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Citrulline Content of Fruit Flesh and Rind Parts in Different Watermelon Lines

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ABSTRACT

Citrulline is a natural antioxidant and an amino acid found in watermelon, which is 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 highest value of citrulline was obtained for the genotype with a very light green ground skin color (3.37 g kg^{-1}), while the lowest value was obtained from the genotype with a very dark green ground skin color (2.0 g kg^{-1}). Regarding the fruit parts, the highest value was in the third part (3.72 g kg^{-1}), while the lowest value belonged to the second part (2.00 g kg^{-1}). Higher citrulline values were found in the fruit flesh (3.10 g kg^{-1}) than in the fruit rind (2.40 g kg^{-1}).

Keywords: *Citrullus lanatus,* Citrulline, Watermelon ground skin color.

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

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

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al. (2007) indicated watermelon as a potent source of both arginine and citrulline.

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

Watermelon is the richest known source of citrulline, and this amino acid plays a vital role in drought tolerance (Yokota *et al*., 2002; Rimando *et al*., 2005). Citrulline functions as a hydroxyl radical scavenger and may shield plants from oxidative stress brought on by dryness (Akashi *et al*., 2001). However, neither the effects of production conditions nor the differences between cultivars have been sufficiently investigated (Davis *et al*., 2011). According to Fish and Bruton (2010), one cultivar produced in two locations showed no change in the amount of L-citrulline in the flesh. Tarazona-Díaz *et al*. (2011) observed a mean citrulline concentration of 2.33 mg g^{-1} in watermelon flesh based on five lines (four of which were triploid seedless cultivars) grown in a single location. The authors also demonstrated that the seeded cultivar had the lowest L-citrulline content in flesh tissue. An earlier study found that 14 watermelon cultivars ranged from 0.5 to 3.6 mg g^{-1} in terms of the fresh weight of citrulline, with an average concentration of 2.4 mg g^{-1} (Rimando and Perkins-Veazie, 2005). The authors claimed that red-fleshed fruit contained less L-citrulline than yellow or orange fruit. Still, since only a small sample size (three fruits for each variety) was used, it was difficult to determine the influence of genotype and environment on the *L*-citrulline concentration in those fruits. According to Liu *et al*. (2010), nine induced autotriploid hybrid watermelons produced in greenhouses showed greater *L*-citrulline levels than their diploid and induced autotetraploid parents. In Fish and Bruton (2010) and Liu *et al*. (2010) studies, L-citrulline levels peaked at peak maturity.

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

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

MATERIALS AND METHODS

Materials

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

These pure lines, which can be commercially parented to hybrids, are

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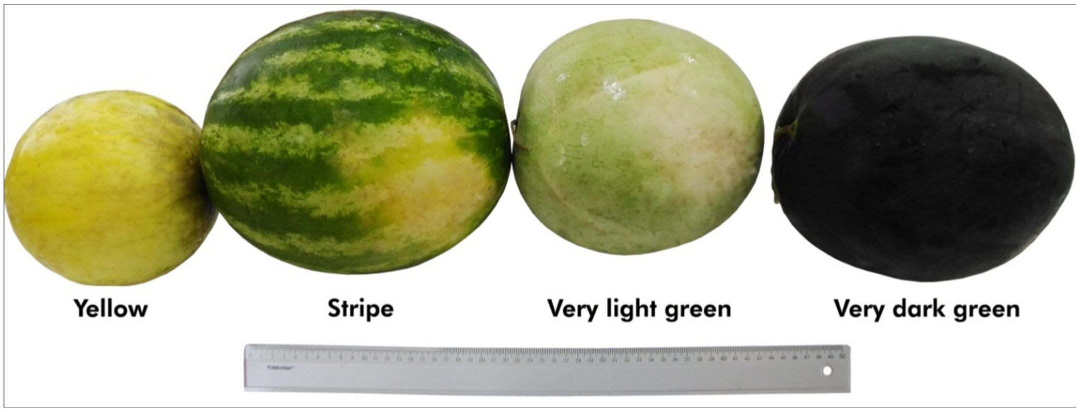

Figure 1. Watermelon pure lines of different ground colors used in the experiment.

diploid and self at least six times (Table 1). Apart from skin-ground characteristics, they do not have many different characteristics from the other pure lines in the gene pool.

Method

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

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

According to the soil analysis, pure fertilizers in the amounts of 140–160 kg N ha^{-1}, 80–100 kg P_2O_5 ha^{-1}, and 60–80 kg

Table 1. Lines selected for trial and their features.

Line	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Skin thickness (cm)	Brix (%)
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

Table 2. Climatic data of the trial area.

Climate parameters	March	April	May	June	July
Max. temperature (^oC)	23.5	28.2	30.0	34.2	40.5
Min. temperature (^oC)	2.6	6.1	2.0	14.7	19.3
Average temperature (^oC)	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÷m²)	211.6	76.4	12.8	0.2	0.0

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

Analysis	Limit values	Analysis results (0–30 cm)
Texture (100 g mL^{-1})	30–50	48.00 (Loamy)
Total calcitic ($CaCO_3$%)	5–15	40 (High calcareous)
Salinity EC dS m^{-1} (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^{-1})	244–300	70.60 (Very low)
Available phosphorus (mg kg^{-1})	20–40	21.30 (Optimum)

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

Application time	Applied fertilizers and their amounts
During soil preparation	90 kg P_2O_5 ha^{-1}
During planting (Stage 1)	50 kg N ha^{-1} and 35 kg K_2O ha^{-1}
Stage when the female flower is seen (Stage 2)	50 kg N ha^{-1} and 35 kg K_2O ha^{-1}
When the fruits reach the size of an apple (Stage 3)	50 kg N ha^{-1} and 35 kg K_2O ha^{-1}

K_2O ha^{-1} were used (Güçdemir, 2012). Drip irrigation was used to apply the fertilizers. All phosphorus was given during soil preparation. Nitrogen and potassium were divided into three parts according to the three growth stages of the watermelon: planting stage; the period when the first female flowers opened, and when the fruits reached the size of an apple (Table 4).

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

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

Citrulline Analysis

Citrulline determination was done according to Jayaprakasha *et al*. (2011) and Tarazona-Díaz *et al*. (2011) methods, which were modified by Aras *et al*. (2021). In brief, 5 g of watermelon sample was kept in 4 mL of 0.2M acetic acid for one night and centrifuged at 5,000×*g* for 1 minute. The sample was filtered and vialed with a 0.45-µ filter and analyzed at 207 nm in a 0.3 mM o-phosphoric acid mobile phase with a flow rate of 0.7 mL min^{-1} in HPLC (Shimadzu LC-20AD, Japan).

Statistical Analysis

Three replications of the experiment were set up using a randomized plot design in the field. In each replication, three fruits were used. JMP statistical software (JMP, Version 7, SAS Institute Inc., Cary, NC, 1989–2007, NC 27513-2414, USA) was used to analyze the data statistically. Significant differences among groups were determined using the Student's t-test for pairwise comparison and the Tukey test for multiple comparisons ($P \leq 0.05$). The numbers used in the table are values without logarithmic transformation.

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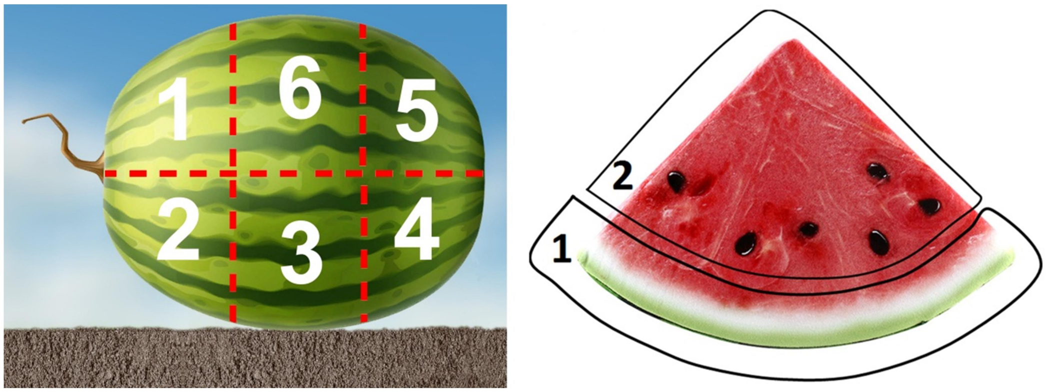

Figure 2. Locations of samples taken from different parts of the fruit (left); section 1 (rind) and section 2 (flesh) samples were taken for citrulline analysis.

As a result of statistical analysis, the Coefficient of Variation (CV) was 25.98. Due to the high CV, logarithmic transformation was applied to the numbers, and statistical analysis was performed again. These letterings were also used in the groupings obtained.

RESULTS AND DISCUSSION

The highest citrulline value in watermelon lines was obtained from those with very light green ground skin colors (3.37 g kg^{-1}), while the lowest value was obtained from very dark green ground skin colors (2.0 g kg^{-1}). For the fruit parts, the highest value was taken from the third part (3.72 g kg^{-1}), while the lowest value came from the second (2.00 g kg^{-1}). When the fruit flesh and rind were compared, higher citrulline values were found in the flesh (3.10) than in the rind (2.40 g kg^{-1}) (Table 3). Akashi *et al*. (2016) and Dubey (2021) detected higher citrulline levels in the skin than in the flesh. Our findings show a higher concentration of citrulline in the fruit flesh. L-citrulline levels in three distinct kinds of watermelon juice and rinds were measured by Jayaprakasha *et al*. (2011). Compared to watermelon juice, which only contained 11.25–16.73 mg g^{-1} dry weight of L-citrulline, rinds had 13.95–28.46 mg g^{-1} dry weight. According to previous studies, each liter of unpasteurized watermelon juice contains 2.33 g of citrulline (Tarazona-Díaz *et al*., 2013; Bailey *et al*., 2016). Ridwan (2018) examined the *L*-citrulline content of watermelons (flesh and rind) grown and consumed in Malaysia and found that it was higher in the rind of red watermelon juice extract (45.02 mg/g) than in the flesh (43.81 mg g^{-1}). Similar trends were also observed in yellow crimson watermelon juice extract (16.61 mg/g in the rind and 15.77 mg g^{-1} in the flesh) of the same fruit. Casacchia *et al*. (2020) investigated bioactive compounds obtained from watermelon pulp and rind using nine distinct watermelon cultivars of various origins. The concentration of L-citrulline in fresh rind was substantially higher than that of fresh pulp, except for watermelons from Santana, Romania and Latina, Italy, which contained 2.6 mg g^{-1} of *L*-citrulline in their fresh rind. In our research, we obtained findings ranging from 0.80 to 4.95. The broad range of ground colors in our study can be attributed to the distinct portions of different watermelons. *L*-citrulline concentration can vary depending on several environmental (such as exposure to drought stress and high light intensity) and physiological factors (e.g., cultivar, genotype, flesh color, and fruit anatomy) (Hartman *et al*., 2019). According to the data obtained in our study, this conclusion has also been reached; still, there is no clear

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information about the relationship between the ground color of the shell and watermelon content. For this reason, this lack of relevant data needs to be investigated with more varieties.

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

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

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

Some studies indicate that watermelon juice is a good source of citrulline (Mandel *et al*., 2005; Collins *et al*., 2007). Rimando and Perkins-Veazie (2005) sampled six diploid and eight triploid varieties of the 14 different watermelon varieties' mesocarp (flesh) and reported that diploid (seeded) or triploid (seedless) watermelons exhibited a somewhat lower average citrulline content. The citrulline concentration in the seeded and seedless varieties was comparable (16.6 and 20.3 mg g^{-1} dwt, respectively) and ranged from 3.9 to 28.5 mg g^{-1} dry weight (dwt). On average, triploid watermelons had slightly more citrulline than diploid watermelons. Due to the higher dry weight of the seedless variety, this difference was more significant than the fresh weight basis. According to a recent study, rind contains a low phenolic concentration and high citrulline content (3.34 and 2.33 g kg^{-1}, respectively) (Kumar *et al*., 2021). In another study, although watermelon skin had the highest total phenolic content and a greater antioxidant potential and radical scavenging activity than watermelon flesh, the latter had the highest citrulline amount (Gu *et al*., 2023).

In another study, Aras *et al*. (2021) developed 55 watermelon hybrids (striped and dark green) to observe their bioactive properties (total carotenoid, lycopene, carotene, ascorbic acid, total phenol, antioxidant activity, citrulline, pectin methylesterase, chitinase, fructose, glucose, sucrose, and total soluble solid) in open field conditions, in 2017 and 2018. In 2017, the 187×80 (striped) hybrid had the highest citrulline value (6.07 g kg^{-1}), while the 138-Y×91 (striped) hybrid had the lowest (0.31 g kg^{-1}). In 2018, the 138-Y×91 (striped) hybrid had the highest citrulline value (9.68 g kg^{-1}), while the 138-Y×80 (striped) hybrid had the lowest (0.51 g kg^{-1}). As can be seen from this study, different hybrids in terms of citrulline have come to the fore over the years, and these results showed that

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Table 5. Amount of citrulline (g kg^{-1}) in the rind and flesh of six different parts of watermelons 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	0.80 i	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		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		3.72 A
	2	4.85 ab	1.89 e-i	4.79 a-c	4.95 a		
4	1	3.14 a-h	1.67 f-i	4.08 a-e	2.60 b-i	Fruit 3.10 A	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		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		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	3.37 A	2.72 B		
		Line×Part (Interaction)					
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	4.74 a	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		
		Line×Section (Interaction)					
	1	2.52 cd	1.45 e	3.42 a	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	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0024	0.0042	0.0272
CV (%5): 0.05							

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citrulline is affected by environmental conditions.

Citrulline synthesis and function in plants are complicated. Numerous investigations on the physiological and environmental factors in watermelon indicate possible effects on the abundance of citrulline (Hartman *et al*., 2019). In response to drought and high light-intensity stress, citrulline accumulates dramatically in the leaves of *Cucumis melo* and several other *Citrullus* species (Akashi *et al*., 2001; Kawasaki *et al*., 2000). This implies that citrulline functions in osmotic control, scavenging of reactive oxygen species, and possible application as a biomarker in selecting resilient crop plants. These occurrences are supported by the fact that, under abiotic and biotic stress, some genes in watermelons are activated (such as glutamine acyl transferases) while others are downregulated (Guo *et al*., 2013). When stress, such as a high CO_2 level, was diminished in cucumbers under drought stress, citrulline and proline metabolism were downregulated (Hartman *et al*., 2019).

CONCLUSIONS

This study investigated the proportion of citrulline in different parts of watermelons with different ground skin colors in the flesh and the rind of the fruit. The highest value of citrulline was obtained for the genotype with a very light green ground skin color (3.37 g kg^{-1}), while the lowest value was obtained from the genotype with a very dark green ground skin color (2.0 g kg^{-1}). Higher citrulline values were found in the fruit flesh (3.10 g kg^{-1}) than in the rind (2.40 g kg^{-1}). In many previous studies, the citrulline content in the peel part of the watermelon was higher than in the pulp; however, in our study, the citrulline content in the fruit flesh was higher. Since consumers typically consume the fruit flesh, they can easily get citrulline into their bodies. Different growing conditions can affect the bioactive properties of the fruit. For this reason, studies should be repeated and tested under different growing conditions.

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محتوای سیترولین قسمت های گوشت و پوست میوه در لاین‌های مختلف هندوانه

ویسل ارس

چکیده

سیترولین یک آنتی اکسیدان طبیعی و یک اسید آمینه موجود در هندوانه است که برای گوشت میوه آن مصرف می شود. در این پژوهش، محتوای سیترولین در قسمت‌های مختلف گوشت و پوست میوه چهار لاین هندوانه با رنگ‌های پوستی متفاوت تعیین شد. میوه ها به شش قسمت تقسیم شدند و از پوست و پالپ میوه برای سیترولین در هر قسمت نمونه برداری شد.بیشترین مقدار سیترولین برای ژنوتیپ با زمینه رنگ پوست سبز بسیار روشن (۳/۳۷ گرم بر کیلوگرم) و کمترین مقدار مربوط به ژنوتیپ با رنگ پوست سبز بسیار تیره (۲/۰ گرم بر کیلوگرم) به دست آمد. در بخش میوه بیشترین مقدار مربوط به قسمت سوم (۳/۷۲ گرم بر کیلوگرم) و کمترین مقدار مربوط به قسمت دوم (۲/۰۰ گرم بر کیلوگرم) بود. مقادیر بالاتر سیترولین در گوشت میوه (۳.۱۰ گرم بر کیلوگرم) نسبت به پوست میوه (۲.۴۰ گرم بر کیلوگرم) یافت شد.

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