

**The citrulline content of watermelon lines differs in their fruit flesh and rind parts**

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**Abstract**

Citrulline is a natural antioxidant and an amino acid. The watermelon is a fruit that is generally eaten for its fruit flesh. In this study, citrulline content was determined in different parts of the fruit flesh and rind of four watermelon lines with varying skin colors. The fruits were divided into six parts, and samples were taken from the rind and fruit pulp for the citrulline in each part. The results showed that the highest value of citrulline was obtained for the genotype with a very light green ground skin color (3.37 g/kg), while the lowest value was obtained from the genotype with a very dark green ground skin color (2.0 g/kg). Regarding the fruit parts, the highest value was taken from the 3rd part (3.72 g/kg), while the lowest value came from the 2nd part (2.00 g/kg). Higher citrulline values were found in the fruit flesh (3.10 g/kg) than in the fruit rind (2.40 g/kg).

**Keywords:** watermelon, fruit, citrulline, ground skin color, different parts of the flesh fruit.

**1. Introduction**

Watermelons are economically important worldwide, with a global production of 101.6 million tons. China (60.1 million tons) is the largest producer country, followed by Türkiye (3.5 million tons), India (3.3 million tons), Brazil (2.1 million tons), Algeria (2.1 million tons), and several other countries (29.8 million tons) (FAO 2021).

The watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai (2n = 22)) is one of the significant horticultural crops in the Cucurbitaceae family. It is typically consumed in fruit salads, desserts, or drinks. **A wide range of phenotypic characteristics, including fruit size, flesh color, rind pattern, and also disease resistance and flesh sweetness, are observed between cultivars (Chikh-Rouhou et al. 2019). Each growing region has a unique set of cultivars that are widely grown and are suited for cultivation in the local environment (Wehner, 2008; Chikh-Rouhou and Garcés-Claver. 2021).** Numerous nutrients and

35 bioactive substances, such as vitamins, lycopene, citrulline, and phenolic compounds, are found  
36 in watermelon (Romdhane et al. 2017). It is a natural source of citrulline, an amino acid,  
37 phenolic compounds, and carotenoid components, including lycopene, which has antioxidant  
38 properties (Rimando and Perkins-Veazie 2005; Aguilo-Aguayo et al. 2010; Sun et al. 2010;  
39 Joshi et al. 2019). Watermelon is freshly consumed by many worldwide, partly due to its low-  
40 calorie content and the fact that it is highly nutritious and thirst-quenching (Watt and Merrill  
41 1975; Sari et al. 2021).

42 Our body converts citrulline into arginine, an essential amino acid. This critical amino acid  
43 plays a significant role in the immunological, gastrointestinal, respiratory, pulmonary, renal,  
44 and hepatic systems, as well as aiding in the healing of wounds (Wu et al. 2000; Flynn et al.  
45 2002; Collins et al. 2007). In addition, citrulline has a potential role in vasodilation and  
46 cardiovascular functions, as arginine is a conditionally essential amino acid related to the NO  
47 system (Levine et al. 2012; Hong et al. 2015). Mandel et al. (2005) and Collins et al. (2007)  
48 indicated that watermelon is a potent source of both arginine and citrulline.

49 Recent studies have shown that citrulline and arginine profiles are important in combatting  
50 cancer (Fekkes et al. 2007; Yoon et al. 2007; Bowles et al. 2008; Lam et al. 2009; Schnader et  
51 al. 2009; Di et al. 2022), heart disease (Tang et al. 2009; Hong et al. 2015), acute hydrocephalus  
52 (Perez-Neri et al. 2007), minor intestine diseases, blood poisoning, trauma, and pulmonary  
53 hypertension (Papaida et al. 2007; Beyer et al. 2008; Santarpia et al. 2008) shows that it is useful  
54 in the healing of various diseases.

55 The watermelon is the richest known source of citrulline, and this amino acid plays a vital role  
56 in drought tolerance (Yokota et al. 2002; Rimando et al. 2005). Citrulline functions as a  
57 hydroxyl radical scavenger and may shield plants from oxidative stress brought on by dryness  
58 (Akashi et al. 2001). However, neither the effects of production conditions nor the differences  
59 between cultivars have been sufficiently investigated (Davis et al. 2011). According to Fish and  
60 Bruton (2010), one cultivar produced in two locations showed no change in the amount of L-  
61 citrulline in the flesh. Tarazona-Díaz et al. (2011) observed a mean citrulline concentration of  
62 2.33 mg/g in watermelon flesh based on five lines (four of which were triploid seedless  
63 cultivars) grown in a single location. The authors also demonstrated that the seeded cultivar had  
64 the lowest L-citrulline content in flesh tissue. An earlier study found that 14 watermelon  
65 cultivars ranged from 0.5 to 3.6 mg/g in terms of the fresh weight of citrulline, with an average  
66 concentration of 2.4 mg/g (Rimando and Perkins-Veazie 2005). The authors claimed that red-  
67 fleshed fruit contained less L-citrulline than yellow or orange fruit. Still, since only a small  
68 sample size (three fruits for each variety) was used, it would have been difficult to determine

69 the influence of genotype and environment on the L-citrulline concentration in those fruits.  
70 According to Liu et al. (2010), nine induced autotriploid hybrid watermelons produced in  
71 greenhouses showed greater L-citrulline levels than their diploid and induced autotetraploid  
72 parents. In Fish and Bruton's (2010) and Liu et al.'s (2010) studies, L-citrulline levels peaked  
73 at peak maturity.

74 Previous studies found a higher amount of citrulline in the watermelon rind. (Rimando et al.  
75 2005; Jayaprakasha et al. 2011; Tarazona-Díaz et al. 2011; Akashi et al. 2016; Dubey 2021).  
76 Rimando et al. (2005) and Kumar et al. (2012) stated that the rinds make up about 30% of the  
77 watermelon's total weight, while the flesh accounts for about 70% of total weight; conversely,  
78 Chakrabarty et al. (2020) and Zamuz et al. (2021) indicated that the rinds and seeds constitute  
79 approximately 40% of the total fruit weight and the flesh makes up approximately 60% of the  
80 fruit. However, the rind is not typically consumed. Although watermelon rind and skin are  
81 typically discarded as by-products, they have a similar or higher total phenolic and citrulline  
82 content than the flesh, indicating that they have excellent antioxidant properties (Tarazona-Díaz  
83 et al. 2011; Din et al. 2022).

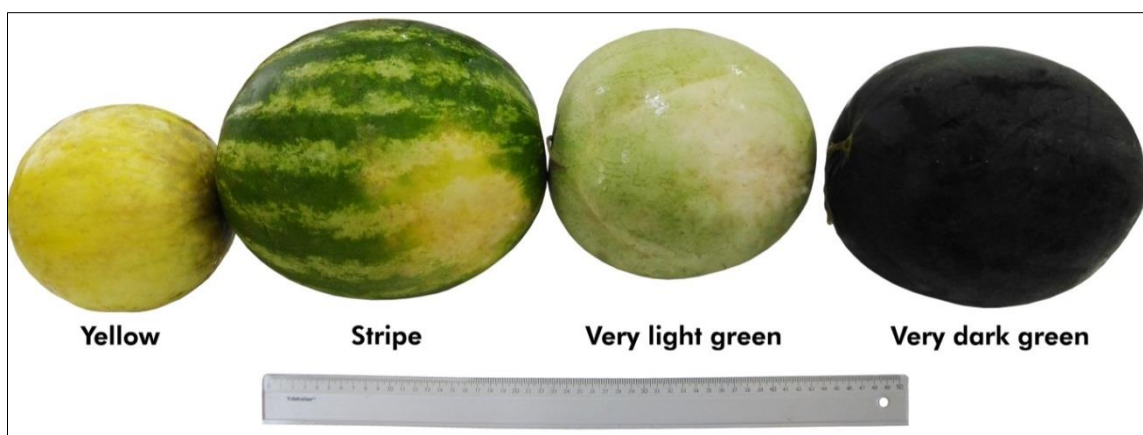
84 Thus, numerous studies have been conducted on citrulline's therapeutic properties in  
85 watermelon (Bahri et al. 2013; Rashid et al. 2020). However, little is known about the citrulline  
86 contents of various ground skin colors and fruit parts. Our study investigated the determination  
87 of citrulline in different parts of watermelon cultivars with different ground skin colors, both in  
88 the flesh of the fruit and the rind.

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## 90 **2. Materials and methods**

### 91 **2.1. Materials**

92 Watermelon pure lines with different ground colors (yellow, stripe, very light green, and very  
93 dark green) (Figure 1) in the Alata Horticultural Research Institute gene pool were used.



94 **Figure 1.** Watermelon pure lines of different ground colors used in the experiment.

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 96 These pure lines, which can be commercially parented to hybrids, are diploid and self at least  
 97 six times (Table 1). Apart from skin-ground characteristics, they do not have many different  
 98 characteristics from the other pure lines in the gene pool.

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**Table 1.** Lines selected for trial and their features.

	<b>Fruit weight (g)</b>	<b>Fruit length (cm)</b>	<b>Fruit diameter (cm)</b>	<b>Skin thickness (cm)</b>	<b>Brix (%)</b>
Very light green	6,890	21.50	20.50	0.90	9.6
Stripe	9,210	26.80	22.70	1.40	10.6
Very dark green	8,350	26.00	21.20	1.10	9.9
Yellow	4,310	18.50	17.10	0.70	8.0

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## 104 **2.2. Method**

105 The study was carried out at the Alata Horticultural Research Institute, part of the Ministry of  
 106 Agriculture and Forestry, in an open field at 36°38'08.3' N and 34°21'00.5" E (Erdemli,  
 107 Mersin, Türkiye). Seed sowing started on March 4, 2017, land preparation began on April 6,  
 108 2017, and planting in the field was carried out on April 11, 2017. The climate values of the field  
 109 when the research was conducted are given in Table 2.

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**Table 2.** Climatic data of the trial area.

Climate parameters	March	April	May	June	July
Max. temperature (°C)	23.5	28.2	30.0	34.2	40.5
Min. temperature (°C)	2.6	6.1	2.0	14.7	19.3
Average temperature (°C)	13.4	16.8	20.0	24.6	28.8
Max. humidity (%)	90.5	88.8	84.0	81.3	80.4
Min. humidity (%)	39.6	41.4	59.0	61.5	43.5
Average humidity (%)	69.9	67.2	75.8	75.0	71.4
Precipitation (mm = kg ÷ m <sup>2</sup> )	211.6	76.4	12.8	0.2	0.0

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113 The planting was carried out in an open area on banks with a width of 70 cm and a height of  
 114 40 cm, covered with black mulch, in a single row at 40-cm intervals, 9 cm from each pure line.  
 115 The soil pH value of the parcel where the study was carried out was 7.71, and it had a loamy  
 116 texture. Soil analysis results are given in Table 3.

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**Table 3.** Results of the parcel's soil analysis where the study was conducted.

<b>Analysis</b>	<b>Limit values</b>	<b>Analysis results (0–30 cm)</b>
Texture (100 g/mL)	30–50	48.00 (loamy)
Total calcitic (CaCO <sub>3</sub> %)	5–15	40 (high calcareous)
Salinity E.C. ds/m (25 °C)	0-0.8	0.32 (slightly salty)
Organic matter (%)	3–4	2.20 (deficient)
pH 1: 2.5	6.0–7.0	7.71 (slightly alkali)
Available potassium (mg/kg)	244–300	70.60 (very low)

Receivable phosphorus (mg/kg)	20–40	21.30 (optimum)
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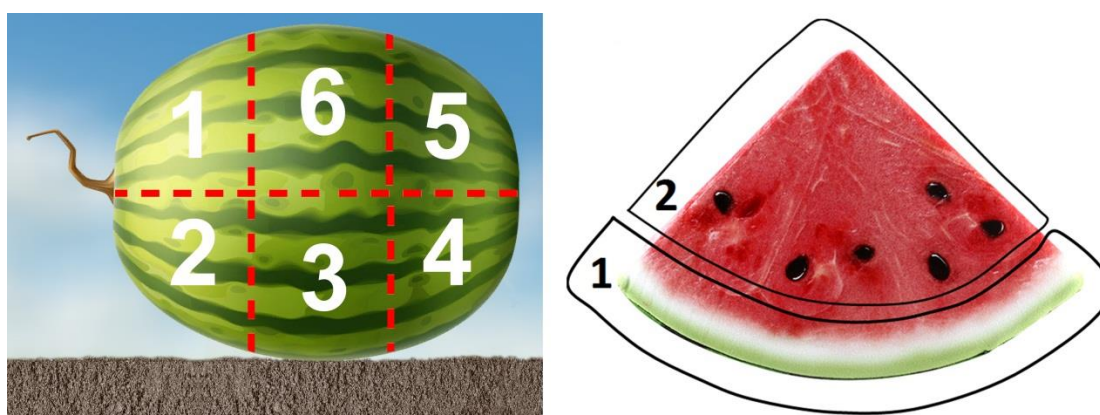
119 According to the findings of the soil study, pure fertilizers in the amounts of 140–160 kg N/ha,  
 120 80–100 kg P<sub>2</sub>O<sub>5</sub>/ha, and 60–80 kg K<sub>2</sub>O/ha were used (Güçdemir 2012). Drip irrigation was used  
 121 to apply the fertilizers. All phosphorus was given during soil preparation. Nitrogen and  
 122 potassium were divided into three parts according to the three growth stages of the watermelon.  
 123 The planting stage is the first stage; the period when the first female flower opens is the second,  
 124 and the third is when the fruits reach the size of an apple (Table 4).  
 125

126 **Table 4.** Application times and fertilizers amounts used throughout the trial.

Application time	Applied fertilizers and their amounts
During soil preparation	90 kg P <sub>2</sub> O <sub>5</sub> /ha
During planting (Stage 1)	50 kg N/ha and 35 kg K <sub>2</sub> O/ha
Stage when the female flower is seen (Stage 2)	50 kg N/ha and 35 kg K <sub>2</sub> O/ha
When the fruits reach the size of an apple (Stage 3)	50 kg N/ha and 35 kg K <sub>2</sub> O/ha

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 129 Regular pesticide application for diseases and pests was carried out, along with mechanical  
 130 weeding and trimming. Mechanical and manual methods were used for weed control. When the  
 131 tendrils and auricles of the fruits were dry, they were harvested on 4 July 2017 and brought to  
 132 cold storage. Citrulline analysis was then performed by taking the fruits (three replications per  
 133 pure line and three fruits per replication) from cold air storage at 4 °C and 90–95% relative  
 134 humidity.

135 The fruit samples of the pure lines with different ground colors (yellow, stripe, very light  
 136 green, and very dark green) were brought to the laboratory, and six different parts of each fruit  
 137 were taken for citrulline analysis. In addition, samples were taken from the part close to the rind  
 138 and flesh of the six parts. The order in which fruit samples were taken is given in Figure 2.



139 **Figure 2.** Locations of samples taken from different parts of the fruit (left); section 1 (rind) and  
 140 section 2 (flesh) samples were taken for citrulline analysis.

### 141 2.3. Citrulline analysis

143 Citrulline determination was done according to Jayaprakasha et al. (2011) and Tarazona-Díaz  
144 et al.'s (2011) methods, which were modified by Aras et al. (2021). In brief, 5 g of watermelon  
145 sample was kept in 4 mL of 0.2 M acetic acid for one night and centrifuged at  $5000 \times g$  for 1  
146 minute. The sample was filtered and vialled with a 0.45- $\mu$  filter and analyzed at 207 nm in a  
147 0.3mM o-phosphoric acid mobile phase with a flow rate of 0.7 mL min<sup>-1</sup> in HPLC (Shimadzu  
148 LC-20AD, Japan).

#### 149 **2.4. Statistical analysis**

150 Three replications of the experiment were set up using a randomized plot design in the field.  
151 In each replication, three fruits were used. JMP statistical software (JMP, Version 7, SAS  
152 Institute Inc., Cary, NC, 1989–2007, NC 27513-2414, USA) was used to analyze the data  
153 statistically. Significant differences among groups were determined using the Student's t-test  
154 for pairwise comparison and the Tukey test for multiple comparisons ( $p \leq 0.05$ ). The numbers  
155 used in the table are values without logarithmic transformation. As a result of statistical  
156 analysis, the coefficient of variation (CV) was 25.98. Due to the high CV, logarithmic  
157 transformation was applied to the numbers, and statistical analysis was performed again. These  
158 letterings were also used in the groupings obtained.

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### 160 **3. Result and discussion**

161 The highest citrulline value in watermelon lines was obtained from those with very light green  
162 ground skin colors (3.37 g/kg), while the lowest value was obtained from very dark green  
163 ground skin colors (2.0 g/kg). For the fruit parts, the highest value was taken from the 3rd part  
164 (3.72 g/kg), while the lowest value came from the 2nd (2.00 g/kg). When the fruit flesh and  
165 rind were compared, higher citrulline values were found in the flesh (3.10) than in the rind (2.40  
166 g/kg) (Table 3). Akashi et al. (2016) and Dubey (2021) detected higher citrulline levels in the  
167 skin than in the flesh. Our findings show a higher concentration of citrulline in the fruit flesh.  
168 L-citrulline levels in three distinct kinds of watermelon juice and rinds were measured by  
169 Jayaprakasha et al. (2011). Compared to watermelon juice, which only contained 11.25–16.73  
170 mg/g dry weight of L-citrulline, rinds had 13.95–28.46 mg/g dry weight. According to previous  
171 studies, each liter of unpasteurized watermelon juice contains 2.33 g of citrulline (Tarazona-  
172 Díaz et al. 2013; Bailey et al. 2016). Ridwan (2018) examined the L-citrulline content of  
173 watermelons (flesh and rind) grown and consumed in Malaysia and found that it was higher in  
174 the rind of red watermelon juice extract (45.02 mg/g) than in the flesh (43.81 mg/g). Similar  
175 trends were also observed in yellow crimson watermelon juice extract (16.61 mg/g in the rind  
176 and 15.77 mg/g in the flesh) of the same fruit. Casacchia et al. (2020) investigated bioactive

177 compounds obtained from watermelon pulp and rind using nine distinct watermelon cultivars  
 178 of various origins. The concentration of L-citrulline in fresh rind was substantially higher than  
 179 that of fresh pulp, except for watermelons from Santana, Romania and Latina, Italy, which  
 180 contained 2.6 mg/g of L-citrulline in their fresh rind. In our research, we obtained findings  
 181 ranging from 0.80 to 4.95. The broad range of ground colors in our study can be attributed to  
 182 the distinct portions of different watermelons. L-citrulline concentration can vary depending on  
 183 several environmental (such as exposure to drought stress and high light intensity) and  
 184 physiological factors (e.g., cultivar, genotype, flesh color, and fruit anatomy) (Hartman et al.  
 185 2019). According to the data obtained in our study, this conclusion has also been reached; still,  
 186 there is no clear information about the relationship between the ground color of the shell and  
 187 watermelon content. For this reason, this lack of relevant data needs to be investigated with  
 188 more varieties.

189 Lines  $\times$  parts  $\times$  sections interaction was found to be statistically significant. The highest value  
 190 was obtained from the fruit flesh (4.95) of the 3rd part of the fruit at the striped line of the  
 191 ground colors of the skin, while the lowest value was taken from the fruit rind (0.80) of the 1st  
 192 part of the fruit at the striped line of the ground colors of the rind. In terms of the lines  $\times$  parts  
 193 interaction, the highest value was obtained from the 3rd part of the fruit flesh (4.74) of the very  
 194 light green of the ground colors of the skin, while the lowest values were taken from the 5th  
 195 part (1.51) and 2nd part (1.58) of the fruit of the very dark green line of the ground colors of  
 196 the skin. Based on the lines  $\times$  sections interaction, the highest value was obtained from the rind  
 197 (3.42) of the very light green of the ground colors of the skin, while the lowest value was taken  
 198 from the rind (1.45) of the fruit of the black line ground colors of the skin (Table 5).

199  
 200 **Table 5.** Amount of citrulline (g/kg) in the rind and flesh of six different parts of watermelons  
 201 with different ground skin colors.

Parts of the fruit	Section	Ground skin colors				Average (section)	Average (part of fruit)
		Yellow	Very dark green	Very light green	Stripe		
1	1	2.25 e-i	1.20 g-i	4.40 a-e	<b>0.80 i</b>	Rind 2.40 B	2.76 B
	2	2.51 c-i	3.64 a-g	4.40 a-e	2.90 a-i		
2	1	1.83 e-i	1.46 f-i	2.04 e-i	1.64 f-i	Rind 2.40 B	2.00 C
	2	2.20 e-i	1.69 f-i	2.04 e-i	3.07 a-i		
3	1	2.93 a-i	1.47 f-i	4.69 a-d	4.33 a-e	Fruit <b>3.10 A</b>	<b>3.72 A</b>
	2	4.85 ab	1.89 e-i	4.79 a-c	<b>4.95 a</b>		
4	1	3.14 a-h	1.67 f-i	4.08 a-e	2.60 b-i	Fruit <b>3.10 A</b>	2.91 B
	2	3.14 a-h	2.73 a-i	2.90 a-i	3.05 a-i		
5	1	4.11 a-h	1.54 f-i	2.38 d-i	2.13 e-i	Fruit <b>3.10 A</b>	2.58 BC
	2	3.86 a-f	1.47 f-i	3.06 a-i	2.07 e-i		
6	1	0.99 hi	1.36 f-i	2.93 a-i	1.70 f-i	Fruit <b>3.10 A</b>	2.51 BC
	2	3.03 a-i	3.90 a-i	2.78 a-i	3.41 a-g		
Average (ground colors of the skin)		2.89 B	2.00 C	<b>3.37 A</b>	2.72 B		

<b>Line × Part (interaction)</b>						
1	2.38 d-h	2.42 d-h	4.40 abc	1.85 gh		
2	2.01 fgh	1.58 h	2.04 fgh	2.36 d-h		
3	3.84 a-d	1.68 gh	<b>4.74 a</b>	4.64 ab		
4	3.14 b-g	2.20 e-h	3.49 a-f	2.82 c-h		
5	3.98 a-e	1.51 h	2.72 d-h	2.10 e-h		
6	2.01 fgh	2.63 c-h	2.86 c-h	2.55 d-h		
<b>Line × Section (interaction)</b>						
1	2.52 cd	1.45 e	<b>3.42 a</b>	2.20 d		
2	3.26 abc	2.55 bcd	3.33 ab	3.24 abc		
Lines	Parts	Sections	Lines × Parts	Lines × Sections	Parts × Sections	Lines × Parts × Sections
Prob > f	<.0001	<.0001	<.0001	0.0024	0.0042	0.0272
CV (%5):	0.05					

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203 The mean values given in different capital letters in the same column and row and the mean  
 204 values of the interaction in the middle of the table with lower case letters were statistically  
 205 significant; those without lettering were found to be insignificant ( $p < 0.05$ ).

206 According to Koga and Ohtake (1914) and Wada (1930), citrulline was the first derivate in  
 207 watermelon juice (Nguyen et al. 2018). It was later discovered by Inatomi et al. (1969) in seeds  
 208 and other watermelon fruit parts. The authors could not determine whether citrulline was  
 209 produced in the fruit or carried there from other plant parts. Citrulline has been found in  
 210 watermelon flesh and rind, although reports on the substance were based on colorimetric tests;  
 211 thus, the results may have been overstated (Rimando and Perkins-Veazie 2005). The citrulline  
 212 extraction method (filtered or sonication) and the chemicals used for extraction (MeOH or HCl)  
 213 affect the citrulline yield. Our methods are similar to the acid filtration and extraction used by  
 214 Rimando and Perkins-Veazie (2005).

215 Some studies indicate that watermelon juice is a good source of citrulline (Mandel et al. 2005;  
 216 Collins et al. 2007). Rimando and Perkins-Veazie (2005) sampled six diploid and eight triploid  
 217 varieties of the 14 different watermelon varieties' mesocarp (flesh) and reported that diploid  
 218 (seeded) or triploid (seedless) watermelons exhibited a somewhat lower average citrulline  
 219 content. The citrulline concentration in the seeded and seedless varieties was comparable (16.6  
 220 and 20.3 mg/g dwt, respectively) and ranged from 3.9 to 28.5 mg/g dry weight (dwt). On  
 221 average, triploid watermelons had slightly more citrulline than diploid watermelons. Due to the  
 222 higher dry weight of the seedless variety, this difference was more significant than the fresh  
 223 weight basis. According to a recent study, rind contains a low phenolic concentration and high  
 224 citrulline content (3.34 and 2.33 g/kg, respectively) (Kumar et al. 2021). In another study,  
 225 although watermelon skin had the largest total phenolic content and a greater antioxidant  
 226 potential and radical scavenging activity than watermelon flesh, the latter had the highest  
 227 citrulline amount (Gu et al. 2023).



228 In another study, Aras et al. (2021) developed 55 watermelon hybrids (striped and dark green)  
229 to observe their bioactive properties (total carotenoid, lycopene, carotene, ascorbic acid, total  
230 phenol, antioxidant activity, citrulline, pectin methylesterase, chitinase, fructose, glucose,  
231 sucrose, and total soluble solid) in open field conditions in 2017 and 2018. In 2017, the 187 ×  
232 80 (striped) hybrid had the highest citrulline value (6.07 g/kg), while the 138-Y × 91 (striped)  
233 hybrid had the lowest (0.31 g/kg). In 2018, the 138-Y × 91 (striped) hybrid had the highest  
234 citrulline value (9.68 g/kg), while the 138-Y × 80 (striped) hybrid had the lowest (0.51 g/kg).  
235 As can be seen from this study, different hybrids in terms of citrulline have come to the fore  
236 over the years, and these results showed that citrulline is affected by environmental conditions.

237 Citrulline synthesis and function in plants are complicated. Numerous investigations on the  
238 physiological and environmental factors in watermelon indicate possible effects on the  
239 abundance of citrulline (Hartman et al. 2019). In response to drought and high light-intensity  
240 stress, citrulline accumulates dramatically in the leaves of *Cucumis melo* and several other  
241 *Citrullus* species (Akashi et al. 2001; Kawasaki et al. 2000). This implies that citrulline  
242 functions in osmotic control, scavenging of reactive oxygen species, and possible application  
243 as a biomarker in selecting resilient crop plants. These occurrences are supported by the fact  
244 that under abiotic and biotic stress, some genes in watermelons are activated (such as glutamine  
245 acyl transferases) while others are downregulated (Guo et al. 2013). When stress, such as a high  
246 CO level, was diminished in cucumbers under drought stress, citrulline and proline metabolism  
247 were downregulated (Hartman et al. 2019).

#### 248 249 **4. Conclusion**

250 This study investigated the proportion of citrulline in different parts of watermelons with  
251 different ground skin colors in the flesh of the fruit and the rind. The highest value of citrulline  
252 was obtained for the genotype with a very light green ground skin color (3.37 g/kg), while the  
253 lowest value was obtained from the genotype with a very dark green ground skin color (2.0  
254 g/kg). Higher citrulline values were found in the fruit flesh (3.10 g/kg) than in the rind (2.40  
255 g/kg). In many previous studies, the citrulline content in the peel part of the watermelon was  
256 shown to be higher than in the pulp; however, in our study, the citrulline content in the fruit  
257 flesh was higher. Since consumers typically consume the fruit flesh, they can easily get  
258 citrulline into their bodies. Different growing conditions can affect the bioactive properties of  
259 the fruit. For this reason, studies should be repeated and tested under different growing  
260 conditions.

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