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Vitamin C and reducing sugars in the world collection of *Capsicum baccatum* L. genotypes

Venu Perla [a], Padma Nimmakayala [a,*], Marjan Nadimi [a], Suresh Alaparthi [a], Gerald R. Hankins [a], Andreas W. Ebert [b], Umesh K. Reddy [a]

[a] Gus R. Douglass Land-Grant Institute and Department of Biology, West Virginia State University, Institute, WV 25112, USA
[b] AVRDC – The World Vegetable Center, P.O. Box 42, Shanhua, Tainan 74199, Taiwan

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ABSTRACT

This study aimed to analyze 123 genotypes of *Capsicum baccatum* L. originating from 22 countries, at two stages of fruit development, for vitamin C content and its relationship with reducing sugars in fruit pericarp. Among the parametric population, vitamin C and reducing sugar concentrations ranged between 2.54 to 50.44 and 41–700 mg g^{-1} DW of pericarp, respectively. Overall, 14 genotypes accumulated 50–500% of the RDA of vitamin C in each 2 g of fruit pericarp on a dry weight basis. Compared with ripened fruits, matured (unripened) fruits contained higher vitamin C and lower reducing sugars. About 44% variation in the vitamin C content could be ascribed to levels of reducing sugars. For the first time, this study provides comprehensive data on vitamin C in the world collection of *C. baccatum* genotypes that could serve as a key resource for food research in future.

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1. Introduction

L-ascorbic acid, otherwise known as vitamin C, is commonly in fruits and vegetables. The importance of vitamin C in human health has been reviewed elsewhere (Naidu, 2003; Wahyuni, Ballester, Sudarmonowati, Bino, & Bovy, 2013). Vitamin C is known to have a key role in the maintenance of collagen, a major body structural protein (Levin, 1986), wound repair and healing process (Naidu, 2003; Shukla, 1969), synthesis of muscle carnitine, which is important for fatty acid transport and energy production (Hulse, Ellis, & Henderson, 1978), dietary absorption of iron (Hallberg, 1981), and prevention or relief from the common cold (Pauling, 1970). Although inconclusive, the role of vitamin C in reduced risk of cardiovascular disease and certain cancers cannot be ignored (Naidu, 2003). It has been shown in vitro that the effects of low dose pesticides on cell viability and reactive oxygen species production can be minimized by quantities of vitamin C equivalent to the recommended daily allowance (RDA) (Perla, Perrin, & Greenlee, 2008). The RDA for vitamin C, set by the US Food and Nutrition Board, for adult men is 90 mg/day and for adult women is 75 mg/day. However, the tolerable Upper intake Level (UL) of vitamin C for adults is 2 g/day (US Food and Nutrition Board, 2000).

The amount of vitamin C available in vegetables for human consumption varies widely. In one study, red and green chilli (*Capsicum annum* var. *longum*), kale (*Brassica oleracea* L. var. *alboglabra* L. *H. Bailey*), and red cabbage (*Brassica oleracea* var. *capitata* L. (*f. Rubra*)) were recorded as containing very high levels of ascorbic acid among 66 vegetables tested. In fact, the highest amount of vitamin C (>2 mg/g FW) was recorded in red chilli (Isabelle et al., 2010). In another study, 32 accessions belong to four pepper species, viz. *Capsicum annuum, Capsicum frutescens, Capsicum chinense and C. baccatum*, vitamin C levels ranged from 20.45 (*C. baccatum*) to 205.94 mg/100 g FW (*C. annuum*) (Wahyuni, Ballestera, Sudarmonowatib, Binoa, & Bovya, 2011). Similar studies on 63 to 216 accessions of *C. chinense*, obtained from North, Central, and South America, contained up to 1466 mg vitamin C/100 g FW (Antonious, Lobel, Kochhar, Berke, & Jarret, 2009; Jarret, Berke, Baldwin, & Antonious, 2009). Estimated daily intake of fresh *Capsicum* fruits by Americans was about 22 g in 2014 (Wells, Bond, & Thornsbury, 2015). Several pepper genotypes are able to supply between 50% to more than 100% of the recommended daily intake (RDI) of vitamin C (Howard, Talcott, Brenes, & Villalon, 2000; Wahyuni et al., 2013). These findings suggest there is a scope for further exploration of *Capsicum* species for vitamin C supply.

* Corresponding author.
E-mail addresses: venuperla@yahoo.com (V. Perla), padma@wvstateu.edu (P. Nimmakayala), marjan.nadimi51@gmail.com (M. Nadimi), salaparthi@wvstateu.edu (S. Alaparthi), ghankins@wvstateu.edu (G.R. Hankins), andreas.ebert@worldveg.org (A.W. Ebert), ureddy@wvstateu.edu (U.K. Reddy).

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Hancock and Viola (2005) and Gest, Gautier, and Stevens (2013) have reviewed various aspects of vitamin C biosynthesis in plants. Vitamin C synthesis in plants involves myo-inositol, L-gulose, L-galactose and/or D-galacturonic acid pathways. Not all the enzymes in these pathways have been identified in plants. However, L-gulose and L-galactose pathways are routed from GDP-D-mannose. Many biosynthetic pathways for vitamin C in higher plants might have a role in tissue- and organ-specific differences (Hancock & Viola, 2005). The majority of plants and animals synthesize ascorbic acid from D-glucose or D-galactose (Naidu, 2003), both reducing sugars. It is possible that the GDP-mannose pathway is the major vitamin C biosynthesis pathway in Arabidopsis (Dowdle, Ishikawa, Gatzek, Rolinski, & Smirnoff, 2007), but an alternative D-galacturonic acid pathway also exists in strawberry (Agius et al., 2003) and tomato (Badejo et al., 2012). These pathways might regulate vitamin C levels, with other pathways, or exhibit stage-specific response in these fruits (Badejo et al., 2012; Cruz-Rus, Amaya, Sanchez-Sevilla, Botella, & Valpuesta, 2011). Marín, Ferreres, Tomás-Barberán, and Gil (2004) reported that in sweet peppers (*C. annuum* L.), vitamin C accumulation increased with maturity and reached the highest levels in red ripened fruits. In another study, however, vitamin C levels in tomato and bell pepper (*C. annuum*) fruits decreased 74 and 51 days, respectively, after the fruit set (Yahiaa, Contreras-Padillaa, & Gonzalez-Aguilarb, 2001). From these reports, it is not clear whether vitamin C accumulation in pepper fruits is specific to genotype or species. Investigation of a large number of genotypes in a species may reveal the true pattern of vitamin C accumulation in this species.

Earlier studies on vitamin C content in *Capsicum* species were limited to few genotypes, regions or continents. Controversial reports exists on the stage in fruit development at which highest vitamin C is accumulated. Similarly, the relationship between reducing sugars and vitamin C in *C. baccatum* genotypes, which are widespread throughout the South America, is also not clear. Thus, the objective of this study was to analyze vitamin C in the world collection of 123 *C. baccatum* genotypes, and understand better the relationships between vitamin C and reducing sugars concentrations and fruit development. In this study, fully matured (un-ripened) and ripened fruit, which are the two major stages in harvest and consumption, were analyzed for vitamin C and reducing sugars, specifically D-glucose and D-galactose. We also attempted to identify the genotypes that might be able to supply at least half the RDA of vitamin C in human diet.

2. Materials and methods

2.1. Collection of fruit samples

Matured unripe and ripened fruits from 123 genotypes of *C. baccatum* were examined in this study. These genotypes were obtained previously from the Asian Vegetable Research and Development Center (AVRDC), Taiwan. This collection represents 22 countries, viz. Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, France, Germany, Guatemala, Guyana, India, Jamaica, Kenya, Mexico, Netherlands, Paraguay, Peru, UK, USA and Zambia. All these genotypes were grown in a field at Sissonville (WV, USA) during the summer of 2013 using standard cultural practices. Three plants were selected randomly from each genotype for sample collection. For each stage approximately 300 g (not less than five fruits when fruit size was bigger) of fruits were collected from each plant in a plastic zipper bag. Three zipper bags of fruits collected from three independent plants in each genotype were kept in a larger zipper bag and transferred to laboratory on ice.

Fruits from each zipper bag dipped in sufficient liquid nitrogen until frozen and returned to the same zipper bag before being stored at −80 °C for further analysis.

2.2. PBS extraction

From stored samples, approximately 20 g of fruits (not less than five fruits when size was bigger) from each plant were used to isolate pericarp. A small portion of the pericarp from each fruit was pooled to make up 2.5 g. This pooled sample was ground to fine paste with sand in a pestle and mortar, and mixed with 5 ml of ice cold phosphate buffered saline (PBS; without calcium chloride; without magnesium chloride; pH 7.4) (Life Technologies, Grand Island, USA). This mixture was centrifuged at 5000 x g at 5–8 °C for 5 min, and the supernatant collected and stored at −80 °C.

2.3. Estimation of pericarp dry matter

After separating the pericarp for extraction, remainder from all fruit of the same genotype was pooled, weighed, and dried in an oven at 65 ± 2 °C for 72 h. After drying, samples were weighed and the percentage dry matter estimated.

2.4. Estimation of vitamin C

Vitamin C (L-ascorbic acid) in extracted samples was estimated using a protocol adopted from Kapur et al. (2012) with modifications. Briefly, extracts were thawed on equal amounts of ice and water, and kept on ice until use. Samples were vortexed before and after every step in the procedure. 500 μL was mixed with an equal amount of 3% metphosphoric acid (Sigma–Aldrich, St. Louis, USA) and the mixture centrifuged at 10,000×*g* for 15 min in a table top microcentrifuge (eppendorf, Hauppauge, USA). 280 μL of the supernatant was collected and mixed with 14 μL of bromine concentrate (0.05 mol L^{-1} commercial solution; Sigma–Aldrich). To this solution, 14 μL of 4% thiourea (Sigma–Aldrich) was added and mixed well. Then, 70 μL of 2% DNPH (2,4-dinitrophenylhydrazine) (Sigma–Aldrich) was added and the mixture incubated at 35 ± 2 °C for 3 h. After incubation, the samples were kept on ice and 350 μL of cold sulfuric acid (85%) (Sigma–Aldrich) added. After 5 min, 80 μL was transferred to a glass 96 well plate (Cayman Chemical, Ann Arbor, USA), and the absorbance read at 520 nm in a microplate reader (Synergy HT™, BioTek Instruments, Winooski, USA).

Metphosphoric acid (3%) was prepared by dissolving 15 g of metphosphoric acid in 40 mL of glacial acetic acid (Sigma–Aldrich) and made up to 500 mL with distilled water. Sulfuric acid (4.5 mol L^{-1}) was used as a solvent for thiourea and DNPH solutions. DNPH solution was filtered using glass microfiber filters (GE Healthcare Bio-Sciences, Pittsburgh, USA). Freshly prepared ascorbic acid in PBS was used as a standard.

2.5. Estimation of reducing sugars

Reducing sugars in the samples were estimated using a protocol adopted from Perla, Holm, and Jayanty (2012). Glucose (Sigma–Aldrich) in PBS was used as a standard.

2.6. Statistical analysis

Unless otherwise stated, all the experiments were conducted with six replicates, and data are presented as mean ± standard deviation. Statistical analysis was performed using SAS® software (Version 9.4, Cary, NC) using BASE SAS, DATA step, PROC IMPORT, PROC CONTENTS, PROC SORT, PROC TRANSPOSE, PROC FORMAT, PROC CORR, PROC REG, PROC UNIVARIATE, PROC SGSCATTER,