5	1.8	2.8
15034	light orange	60.4	0.6	0.9
15031	red	54.1	1.1	1.9

\*Each value represents the mean of triplicate determination; DP= standard deviation; CV (%)= coefficient of variation (\*cada valor é média de determinações em triplicata; DP= desvio padrão; CV (%)= coeficiente de variação); light red (vermelho claro); red (vermelho); light orange (laranja pálido); dark red (vermelho escuro); orange (laranja).

## RESULTS AND DISCUSSION

The HPLC analytical methodology used in this work (Fontanaz *et al.*, 2006) showed extract stability and enabled the determination of vitamin C interference-free from colorful compounds or from other antioxidants (Figure 1). In Figure 1 only the chromatogram of accession CNPH '15027' was presented because the chromatograms of all the evaluated accessions showed the same chromatographic profile. The extraction solution used in HPLC method for analysis (with TCEP/TCA) showed the advantage of higher stability, when stored under refrigeration and protected from the light, if compared to the extraction using only acid solution (TCA).

The vitamin C contents ranged from 54.1 to 129.8 mg/100 g (Table 1). Based on vitamin C content, four heterogeneous groups of diversity were formed (Figure 2). The contents of the first group ranged from 116.2 to 129.8 mg/100 g, with average of  $123.5 \pm 8.9$  mg/100 g; the second group ranged from 94.0 to 104.6 mg/100 g, with average of  $98.1 \pm 6.2$  mg/100 g; the third group ranged from 76.6 to 90.0 mg/100 g, with average of  $82.0 \pm 4.8$  mg/100 g; the

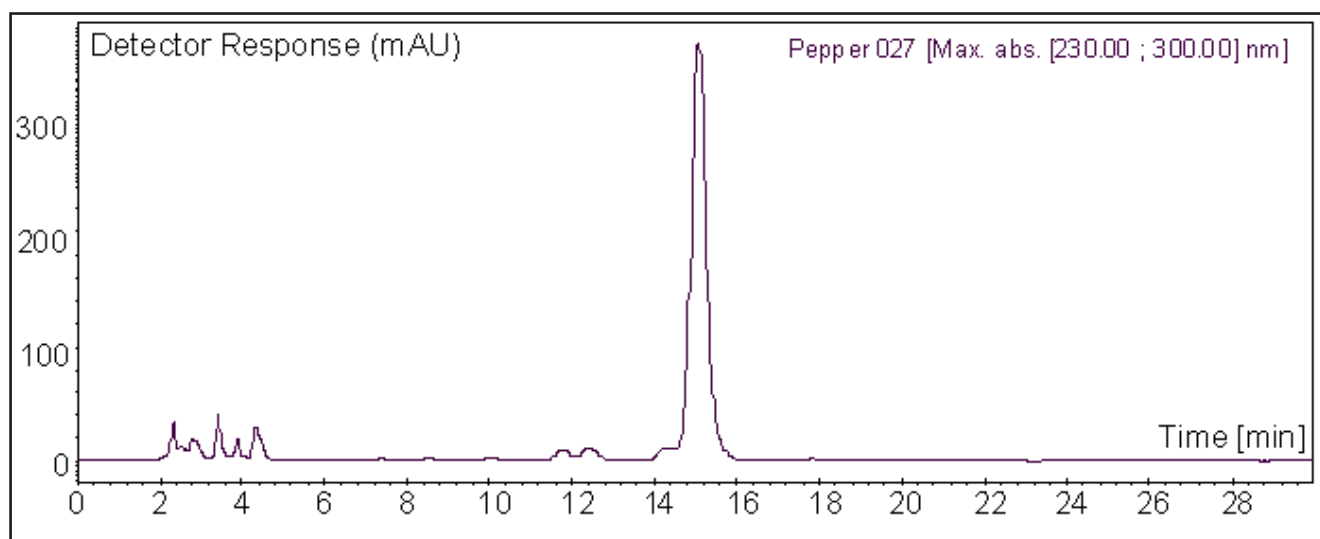
fourth group ranged from 54.1 to 66.6 mg/100 g, with average of  $61.4 \pm 5.4$  mg/100 g.

According to Lutz & Freitas (2008), the vitamin C content in Brazilian peppers, like "dedo-de-moça" (*C. baccatum*), biquinho, cumari-do-Pará, murupi (*C. chinense*), de-cheiro, debode, malagueta (*C. frutescens*) and jalapeño (*C. annuum*) ranges from 52.0 mg/100 g (jalapeño and dedo-de-moça) to 134.0 mg/100 g (murupi); the ascorbic acid contents determined in 17 accessions of *Capsicum* Breeding Program of Embrapa Vegetable Crops, Brasília, through the titrimetric method of Tillmans, ranged from 22.0 to 148.0 mg/100 g. The evaluation of a green and red cultivar of *C. chinense* var. Habanero grown in Santa Maria, Rio Grande do Sul state, showed 10.4 and 15.3 mg/100 g of vitamin C, respectively (Oboh *et al.*, 2007). For peppers grown in the United States, two Habanero cultivars (cv. Francisca and cv. Red Savina) showed vitamin C contents of 122.0 and 115.0 mg/100 g, respectively (Howard *et al.*, 2000). However, an extensive study of North American Germplasm, consisting of 216 different *C. chinense* accessions from North, South and Central America (Jarret *et al.*, 2009) showed a greater

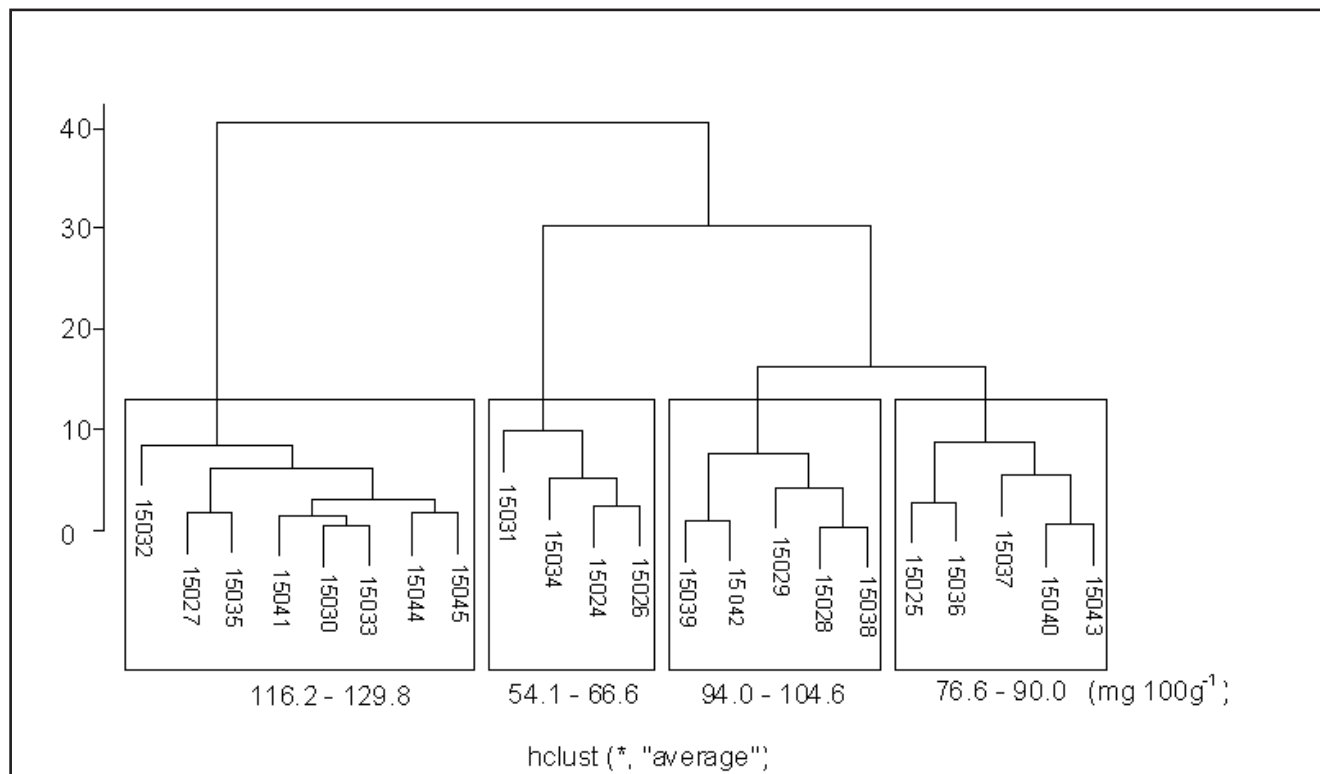
range of variation for ascorbic acid contents (30.0 to 1466.0 mg/100 g, with average of 391 mg/100 g) and pointed that Habanero varietal group can be further improved with respect to that trait.

The ascorbic acid and vitamin C, besides nutritional potential, contain antioxidant properties and it is present in high concentrations in several types of peppers. Maturation and storage conditions can also affect the stability and accumulation of vitamin C in fruits of *Capsicum*, besides genetic diversity (Howard *et al.*, 2000; Jimenez *et al.*, 2003). However, all the peppers used in this experiment were grown under the same controlled field conditions, allowing, this way, a comparative analysis.

The results showed variation in vitamin C contents in the accessions of this collection of *C. chinense* Habanero varietal group and also indicated potential to improve this variety for this vitamin. Some accessions showed vitamin C above the current daily recommendation for human diet, which is between 75 and 90 mg (Chen *et al.*, 2003). Thus, the *in natura* consumption or the consumption in spicy sauces with higher antioxidants contents



**Figure 1.** Ascorbic acid determination in *C. chinense* (access 'CNPH 15027'), using LiChospher column RP-18.5  $\mu\text{m}$ , 4.6x250 mm; mobile phase: 1.6 g of decilamine: 80 mL of acetonitrile: 100 mL of 0.25 M sodium acetate pH 5.4: water qsp 1000 mL; 50 mg TCEP-HCl in the final solution; flow: 1 mL/min; detection: 265 nm [cromatograma (CLAE) de determinação de vitamina C (ácido ascórbico) total em *C. chinense* (acesso 'CNPH 15027'), usando coluna LiChospher RP-18,5  $\mu\text{m}$ , 4,6x250 mm; fase móvel: 1,6 g de decilamina: 80 mL de acetonitrila: 100 mL de acetato de sódio 0,25 M pH 5,4: água qsp 1000 mL; 50 mg de TCEP-HCl na solução final; fluxo: 1 mL/min; detecção: 265 nm (HPLC chromatogram for total vitamin C)]. Brasília, Embrapa Recursos Genéticos e Biotecnologia, 2009.



**Figure 2.** Cluster analysis of 22 accessions of *C. chinense* Habanero originating from the breeding program of Embrapa Vegetables, conducted under controlled conditions in field, based on the matrix of standardized mean Euclidean distances, according to the levels of vitamin C (análise de agrupamento de 22 acessos de *C. chinense* do grupo Habanero, procedentes do programa de melhoramento genético da Embrapa Hortaliças, conduzidos em condições controladas de campo, com base na matriz de distâncias euclidianas médias padronizadas, calculadas com base nos teores de vitamina C). Brasília, Embrapa Recursos Genéticos e Biotecnologia, 2009.

may contribute to these daily values to be achieved in a larger number of consumers.

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