horticulturae

Article

Citrulline and Arginine Content of Taxa of Cucurbitaceae

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Received: 13 December 2018; Accepted: 28 February 2019; Published: 4 March 2019

Abstract: Watermelon is the most significant, natural plant source of L-citrulline, a non-proteinaceous amino acid that benefits cardiovascular health and increases vasodilation in many tissues of the body. Watermelon is a member of the Cucurbitaceae, which includes squash, melon, pumpkin, and cucumber. It is possible that other cucurbits could be good sources of citrulline or of arginine, its direct precursor. Twenty-one cultigens were evaluated in triplicate at two locations in North Carolina to estimate citrulline and arginine amounts and variation due to cultigen, replication, and environment. Cultigens containing the highest amount of citrulline (based on LS means) in g/kg fresh weight were 'Crimson Sweet' watermelon (2.85), 'Dixielee' watermelon (2.43), casaba-type melon (0.86), mouse melon (0.64), and horned melon rind (0.45). Additionally, mouse melon, horned melon, and bitter gourd (arils) may be interesting sources of arginine-family amino acids, perhaps because of their large seed and aril content relative to mesocarp.

Keywords: Survey; Cucurbits; *Citrullus lanatus*; phytonutrients; *Cucumis sativus*; *Cucumis melo*; *Melothria scabra*; *Cucumis metuliferus*

1. Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matasum, and Nakai] is a natural plant source of lycopene, citrulline, and arginine, which are phytochemicals associated with reduced risk to several cancers and increase of vasodilation in many tissues of the body [1]. Citrulline, a non- proteinaceous amino acid, is unusually abundant in watermelon, more than any other plant taxon recorded to date [2]. Citrulline has a high nitrogen to carbon ratio, having roles in both plant and human metabolism. In human health, citrulline offers many health benefits, especially in exercise supplementation for muscle performance and recovery [3–5]. While dietary uses have received the most attention, citrulline has been investigated in other human-health fields, including pharmacology [6,7], immunology [8], and neurology [9]. Citrulline is a precursor to the essential amino acid arginine; pharmacological study shows that in diseases related to arginine deficiencies, supplementation with citrulline can be more effective than arginine itself. This effect is due to its more targeted metabolism, which contributes to increased bioavailability, better absorption into the bloodstream and reduced side-effects when administered orally [3,10–13].

The production and role of citrulline in plants is both less studied and more complex than in human systems. Several studies that examined physiological and environmental variables in watermelon suggest potential influences on citrulline abundance. Citrulline accumulates dramatically in the leaves of *Cucumis melo* (melon) and some *Citrullus* species in response to stresses of drought and high light intensity, suggesting a role in osmotic adjustment, radical oxygen species scavenging, and potential use as a biomarker for selecting tolerant cultigens [14–17]. Several genes are upregulated in watermelon during drought stress (including glutamine acyl transferases), and others downregulated, further supporting these ideas [18–20]. In drought-stressed cucumbers, citrulline, and proline metabolism were down-regulated upon alleviation of the stress involving CO_2 levels; citrulline was identified using ultra-high performance liquid chromatography quadrupole-time of flight mass spectrometry (UHPLC-Q-TOF MS) [21].

Citrulline content in domestic watermelon fruit varies with cultivar, ploidy, genotype, flesh color, and fruit anatomy. Rimando and Perkins-Veazie [22] reported that citrulline was most abundant in red watermelon fruit compared to orange and salmon yellow-fleshed fruit on both a fresh and dry weight basis. Seedless and seeded types also differed in citrulline, being higher in seedless (3X) yellow and orange cultivars than in seeded (2X). Canary yellow fruit had the highest citrulline, while there was high variation in red-fleshed watermelon cultivars (0.7–3.5 g/kg FW citrulline, the mean of 1.0, while orange-fleshed cultivars, 'Tendersweet Orange Flesh', and 'Orange Sunshine' had 0.5 and 3.0 g/kg FW citrulline, respectively [22]. Though apparently not causal, it appears that certain carotenoids may prove predictive of citrulline content in some watermelons, especially cultivars with canary yellow-flesh (genotype *CC*). In larger studies with triploid and autotetraploid watermelons and their diploid progenitors, but using thin layer chromatography (TLC), citrulline differences were not significant [23,24]. Among seeded cultivars grown in three locations (Lane OK, Clinton NC, and Kinston NC), citrulline content varied more among fruit within a cultivar than across cultivars or locations [25]. The lack of a location impact on within-cultivar variation (on citrulline content in watermelon) warrants investigation in other cucurbit taxa in the hopes of finding other high citrulline and arginine fruit and vegetables.

Citrulline accumulation appears to peak with ripeness, concomitant with sugars [20,26,27]. Among tissues, watermelon heart (central-most, non-locular), rind, and peel had the greatest citrulline content [26,27]. Joshi and Fernie [28] indicate that citrulline accumulation likely results from decreased catabolism rather than an increase in biosynthesis. Citrulline content may also be higher in seedless fruit due to more cells per unit area of flesh [29], and higher in grafted watermelon, though there are conflicting hypotheses regarding this [30,31].

Apart from watermelon and a handful of fermented foods (e.g., cocoa pulp and fermented soybeans in soy sauce), there are few significant reports on citrulline content in plants and plant products using modern extraction techniques [32,33]. Among several fruits and vegetables studied, Fish [2] found the highest amounts of citrulline and arginine in an accession of *Citrullus* from Botswana watermelon (= 48.94 $gkg^{-1}DW^{-1}$), followed by domestic (dessert) watermelon (195.2 mmol = 34.2 $gkg^{-1}DW^{-1}$), with pumpkin (*Cucurbita pepo* L. subsp. *pepo*), cantaloupe (*Cucumis melo* L. Reticulatus Group), cucumber (*Cucumis sativus* L.), and buffalo gourd (*Cucumis foetidissima* Kunth) falling far behind (4.6 $gkg^{-1}DW^{-1}$, 3.78 $gkg^{-1}DW^{-1}$, 3.31$gkg^{-1}DW^{-1}$, and 2.47 $gkg^{-1}DW^{-1}$, respectively), and undetected in straightneck squash (*Cucurbita pepo* subsp. *texana*) [2]. Though notable, Fish's study focused on technical variation associated with validating a quantitation method, and thus collected multiple samples from a single fruit.

In contrast, the objective of this study was to measure the biological variation in the content of citrulline and arginine of cultigens from multiple species and genera in the Cucurbitaceae freshly harvested from field plantings.

2. Materials and Methods

2.1. Germplasm, Cultivation and Field Design

Cucurbits were selected from genotypes and cultivars common in the United States and Asia. Germplasm spanned seven species including five genera, including *Citrullus lanatus* dessert watermelon), *Cucumis melo* (melon), *Cucumis metuliferus* (horned melon), *Cucumis sativus* (cucumber), *Cucurbita pepo* (squash), *Melothria scabra* (mouse melon), and *Momordica charantia* (bitter gourd) (Table 1).

We were unable to collect reliable data from all winter squash and some horned melon due to susceptibility to downy mildew, so winter squash and several horned melon plots were excluded from the analysis.

Cultigens were evaluated in 2016 at two locations, the Horticultural Crops Research Station in Clinton, NC and the Cunningham Research Station in Kinston, NC. The experiment was a randomized complete block design with three replications. Plants were grown using five-plant plots with 0.6 m spacing in 3.6 m long plots and 1.5 m row spacing. The vines were turned until the initiation of fruit set to prevent them from growing into adjacent plots. Plots were grown using horticultural practices recommended by the North Carolina Extension Service, also including drip irrigation and black plastic mulch [34]. Cucurbits were harvested by groups: Summer squash, winter squash, and all other cultigens.

2.2. Sample Collection

Fruit Harvest and Processing

Watermelon fruit were harvested when ripe (brown tendril, yellow ground spot, largest fruit, full seeds, and red flesh). Squash were harvested unripe, before seed development, according to market guidelines. Cantaloupe were harvested when rind began to yellow and at half-slip or later, according to market guidelines. Pickling melons (*Cucumis melo* Conomon Group) were harvested at approximately a 7.5 cm diameter. Cucumbers were harvested at a 5 cm diameter, and mouse melons when easily detached from the peduncle and fading spots were observed. Horned melons were harvested when the skin turned completely orange. Bitter gourds were harvested at an unripe, green stage before seeds matured, and again when the fruit ripened, and the end of the fruit began to dehisce, exposing the inner, bright-red arils.

All samples were collected and stored individually in zip-seal polyethylene bags. Samples were immediately transferred to coolers and stored on ice (up to 6 hr) before being transferred to a −18 °C freezer (up to 5 months) until blending. The portion of fruit sampled varied with cucurbit taxon and crop and ranged from flesh only to flesh and peel, as described in Table 1.

Ripe watermelon and melon samples were partially thawed at room temperature for 30 min, while whole-fruit samples were partially thawed at 4 °C. Variations or details for the specific fruit type are described in Table 1. Depending on cucurbit taxon and crop, the fruit were blended individually or combined by weight or size (using the middle third of fruit). For fruit considered ripe at the marketable stage (harvest time), SSC (soluble solids content), and pH data were taken to ensure only ripe fruit were sampled and combined (watermelon, most melons). Samples were blended for 45–90 s until homogenized into a consistent slurry, using stainless steel blender cups (50–250 mL) and a Waring Laboratory 7010S 1L 2 Speed Blender w/Timer. Variations or details for the specific cucurbit taxon and crop are described in Table 1.

2.3. Citrulline and Arginine Extraction and Quantification

2.3.1. Extraction

Frozen puree samples were thawed at room temperature and weighed as 0.2 g +/− 0.01 g aliquots. Phosphoric acid (1.2 mL, 0.03 M) was added to samples before vortexing for 1 min. Samples were sonicated (30 min), left at room temperature (10 min), and then centrifuged for 20 min at 4 °C, 5700× *g*; (Eppendorf, Model 5417R). A 1 mL aliquot of supernatant was filtered (17 mm nylon syringe filter, F2513-2, Thermo Scientific) into amber HPLC vials and held at −80 °C until HPLC analysis.

Table 1. Cucurbits evaluated, including seed source and blending method.

Taxon/Market Type	Cucurbit	Seed Source	Blending [y]
Citrullus lanatus			
Watermelon	'Dixielee'	Syngenta	M1
	'Crimson Sweet'	Syngenta	M1
Cucumis melo			
Casaba	'Golden Beauty'	NC BL [z]	M2
Honeydew	'Dulce Nectar'	Park Seeds	M2
	'Snow Mass'	Park Seeds	M2
Muskmelon	'Aphrodite'	Syngenta	M2
	'Athena'	Syngenta	M2
Pickling Melon	Green-Striped	Kitazawa Seed	M3
Sprite Melon (Makuwa group)	White-skinned	NC BL	M2
	Yellow-skinned	NC BL	M2
Cucumis metuliferus			
Horned melon (flesh, rind)		Kitazawa Seed	M4, M5
Cucumis sativus			
Pickling	'Expedition'	Seminis	M3
	'Vlaspik'	Seminis	M3
Slicer	'Dasher II'	Seminis	M3
	'Intimidator'	Seminis	M3
Cucurbita pepo			
Squash, straightneck	'Enterprise'	Park Seeds	M4
	'Goldstar'	Park Seeds	M4
Squash, Zucchini	'Payload'	Stoke Seeds	M3
	'Payroll'	Stoke Seeds	M3
Melothria scabra			
Mouse Melon	-	Park Seeds	M4 [x]
Momordica charantia			
Indian bitter gourd (unripe)			M4
Indian bitter gourd (ripe, arils)		Kitazawa Seed	M6
Indian bitter gourd (ripe, mesocarp)			M4

[z] NC BL = North Carolina Breeding Line. [y] **M1**: Fruit were sampled by cutting transversely and scooping flesh from the innermost portion; samples were combined by weight. **M2**: Fruit were sampled by cutting a section of flesh approximately 2in x 2in opposite the groundspot. Rind was removed before bagging and freezing; samples were combined by weight. **M3**: Whole fruit were harvested and frozen. After thawing, middle thirds of the fruit were sampled, peels were removed, and samples were combined by weight (±0.1 g). **M4**: Whole fruit were sampled and frozen. After thawing, samples were blended whole and combined by weight (±0.1 g, all but mouse melons and bitter gourd arils). **M5**: Flesh was blended to loosen seeds from arils; samples were centrifuged to remove seed debris from the sample. Supernatant was tested. **M6**: All ripe arils were quickly removed from the seeds while still frozen. [x] After freezing, outer cuticle was removed.

2.3.2. Quantification by HPLC

Citrulline and arginine concentrations were determined using the method of Jayaprakasha et al. [35] with modifications. Filtered samples (5 µL) were injected onto a High-Performance Liquid Chromatograph (Hitachi Elite LaChrom) equipped with a photodiode array detector and autosampler. A Gemini 3u C18, 110 A, 250 × 4.6 mm. 00G-4439-EO column and guard column (C18 4 × 2.0; AJO-4286, SecurityGuard Cartridge), (Phenomenex, CA, USA) held at 25 °C and a mobile phase of 15 mM phosphoric acid, 0.5 mL/min was used for peak separation. External standards of arginine and citrulline (Sigma) were used to verify and quantify these amino acid peaks.

2.4. Data Analysis

LS means (least squares means) and standard deviations were generated and simultaneously compared via the procedure for general linear models (PROC GLMMIX) using SAS (SAS Institute, Cary, NC, USA). Locations, replications, and genotypes were all analyzed as random effects. Analysis of variance (ANOVA) was employed to determine the significance of each variance component and their interactions for each of the five traits (soluble solids content, lycopene content, citrulline content, and arginine content, and their combination). Correlations among quality traits were calculated using PROC CORR.

3. Results

All analyses were performed assuming unrelated cultigens (i.e., no grouping by genus, species, kind, or type). The interaction between location and cultigen was significant for citrulline ($P < 0.0001$) and with cultigen ($P < 0.0001$), although location alone was not significant ($P = 0.861$) (Table 2). For arginine, the location x cultigen interaction was not significant ($P = 0.061$), nor was location ($P = 0.2359$), while cultigen was significant ($P < 0.0001$). The same was true when citrulline and arginine were combined ($P_{loc*clt} = 0.383$; $P_{loc*clt} = 0.531$; and $P_{clt} < 0.0001$). For SSC, only cultigen was significant ($P < 0.0001$). Cultigen had the largest mean square for all four traits. We did not include pH in the analysis except for correlations.

Table 2. Analysis of variance for four quality traits studied in 2 locations (loc), 3 replications (rep), and 24 cultigens (clt); all factors were considered to be random.

Source	df [z]	SSC	Arginine	F Values Citrulline	Cit±Arg [y]
Location	1	**14.94** *	204.15	3.4162	154.75
Rep(Loc)	4	2	160.63	111.78	531.45
Cultigen	23	**69.93** *	**3400.69** *	**8048.00** *	**19,736.20** *
Loc x Clt	20	0.95	240.71	**431.66** *	425.26
Error	60	0.84	142.38	111.68	390.12

[z] Degrees of freedom. [y] Combined concentrations of citrulline and arginine.* Indicates significant F value at $P = 0.05$.

Cultigens containing the highest amount of citrulline (based on LS means) in g/kg fresh weight were 'Crimson Sweet' watermelon (2.85), 'Dixielee' watermelon (2.36), casaba-type melon (0.86), mouse melon (0.64), and horned melon rind (0.45) (Table 3). Using least squares means, watermelon was distinctly separated from other cultigens for citrulline, arginine, and citrulline+arginine (cit+arg). LS means showed eight groupings of cultigens for citrulline, three for arginine, and six for cit+arg. Table 4.

Cultigens containing the highest amounts (in g/kg fresh weight) of arginine were 'Crimson Sweet' watermelon (1.47), 'Dixielee' watermelon (1.32), bitter gourd arils (0.66), horned melon rind (0.39), and horned melon flesh (0.38) (Table 3). Grouping by LS means revealed three groups, again, with only watermelon distinctly separated (Table 4). Considering the combined concentrations of citrulline and arginine, cultigens containing the highest amounts (in g/kg) fresh weight were 'Crimson Sweet' watermelon (4.32), 'Dixielee' watermelon (3.70), casaba-type melon (1.04), mouse melon (0.99), and horned melon rind (0.85) (Table 3). Six groups were found using multiple comparisons, watermelons being the only distinct group. Considering citrulline and cit+arg amino acid concentrations, watermelons had the greatest LS means (ranked first and second), at least twice as large compared to the next highest cultigen, which was casaba-type (ranked third). Bitter gourd aril ranked fourth for citrulline (0.66), but last for arginine (0.03). That was the greatest difference between citrulline and arginine rankings (Table 4).

Table 3. Means and standard deviations for five fruit quality traits from 21 cucurbit taxa.

Taxon/Market Type	Cucurbit	DP(ct.) [z]	pH (mean)	SSC (°Brix)	Arginine g(kg)$^{-1}$	Citrulline g(kg)$^{-1}$	Cit+Arg [y] g(kg)$^{-1}$
Citrullus lanatus							
Red-flesh seeded	'Crimson Sweet'	2(6)	5.68	10.6 ± 0.2	1.47 ± 0.1	2.85 ± 0.3	4.33 ± 0.2
	'Dixielee'	3(9)	5.69	11.2 ± 0.6	1.32 ± 0.1	2.37 ± 0.5	3.70 ± 0.4
Cucumis melo							
Casaba	NC BL	2 (20)	6.05	11.0 ± 0.6	0.18 ± 0.05	0.863 ± 0.04	1.04 ± 0.09
Honeydew	'Dulce Nectar'	6 (17)	6.26	11.5 ± 1.3	0.16 ± 0.01	0.163 ± 0.01	0.42 ± 0.09
	'Snow Mass'	5 (14)	6.41	12.9 ± 2.3	0.17 ± 0.05	0.177 ± 0.05	0.46 ± 0.1
Muskmelon	'Aphrodite'	5 (15)	6.39	11.5 ± 1.3	0.12 ± 0.02	0.340 ± 0.07	0.47 ± 0.08
	'Athena'	6 (18)	6.36	7.2 ± 1.5	0.13 ± 0.02	0.410 ± 0.07	0.55 ± 0.09
Pickling Melon	Green-Striped	5(14)	4.58	3.4 ± 0.5	0.10 ± 0.03	0.167 ± 0.06	0.37 ± 0.24
Sprite Melon	White-skinned	2 (20)	6.26	10.7 ± 0.4	0.096 ± 0.00	0.354 ± 0.18	0.45 ± 0.2
	Yellow-skinned	2 (20)	6.4	9.7 ± 0.4	0.094 ± 0.01	0.407 ± 0.2	0.50 ± 0.2
Cucumis metuliferus							
Horned melon, Rind		3 (9)	5.06	4.4 ± 0.05	0.412 ± 0.02	0.493 ± 0.3	0.90 ± 0.5
Horned melon, Flesh		3 (9)	4.34	7.3 ± 1.2	0.390 ± 0.01	0.090 ± 0.05	0.48 ± 0.2
Cucumis sativus							
Pickling	'Expedition'	6 (18)	5.88	2.9 ± 0.3	0.200 ± 0.05	0.211 ± 0.09	0.46 ± 0.2
	'Vlaspik'	6 (18)	5.9	3.0 ± 0.2	0.173 ± 0.03	0.232 ± 0.08	0.41 ± 0.11
Slicer	'Dasher II'	6 (15)	5.73	3.2 ± 0.4	0.263 ± 0.06	0.291 ± 0.1	0.56 ± 0.2
	'Intimidator'	5 (13)	5.86	3.4 ± 0.3	0.298 ± 0.07	0.228 ± 0.06	0.53 ± 0.1
Cucurbita pepo							
Squash, Straightneck	'Enterprise'	6 (16)	6.27	4.3 ± 0.4	0.240 ± 0.04	0.071 ± 0.05	0.31 ± 0.08
	'Goldstar'	6 (18)	6.28	4.5 ± 0.2	0.252 ± 0.04	0.056 ± 0.05	0.27 ± 0.09
Squash, Zucchini	'Payload'	6 (11)	6.37	3.1 ± 0.4	0.347 ± 0.31	0.085 ± 0.06	0.20 ± 0.08
	'Payroll'	6 (17)	6.26	2.9 ± 0.5	0.157 ± 0.08	0.042 ± 0.03	0.43 ± 0.4
Melothria scabra							
Mouse Melon	Mouse Melon	4 (120)	4.46	3.1 ± 0.1	0.346 ± 0.08	0.663 ± 0.2	1.0 ± 0.3
Momordica charantia							
Bitter gourd, Indian (unripe)		6 (18)	4.88	2.0 ± 0.3	0.279 ± 0.02	0.114 ± 0.1	0.39 ± 0.3
Bitter gourd, Indian (ripe, mesocarp)		5 (15)	5.98	3.2 ± 0.2	0.170 ± 0.01	0.051 ± 0.02	0.22 ± 0.2
Bitter gourd, Indian (ripe, arils)		5 (15)	6.02	16.6 ± 2.3	0.680 ± 0.02	0.030 ± 0.02	0.71 ± 0.2

[z] Data points (DP) represent number of plots, whose values are averaged (ct. = count of total fruit sampled over all plots). [y] Combined citrulline and arginine concentrations.

Table 4. LS means for citrulline and arginine content of 21 cucurbit taxa, grouped based on significant differences ($P \leq 0.05$) between LS means.

Citrulline			**Arginine**			**Citrulline + Arginine**		
Cultigen	LS Mean	Group	Cultigen	LS Mean	Group	Cultigen	LS Mean	Group
Crimson Sweet watermelon	2.85	A	Crimson Sweet	1.47	A	Crimson Sweet	4.33	A
Dixielee watermelon	2.36	A	Dixielee	1.32	A	Dixielee	3.69	A
Casaba-type melon	0.86	B	Bitter Gourd Ripe Aril	0.66	B	Casaba-type	1.04	B
Mouse Melon	0.64	BC	Horned Melon Rind	0.39	BC	Mouse Melon	0.99	B
Horned Melon Rind	0.45	BCD	Horned Melon Flesh	0.38	BC	Horned Melon Rind	0.85	BC
Athena muskmelon	0.40	BCD	Mouse Melon	0.34	BC	Bitter Gourd Ripe Aril	0.69	BCD
Sprite Melon, yellow	0.39	BCDE	Payroll	0.30	C	Dasher II	0.54	BCDE
Sprite Melon, white	0.34	BCDEF	Intimidator	0.30	C	Athena	0.54	BCDE
Aphrodite muskmelon	0.33	BCDEF	Dasher II	0.26	C	Intimidator	0.52	BCDEF
Dasher II cucumber	0.28	CDEF	Bitter Gourd Unripe	0.26	C	Sprite Melon, Yellow	0.49	BCDEF
Snow Mass honeydew	0.27	CDEF	Goldstar	0.24	C	Horned Melon Flesh	0.47	BCDEF
Dulce Nectar honeydew	0.25	DEF	Enterprise	0.24	C	Aphrodite	0.47	BCDEF
Pickling Melon	0.23	DEFG	Expedition	0.21	C	Snow Mass	0.45	BCDEF
Expedition cucumber	0.23	DEFG	Casaba-type	0.18	C	Sprite Melon, White	0.44	BCDEF
Intimidator cucumber	0.23	DEFG	Snow Mass	0.17	C	Expedition	0.44	BCDEF
Vlaspik cucumber	0.23	EFGH	Vlaspik	0.17	C	Dulce Nectar	0.41	BCDEF
Bitter Gourd Unripe	0.09	EFGH	Dulce Nectar	0.16	C	Vlaspik	0.40	BCDEF
Horned Melon Flesh	0.09	EFGH	Bitter Gourd Ripe Rind	0.15	C	Payroll	0.38	BCDEF
Payroll zucchini	0.07	FGH	Payload	0.15	C	Bitter Gourd Unripe	0.36	CDEF
Enterprise straightneck	0.06	FGH	Athena	0.13	C	Pickling Melon	0.34	CDEF
Bitter Gourd Ripe Rind	0.05	GH	Aphrodite	0.13	C	Enterprise	0.31	DEF
Payload zucchini	0.04	H	Pickling Melon	0.10	C	Goldstar	0.29	EF
Goldstar straightneck	0.04	H	Sprite Melon, White	0.10	C	Bitter Gourd Ripe Rind	0.21	EF
Bitter Gourd Ripe Aril	0.03	H	Sprite Melon, Yellow	0.09	C	Payload	0.20	F

Correlations were performed between SSC, pH, citrulline (cit), arginine (arg), and citrulline plus arginine. Significant correlations (other than arg to cit+arg and cit to cit + arg) included cit and arg (0.173), SSC and cit + arg (−0.558), and SSC and cit (−0.22) (Table 5).

Table 5. Pearson correlation coefficients of the five compositional components tested across all 21 cucurbit taxa.

Trait	Cit+Arg [z]	Citrulline	Arginine	Flesh pH
Citrulline	0.67 *	-		
Arginine	0.01	0.17 *	-	
Flesh pH	0.34	0.11	−0.22	-
SSC [y]	−0.56 *	−0.22 *	0.1	−0.29

[z] Combined citrulline and arginine concentrations. [y] Soluble solids content. * Indicates significant at $P = 0.05$.

4. Discussion

Several cucurbits have significant bioactive and nutrient profiles. For example, watermelon has a high concentration of citrulline and lycopene, which impart human health benefits. Citrulline benefits include increased vasodilation, cardiovascular health, and reduced risks for stroke and several cancers [1]. Citrulline is also thought to benefit the plants that synthesize it, by mediating drought and salt stress in plants, while also serving as a radical oxygen species scavenger [14,35,36].

Our data indicate that, among the cucurbits sampled, watermelon is 7–41 fold higher in citrulline than other cucurbits (Table 2). These results echo the smaller study done by Fish (2012). We were not able to see clear trends among cucurbit species. Within *Cucumis melo* types, citrulline ranged from 0.16 to 0.86 gkg^{-1} fresh weight (Table 2). Citrulline content also differed with tissue type in cucurbits. Citrulline content was much higher in horned melon rind than in flesh (Table 2), yet rind and aril contents were similar in ripe bitter gourd. In contrast, the arginine content of bitter gourd arils was much higher than rind. It may be that conversion of arginine to citrulline in bitter melon is inhibited and/or catabolism of citrulline in rind is enhanced [28]. In red-fleshed watermelon, citrulline was reported to be lower in rind than flesh on a fresh (0.8 and 2.0 g/100 g) or dry weight basis (1.6 and 1.8 g/kg) [22]. The difference was greater in fresh than in dry tissue, perhaps because rind has only half of the sugar content of flesh.

Amounts of citrulline in watermelon relative to other cucurbits were similar between Fish (2012) and this study with watermelon having ~10 times more citrulline than cucumbers, six–nine times more than cantaloupe, and 41 times more than squash (in this study). Casaba-type melon was highest in citrulline among the other cucurbits. Mouse melon and horned melon may also be interesting sources of the arginine family of amino acids, perhaps because of their large seed (or aril) content relative to mesocarp. In watermelon, arginine but not citrulline was found in seeds [31]. Several studies on seeds and seedlings indicate that arginine's plethora of roles includes reserves of easily mobilized nitrogen, not as a source of arginine for NOS [19]. Investigation of the physiology of these fruits could be interesting for seed biologists in germination efficacy and plant ecologists for dispersal studies.

The negative correlations of SSC to citrulline and cit+arg across cucurbits contradicts the moderate positive correlations reported previously in a watermelon heritability study [37]. Given the wide array of cultigens, correlations here may be affected by our choice of cucurbit species to test. Investigation of specific genera, species, and types may be warranted to pursue meaningful relationships between the arginine family amino acids and ripeness parameters.

Citrulline and arginine concentrations in cucurbits can be influenced by extraction methods, ripeness, and water activity in fresh fruit and value-added products. In our study, the extraction method employed for all cucurbits was optimized for extraction using watermelon tissue, which is low in cellulose and easy to homogenize. Citrulline is both water and lipid soluble and the best extraction may depend on obtaining the smallest particle size during homogenization. Tissues like horned melon rind, bitter gourd, and zucchini may be difficult to process due to firm flesh and rind, though our study reports relatively high citrulline content in horned melon rind compared to flesh. However, percent recovery could not be estimated because spiked samples were not included, and no base-line level of citrulline is known in this fruit. Additionally, environmental components (e.g., drought stress, light stress) seem to be impactful in citrulline and arginine content in cucurbitaceous crops, but not considered in detail in this study. Physiological ripeness is also an important factor to incorporate, since in watermelon, citrulline content peaks at physiological ripeness [26,27,30]. Apart from bitter gourd, all cucurbits were studied only at marketable stages, which do not always correlate with physiological ripeness (cucumber, squash, and other immature fruits eaten as culinary vegetables). These limitations warrant further investigation, as they may provide a significant barrier to understanding variation and correlation within and among traits of different cucurbit species. Citrulline has been proposed as the central organic nitrogen carrier in Cucurbitaceous species. This idea, coupled with the hypothesized decrease in citrulline catabolism in the fruit with ripening, may help uncover mechanisms of amino acid transport within the plant.

5. Conclusions

In cucurbit fruit sampled at a consumer maturity stage, citrulline content was the highest in watermelon. While citrulline was detected in other cucurbits sampled, content was <1%–14% that of watermelon. Arginine content was highest in watermelon and in the arils of ripe bitter gourd. These results show that citrulline is present in many cucurbits, but only watermelon contains significant amounts, while mouse melon, horned melon, and bitter gourd may be interesting sources of arginine-family amino acids.

Author Contributions: Conceptualization, T.W. and P.P.V.; methodology, J.L.H., P.P.V., G.M.; validation, P.P.V., G.M.; formal analysis, J.L.H.; investigation, J.L.H.; resources, P.P.V., T.W.; data curation, J.L.H.; writing—original draft preparation, J.L.H.; writing—review and editing, J.L.H., T.W., P.P.V., G.M.; visualization, J.L.H., T.C.W.; supervision, J.L.H., P.P.V.; project administration, J.L.H., P.P.V.; funding acquisition, T.C.W., P.P.V.

Funding: This research received no external funding.

Acknowledgments: I thank my advisor and colleagues Todd Wehner, James Daley, Takshay Patel, Brandon Huber, Lauren Arteman, and Emily Silverman for help in the field, and my advisor and colleagues Penelope Perkins-Veazie, Joyce Edwards, Erin Deaton, and interns for help in the laboratory. Additionally, I thank my work-study lab mentees Dessteen, Sicely, and Madison, and Field stations and crews in Castle Hayne, Clinton, and Kinston, NC. Special thanks for Rachel Naegele (USDA-ARS), Consuelo Arellano (NCSU Dept. Horticultural Science), Luis Rivera (Dept. Crop and Soil Science), and Dahlia Nielsen (Dept. Biological Sciences) for individual consultations.

Conflicts of Interest: The authors declare no conflict of interest.

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