

# Citrulline and Arginine in Fruit Flesh of Citron (*Citrullus amarus*) Accessions.

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## Introduction

Citron watermelon (*Citrullus amarus*) is a wild or semi-wild relative of the cultivated watermelon (*Citrullus lanatus* var. *lanatus*). Citron offers a reservoir of genetic diversity and is used for watermelon breeding programs for traits such as fruit firmness, disease resistance and abiotic stress tolerance (Levi et al., 2001; Levi et al., 2007; Guo et al., 2019; Ngwepe et al., 2019; Mandizvo et al., 2021). In particular, the citron PI 296341 has been used as a grafting rootstock for cultivated watermelon and has resistance to *Fusarium oxysporum* f. sp. *niveum*, race 2 and tolerance to *Macrophomina phaseolina* (Edelstein et al., 2014).

Citrulline, an amino acid first identified in watermelon, has been of human health interest for some years. Citrulline is the precursor of arginine, helps remove ammonia in the urea cycle, and boosts the nitric oxide system and vasodilation (Allerton et al. 2018). Among cucurbits, watermelon generally has the highest content of citrulline (Fish and Bruton, 2010; Hartman et al., 2019). Production environment and cultivar can also affect citrulline and arginine content in watermelon (Rimando and Perkins-Veazie, 2005; Davis et al., 2011). Citrulline values of 6.9 to 44.3 and arginine values of 1.8 to 20.7 mg/g dry weight, with ratios of citrulline to arginine ranging from 2:1 to 6:1, were found across 107 watermelon accessions (Assefa et al., 2020).

The citrulline content in citron fruit may be as high or higher than that of watermelon. Fish and Bruton (2010) reported that Botswana watermelon (*C. amarus* PI 542113) contained 45.8 and 7.2 mg/g dry weight (dwt) citrulline and arginine, respectively, compared to 22.1 and 5.8 mg/g dwt for 'Dixilee' watermelon. Johnson et al. (2022) reported citrulline and arginine concentrations of 39.2 and 2.2 mg/g dwt in wild citron from Queensland Australia compared to 22.9 and 8.1 mg/g dwt of in red-fleshed watermelon. Citrulline and arginine accumulate in drought stressed watermelon as a means of preserving nitrogen, acts as an osmolyte (Song et al. 2020), and helps protect tissues from oxidative stress by scavenging free radicals (Akashi et al., 2001).

In this study, fruit from citron watermelon (*Citrullus amarus* or *Citrullus lanatus* var. *citroides*) were utilized to determine variation in soluble solids content (SSC), pH, citrulline and arginine. Most of the accessions used originated from Africa (Table 1). These data may help in identifying accessions higher in citrulline that have potential value for breeding programs.

## Materials and Methods

Ripe fruit of citron accessions were grown at the U.S. Vegetable Lab in Charleston, SC and transported to Kannapolis, NC. Watermelons were cut in half lengthwise (Figure 1A, B) and samples of 50 to 100 g of flesh per fruit were stored in plastic bags at -80°C until used. Remaining flesh was used to determine SSC and pH. Soluble solids content (SSC) from juice was determined by digital refractometer and pH determined by inserting a stainless steel pH probe into the melon flesh as described in Hartman et al. (2019).

Citrulline and arginine content were analyzed using 0.2 g of puree from thawed citron cubes mixed with a 1.2 ml aliquot of phosphoric acid (0.03M) following the method of Hartman et al. (2019). Filtered samples (5 ul) were injected onto a high-performance liquid chromatograph (Hitachi Elite LaChrom) equipped with a photodiode array detector and autosampler. A Gemini 3u C18, 110A, 250x4.6 mm column and guard column (C18 4x2.0 mm) (Phenomenex, CA, USA) were held at 25 °C and a mobile phase of 0.015 M phosphoric acid, 0.5 ml/min was used for peak separation at 195 nm. External standards of arginine and citrulline (Millipore Sigma, USA) were used to verify and quantify the amino acid peaks.

## Results and Discussion

A total of 45 citron accessions were sampled in this study. The %SSC was low (1.1 to 4.6), with an average value of 2.5 and median value of 2.4 (Table 1). The fruit pH values were 4.8 to 5.6, indicating that most samples were near ripe to ripe when compared to the pH range of 5.0–5.6 of ripe watermelon (*Citrullus lanatus*) (Perkins-Veazie et al, 2006). No correlation

was found between citrulline and soluble solids content in this group of citron accessions although Assefa et al. (2024) reported a negative correlation between citrulline content and SSC in watermelon.

Average concentrations of the citron accessions were 54.6 and 11.6 mg/100g fresh weight (fwt) for citrulline and arginine, respectively (Table 1). Substantial variation in both citrulline and arginine contents was found among accessions. Citrulline content ranged from 2.1 to 294.6 mg/100 g fwt, while arginine content ranged from 0.5 to 64.3 mg/100 g fwt, with median values of 34.8 and 7.4, indicating a broad diversity among accessions. The citrulline content of citron in this study was often lower than that reported for watermelon but was higher than that reported for many other cucurbits, such as melon, cucumber, and squash (Hartman et al, 2019).

Five of the citron accessions in this study (PIs 482283, 482308, 482311, 482312, 50035) were reported to be drought resistant (Zhang et al. 2011). These accessions were medium to low in citrulline (21.5-50.1mg/100g fwt) and arginine (4.8-25.9 mg/100g fwt).

We were unable to follow the potential effects of ripeness among the citron accessions in this study; Johnson et al. (2022) indicated that citron could increase 3-fold between green and ripe stages. Assuming that citron has a dry weight near the 9.1% reported for watermelon, (<https://fdc.nal.usda.gov/food-details/2747675/nutrients>), PI 596696 had a citrulline content of 32.4 mg/g dwt, and was most comparable to that of Johnson et al. (2022).

Our study shows that citron can vary widely in citrulline and arginine content, with some accessions similar to that reported in cultivated watermelon. This information may be helpful in crosses of citron and watermelon if higher amounts of citrulline are desired along with disease and insect resistance.

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Figure 1. Variation in citron watermelon exterior appearance (left), and interior appearance (right).

Table 1. Composition of fruit flesh from citron watermelon (*C. lanatus* v. *citroides*) accessions

Accession	Source	%SSC	pH	mg/100g-fwt	
				Arginine	Citrulline
GRIF15896	Russian federation	2.4	5.1	11.9	61.3
GRIF16135	South Africa	4.2	5.2	64.3	153.2
GRIF17032	USA	1.6	4.9	6.8	104.9
PI270564	South Africa	2.7	5.2	17.6	34.5
PI271775	Transvaal, South Africa	2.1	5.0	1.4	37.7
PI295850	South Africa	1.9	5.1	1.9	16.2
PI296334	Limpopo, South Africa	1.2	5.0	2.2	34.4
PI296335	KwaZulu-Natal, South Africa	3.9	5.4	4.7	50.1
PI296337	South Africa	1.4	5.1	1.0	29.1
PI296339	Cape Province, South Africa	3.1	5.0	1.3	15.2
PI296341	Cape Province, South Africa	2.1	5.0	3.6	62.5
PI296343	Cape Province, South Africa	1.7	5.0	0.8	9.6
PI299378	Cape Province, South Africa	2.2	4.8	2.1	30.0
PI299379	Cape Province, South Africa	1.1	4.9	7.4	59.5
PI379243	North Macedonia	1.3	4.8	3.1	27.4

Table 1. (Continued)

Accession	Source	%SSC	pH	Arginine	Citrulline
				mg/100g-fwt	
PI482257	Zimbabwe	2.2	5.2	7.7	87.7
PI482259	Zimbabwe	4.6	5.2	16.6	96.9
PI482267	Zimbabwe	3.2	5.4	12.7	25.5
PI482276	Zimbabwe	2.1	5.2	1.4	5.1
PI482283	Zimbabwe	2.9	4.9	7.2	21.5
PI482293	Zimbabwe	2.6	5.3	13.2	91.3
PI482298	Zimbabwe	2.5	5.2	5.5	34.4
PI482299	Zimbabwe	2.9	5.2	10.3	50.1
PI482308	Zimbabwe	3.3	5.4	15.9	39.1
PI482311	Zimbabwe	2.4	5.3	4.8	32.0
PI482312	Zimbabwe	2.4	5.3	5.9	45.9
PI482326	Zimbabwe	2.9	5.3	3.8	9.0
PI482335	Zimbabwe	2.4	5.1	8.7	34.0
PI482336	Zimbabwe	2.5	5.2	20.6	30.7
PI482342	Zimbabwe	2.9	5.6	5.6	25.8
PI494817	Zambia	2.5	5.0	15.0	34.8
PI500303	Zambia	3.3	5.1	18.9	29.8
PI500308	Zambia	2.2	5.5	25.9	36.3
PI500335	Zambia	2.0	5.1	0.5	2.1
PI532624	Zimbabwe	1.7	5.1	17.9	50.9
PI532659	South Africa	2.4	5.3	1.8	6.7
PI532664	Eswatini	1.5	4.9	9.6	34.9
PI542119	Botswana	2.8	5.3	31.7	97.1
PI596656	South Africa	2.7	5.1	18.5	120.6
PI596659	South Africa	1.9	5.1	1.2	17.9
PI596665	Transvaal, South Africa	2.3	5.1	18.1	62.8
PI596666	Transvaal, South Africa	2.9	5.1	6.3	10.5
PI596668	Transvaal, South Africa	2.7	5.4	32.2	212.6
PI596669	Cape Province, South Africa	2.6	5.3	11.3	89.3
PI596696	Transvaal, South Africa	2.5	5.1	41.1	294.6
Average		2.5	5.2	11.6	54.6
Median		2.4	5.1	7.4	34.8
Standard deviation		0.73	0.18	12.34	55.27
Minimum		1.1	4.8	0.5	2.1
Maximum		4.6	5.6	64.3	294.6