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Optimizing Greenhouse Cucumber Fertigation Through Grafting: Improving Yield, Bioactive Compounds, and Antioxidant Activity

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Abstract: Consumers prefer cucumbers (*Cucumis sativus*) with high antioxidant content, which is often at odds with farmers' goals of maximizing yield. Therefore, this study aims to explore new methods for fertigation and grafting to optimize the yield and quality of cucumbers. In a greenhouse experiment, we tested fertigation with three different nutrient solutions: the standard as a control (CF) and two new formulations (NF1 and NF2). We also examined grafting in three variants: non-grafted (CG), grafting onto *Cucurbita moschata* × *Cucurbita moschata* (G1), and grafting onto *Lagenaria siceraria* (G2). Our results showed that the highest increase in phenolic content in the flesh of cucumber was observed in the NF2 × G1 treatment (↑ 22.4%). In contrast, grafting and the new fertigation methods generally reduced the phenolic content in the peel. Grafting with G1 significantly increased flavonoid content in the flesh (↑ 59.4% and ↑ 77.3%) but significantly decreased it in the peel. The NF2 × G1 treatment achieved the most significant increases in antioxidant activity indicators, DPPH (↑ 25.9%) and FRAP (↑ 39.4%). For farmers seeking to achieve high yields of greenhouse cucumbers, the combination of NF1 × G1 is recommended, as it resulted in the highest yield increase (↑ 45.3%). Consumers are advised to eat cucumbers with the peel, as this study found higher levels of antioxidant compounds in the peel compared to the flesh.

Keywords: vegetable quality; phenolics; rootstock; fruit; *Cucumis sativus*

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1. Introduction

The cucumber (*Cucumis sativus*) is among the most significant vegetable species due to its worldwide cultivation and diverse uses in human nutrition [1–4]. The cucumber fruit can be consumed fresh or processed. Swamy [5] recommends that the fruit should be eaten as a whole, including both the flesh and the peel, to benefit fully from its nutritional content. Ji et al. [6] state that the peel of cucumbers contains significantly more phenolics than the pulp. Additionally, young cucumber leaves can be consumed as salad, and seeds are used as amendments to rice dishes and soups [7].

Nutritionally, a cucumber fruit contains about 95.0% water, 3.6% carbohydrates, and 0.65% protein, which gives it a low caloric value [1]. Cucumber is also rich in vitamins C, E, and B group, including folic and pantothenic acid [1,4,8]. However, cucumbers can also contain potentially harmful compounds such as nitrates and nitrites [9,10].

In recent years, there has been a trend of declining cucumber yields per unit area in greenhouse production, often due to adverse effects of biotic and abiotic factors (e.g., degraded soil or nutrient deficiencies) during the vegetation period. To address this issue, grafting cucumbers onto tolerant rootstocks of other species is being increasingly explored.

Grafting is used to improve the uptake and utilization of water [11] and the uptake of mineral elements [12,13], especially in greenhouse production, in which crop rotation is often absent [14].

Grafting can reduce the need for fungicides and the frequency of irrigation due to a stronger root system and generally increased tolerance of grafted plants [15]. Helaly et al. [16] report that grafting enhanced nutrient uptake, which led to increased photosynthesis under low light and CO_2 conditions. In peppers (*Capsicum annuum*) and tomatoes (*Lycopersicon esculentum*), grafting increased the antioxidant activity of the fruits [17,18]. Additionally, grafted tomatoes showed increased dry-matter content [19,20] and salt stress tolerance [21]. However, grafting can also reduce bioactive compounds by increasing plant tolerance to abiotic stress conditions, leading to reduced synthesis of secondary metabolites such as phenolics and flavonoids [22,23].

In cucumber greenhouse production, inadequate mineral nutrition is a common issue. Standard fertilization protocols for nitrogen (N), phosphorus (P), and potassium (K) are not adopted to the assortment of cucumber cultivars, which has changed significantly over time, leading to different nutrient requirements. Therefore, there is a need to develop new nutrient solutions that can meet these evolving needs and help achieve high yields while enhancing the content of antioxidant compounds in cucumbers.

In cucumber production, under greenhouse conditions, manure or mineral NPK fertilizers are commonly applied in amounts of 8–10 kg/m^2 or 50–70 g/m^2 [24]. According to Arshad et al. [25], the best results in experiments with cucumber were achieved when mineral NPK fertilizers were applied through fertigation systems. Jilani et al. [26] noted that different doses of NPK fertilizers significantly affected cucumber yield, with the highest yield observed in the treatment with 100 kg N/ha, 50 kg K/ha, and 50 kg P/ha. In tomatoes, the application of 60 kg N/ha, 60 kg P/ha, and 60 kg K/ha increased the phenolics content in the fruits [27]. Fertilizing peppers with different rates of N did not significantly alter antioxidant activity as measured via the DPPH test [28]. This highlights the complexity of optimizing fertilization strategies to enhance the nutritional quality of fruit.

Given the importance of cucumbers in global diets, there is a pressing need to refine agricultural practices in order to improve yield and quality. Despite its significance, the existing research is often fragmented, with many studies focusing only on grafting [29–32] or fertilization [25,26,33–35] and rarely exploring the interaction effects of these two factors, mainly when cucumbers are grafted onto intraspecies rootstocks. Furthermore, there is a significant gap in understanding the impact of grafting and fertigation on the bioactive compounds in cucumber peel, which is critical since many people consume only the flesh, while the peel, rich in nutrients, is discarded.

Given these gaps in current knowledge, this study aims to investigate the effects of innovative fertigation formulations and grafting on the yield and antioxidant compounds of flesh and peel of cucumbers. By addressing these research gaps, we aim to provide information that can help farmers achieve higher yields and better-quality cucumbers, ultimately benefiting consumers worldwide.

2. Materials and Methods

The experiment was carried out under controlled conditions in a greenhouse (5.2 × 19.0 m) at the family farm Koprivica [36] in Čelarevo (45.2695° N, 19.5279° E), Serbia. The greenhouse experiment was set up using a split-plot method with a randomized block design in three replications.

The main plots consisted of fertigation with three different nutrient solutions: the standard as a control (CF) and two new formulations (NF1 and NF2) (Table 1).

The subplots involved three grafting protocols: 1. non-grafted cucumber (*Cucumis sativus*) as a control (CG); 2. grafting onto the rootstock Isabella F1 (Wing Seed®, Enkhuizen, The Netherlands) based on *Cucurbita moschata* × *Cucurbita moschata* (G1); 3. grafting onto the rootstock Emphasis F1 (Syngenta®, Basel, Switzerland) based on *Lagenaria siceraria* (G2).

For this research, the G1 rootstock was chosen because it is a new product on the Serbian market, as confirmed by companies that distribute vegetable seeds. The G2 rootstock was selected based on previous positive experiences reported by farmers who used it for grafting watermelon (*Citrullus vulgaris*), where they observed significant improvements in yield and fruit quality.

Table 1. Main plots with fertigation treatments.

Phase	Fertilizer Type	Weigh Fertilizer (g) per Plant/Week (Active Ingredients of Fertilizer (g) per Plant/Week)		
		CF	**NF1**	**NF2**
P_1	NPK 8:16:24 (K-Adriatica S.p.A.®, Loreo, Italy)	8 (0.64 N + 1.28 P + 1.92 K)	0	0
	AN 33.5% N (Petrokemija®, Kutina, Croatia)	5 (1.67 N)	0	0
P_2	NPK 8:16:24 (K-Adriatica S.p.A.®, Loreo, Italy)	4 (0.32 N + 0.64 K + 0.96 P)	0	0
	Ferticare™ 15:30:15 (Yara®, Oslo, Norway)	0	0.5 (0.075 N + 0.15 P + 0.075 K)	1 (0.15 N + 0.30 P + 0.15 K)
	$CaNO_3$ (Van Iperen®, Westmass, The Netherlands)	0	1 (0.155 Ca + 0.263 N)	2 (0.31 Ca + 0.52 N)
P_3	NPK 8:16:24 (K-Adriatica S.p.A.®, Loreo, Italy)	4 (0.32 N + 0.64 K + 0.96 P)	0	0
	NPK 12:11:18 (Yara®, Oslo, Norway)	0	0.5 (0.06 N + 0.055 P + 0.09 K)	1 (0.12 N + 0.11 P + 0.18 K)
	KNO_3 (Yara®, Oslo, Norway)	0	0.5 (0.23 K + 0.065 N)	1 (0.46 K + 0.13 N)
	$CaNO_3$ (Van Iperen®, Westmass, The Netherlands)	0	1 (0.15 Ca + 0.26 N)	2 (0.31 Ca + 0.52 N)
P_4	NPK 8:16:24 (K-Adriatica S.p.A.®, Loreo, Italy)	4 (0.32 N + 0.64 P + 0.96 K)	0	0
	NPK 12:11:18 (Yara®, Oslo, Norway)	0	1.0 (0.12 N + 0.11 P + 0.18 K)	2 (0.24 N + 0.22 P + 0.36 K)
	$CaNO_3$ (Van Iperen®, Westmass, The Netherlands)	0	1.0 (0.15 Ca + 0.26 N)	2 (0.31 Ca + 0.52 N)
P_5	NPK 8:16:24 (K-Adriatica S.p.A.®, Loreo, Italy)	5 (0.4 N + 0.8 P + 1.2 K)	0	0
	Ferticare™ 28:8:16 II (Yara®, Oslo, Norway)	0	1 (0.28 N + 0.08 P + 0.16 K)	2 (0.56 N + 0.16 P + 0.32 K)
	NPK 12:11:18 (Yara®, Oslo, Norway)	0	0.5 (0.06 N + 0.055 P + 0.09 K)	1 (0.12 N + 0.11 P + 0.18 K)
	KNO_3 (Yara®, Oslo, Norway)	0	1 (0.46 K + 0.13 N)	2 (0.92 K + 0.26 N)
	$CaNO_3$ (Van Iperen®, Westmass, The Netherlands)	0	1 (0.15 Ca + 0.26 N)	2 (0.31 Ca + 0.52 N)

CF—standard formulations as a control; NF1—new formulations 1; NF2—new formulations 2; P_1—before transplanting; P_2—after transplanting; P_3—intense growth and flowering; P_4—until first harvest; P_5—during vegetation (after first harvest).

The new fertigation regimes were tailored to match the growth stages of cucumbers in order to optimize nutrient uptake efficiency. Additionally, the fertilizers used in this study

are readily available on the market, ensuring that the findings can be directly applied by farmers to improve their cucumber production.

2.1. Seedling Production

The sowing of the rootstock seeds G1 and G2 was conducted in wooden trays (0.3 × 0.6 m) pre-filled with a mix of black and white peat (0–6 mm and pH 5.5–6.5). G1 was sown four days later, and G2 was sown three days earlier than the cucumber due to the faster growth rate of the scion (cucumber).

The sowing of cucumber seeds (scion) of the variety Centauro F1 (Semillas Fitó®, Barcelona, Spain) was carried out in wooden trays (0.3 × 0.6 m) pre-filled with the substrate. After sowing, treatment with the fungicide Previcur Energy® (Bayer, Leverkusen, Germany) based on propamocarb (530 g/L) + fosetyl-aluminum (310 g/L) was performed at a concentration of 0.20% to prevent damping-off disease (*Pythium* sp.). Afterward, the trays were transferred to a germination chamber set at 25 °C. Germination was recorded after three days, and the trays with seedlings were transferred to the greenhouse, where the temperature was set to 20–22 °C.

Grafting Procedure

Grafting was performed in a shaded (dark) room using the hole insertion grafting technique, which is a standard method in farmers' practice. After grafting, a constant temperature range of 27–30 °C and air humidity of 95% were maintained, which are optimal conditions for proper callus formation. Each subsequent day, the shading was gradually reduced by 10%, and after seven days, the presence of callus at the graft union was confirmed. From the moment of grafting, the seedlings were ready for planting after thirty days.

2.2. Conditions and Agrotechnical Practices in Greenhouse Experiment

The soil substrate in which the cucumbers were grown was medium-humic with 3.3% humus, a pH of 7.2, a $CaCO_3$ content of 4.0%, an N level of 0.16%, K content of 17.2 mg/100 g soil, and electrical conductivity (EC) of 1.73 mS/cm. During the experiment, the greenhouse temperature ranged from 25 to 28 °C.

A week before the start of the experiment, surface cultivation of the soil to a depth of 15 cm was performed using the cultivator Prime VTB 842.057145 by Villager® (Ljubljana, Slovenia). Five days later, drip irrigation tapes by Scarabelli Irrigazione® (Bologna, Italy) were installed for fertigation. The diameter of the drip tapes was 16 mm, the spacing between drippers was 10 cm, the wall thickness was 0.6 mm, and the capacity was 10 L/h/m. Afterwards, UV-stabilized black plastic film (20 µm thickness) by Daios Plastic® (Veria, Greece) was laid over the entire area. Seedling planting was carried out with row spacing of 1.5 m and plant spacing of 0.4 m within the row.

In accordance with the growth and development stages of the cucumber (Table 1), fertigation was carried out once a week using a Venturi tube by Palaplast S.A. (Thessaloniki, Greece) connected to an electric pump with a power of 3 kW and an outlet pipe diameter of 2 inches. A 24 m deep well (45.1554° N, 19.3131° E) was used as the water source.

Cucumber plants were trained using a single-stem method, with two adjacent plants alternately trained in a "V" pattern. During the growing season, regular measures were taken to protect plants against diseases and insects (Table 2).

Table 2. Pesticides used during the experiment.

Pesticides	Commercial Name	Active Ingredient	Application Rate	Target Diseases/Pests
Fungicides	Quadris® (Syngenta, Basel, Switzerland)	Azoxystrobin 250 g/L	7.5 mL/10 L water	*Pseudoperonospora cubensis*; *Erysiphae cichoracearum*
	Switch® 62.5 WG (Syngenta, Basel, Switzerland)	Cyprodinil 375 g/kg + fludioxonil 250 g/kg	10 g/10 L water	*Botrytis cinerea*
Insecticides	Actara® 25 WG (Syngenta, Basel, Switzerland)	Thiamethoxam 250 g/kg	3.5 g/10 L water	*Thripidae*; *Aphididae*
	Teppeki® (Belchim, Londerzeel, Belgium)	Flonicamid 500 g/kg	1.5 g/10 L water	*Trialeurodes vaporariorum*
	Apollo® 50 SC (Adama, Beijing China)	Clofentezine 500 g/L	3 mL/10 L water	*Tetranychus urticae*

2.3. Measurement Procedures

2.3.1. Yield Measurement

The yield of cucumbers was determined by harvesting and weighing all fruits during the growing season, and it was calculated according to the following formula:

$$\text{Yield } (\text{t/ha}) = \frac{\text{Yield per net plot } (\text{kg}) \times 10}{\text{Net plot size } (\text{m}^2)} \quad (1)$$

2.3.2. Sample Size for Quality Assessment

The quality of the cucumber parameters was measured using an average sample of fifteen fruits, randomly selected from five plants in each replicate.

2.3.3. Dry Matter

The fruit samples were peeled, and the peel and flesh were separated to create average samples for each treatment in order to determine the dry-matter content (DM). DM was determined by drying cucumber samples at 105 °C until a constant weight [37] was achieved using an SLW 240 ECO drying oven (Pol-Eko®, Wodzislaw Slaski, Poland). The dry-matter analysis was performed in triplicate and calculated using the following formula:

$$\text{Dry matter } (\%) = \frac{\text{Weight of dry sample } (\text{g})}{\text{Weight of fresh sample } (\text{g})} \times 100 \quad (2)$$

2.3.4. Total Phenolics Content

Total phenolics were determined according to the Singleton and Rossi [38] spectrophotometric Folin–Ciocalteu method. The total phenolics were determined using a gallic acid (GAE) calibration curve and expressed as milligrams of gallic acid equivalents per 100 g of dry matter (mg GAE/100 g DM). The analysis of phenolics was conducted in triplicate.

2.3.5. Total Flavonoid Content

Total flavonoids were determined according to the Harborne [39] spectrophotometric method using aluminum chloride. The results were expressed as mg of catechin (CE) equivalents per 100 g of DM (mg CE/100 g DM) using the equation obtained from the catechin standard diagram. The analysis of flavonoids was conducted in triplicate.

2.3.6. Antioxidant Activity Tests (DPPH, FRAP, and ABTS)

Spectrophotometric measurements were performed using a spectrophotometer UV/VIS LLG-uniSPEC 2 (Lab Logistics Group GmbH, Meckenheim, Germany). The DPPH method (the modified Brand-Williams method [40]) is based on the ability of samples to neutralize DPPH radicals. A FRAP (ferric ion reducing antioxidant power) test was performed using

the modified Benzie and Strain [41] method. The method is based on the measurement of the sample's ability to reduce Fe^{3+} ions. The ABTS test was performed according to Re et al. [42]. A detailed description of the conducted methods is presented in the paper by Vakula et al. [43]. The analysis of antioxidant indicators was conducted in triplicate.

2.4. Statistical Data Analysis

To examine the effects of fertigation and grafting on cucumber yield and quality, an analysis of variance (ANOVA) was used. The significance of differences between means was assessed using the LSD test at a significance level of $p < 0.05$. Prior to performing the ANOVA, the normality of distribution was examined using the Kruskal–Wallis test.

The relationship between phenolics, flavonoid content, and antioxidant activity was assessed through regression analysis. Statistical analysis was conducted using the Statistica® 14 software from TIBCO® Software Inc. (Palo Alto, CA, USA). To better understand the impact of fertigation and grafting on cucumbers, the relative change (%) of treatments compared to the control was calculated using the following formula [44]:

$$Relative\ change(\%) = \frac{\text{Treatment}}{\text{Control}} * 100 - 100 \quad (3)$$

3. Results

3.1. Yield of Cucumber

Grafting significantly influenced the cucumber yield (Figure 1). The highest fruit yield was observed in G1 (32.39 t/ha), followed by a slightly lower yield in G2 (30.28 t/ha). Both treatments showed a significant increase compared to CG, with G1 exhibiting a 16.8% higher yield and G2 a 9.2% higher yield. On average, for fertilization, the highest yield was achieved in NF1 (32.36 t/ha), while the lowest was recorded in CG (28.66 t/ha). The difference of 12.9% between them is statistically significant (Figure 1).

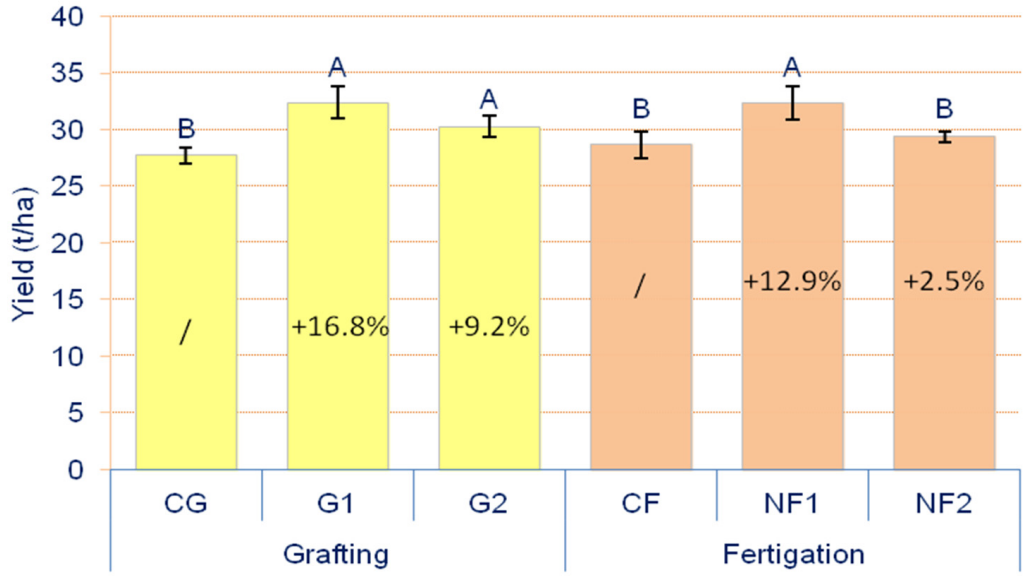


Figure 1. The effect of grafting and fertigation on cucumber yield (t/ha). Different letters denote statistical significance based on the LSD test at $p < 0.05$. The lines on the bars represent the standard error of the mean. The numbers in the bars represent the relative change (%) compared to CG or CF. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

On CF plots, the most significant yield increase occurred with the CF × G1 combination, reaching 30.18 t/ha, which was 17.4% higher compared to CF × CG (25.71 t/ha), and

this difference is statistically significant (Figure 2). For NF1, the highest yield was observed in the NF1 × G1 treatment (37.35 t/ha), while the lowest yield was noted in NF1 × CG (28.03 t/ha), with a statistically significant difference. On the NF2 plot, the highest yield was obtained with NF2 × G1 (29.65 t/ha) and the lowest with NF2 × G2 (29.06 t/ha). Both grafting treatments for NF2 showed no significant difference compared to NF2 × CG (29.42 t/ha).

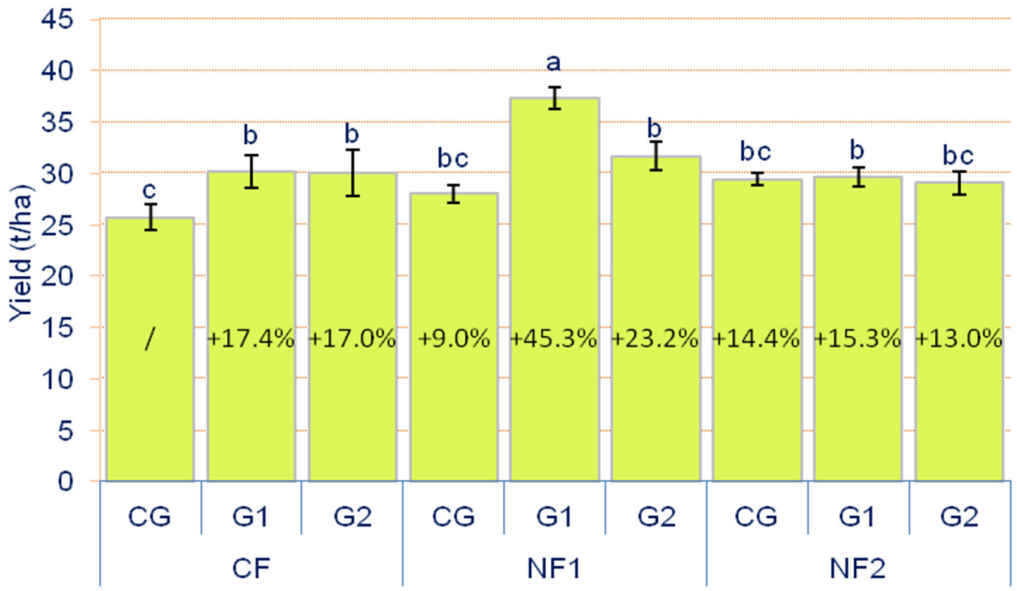


Figure 2. The interaction effect of grafting and fertigation. The different letters denote statistical significance based on the LSD test at $p < 0.05$. The lines on the bars represent the standard error of the mean. The numbers in the bars represent the relative change (%) compared to CG or CF. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

3.2. Dry Matter (DM)

Grafting and fertilization significantly influenced the DM content in the flesh and peel of cucumber fruit (Table 3). On the CF plot, the highest DM content in the flesh was found in CF × CG (4.29%), while the lowest was measured in CF × G2 (3.85%), and their difference was statistically significant. For the NF1 treatment, the highest DM content was achieved in the NF1 × G1 (3.80%) and the lowest in CG × NF1 (3.57%), but their difference was not statistically significant. For NF2, the highest DM in the flesh was observed in NF2 × CG (3.77%), while the lowest was measured in NF2 × G1 (3.38%), and their difference of 0.39% was statistically significant.

Under the CF plots, the highest DM content in the peel was observed in the CF × G1 treatment (6.60%), while the lowest was in CF × CG (5.53%), and their difference was significant. Examining the NF1 revealed that the highest DM content in the peel was found in the NF1 × G1 treatment (6.83%), while the lowest was noticed in NF1 × CG (5.59%), and their difference was significant. On the NF2 plot, the highest DM content was measured in the NF2 × G2 treatment (6.14%), while the lowest was in NF2 × G1 (5.32%), with their difference being statistically significant.

Table 3. Effect of fertigation and grafting on DM content in cucumber flesh and peel (%) *.

Fertigation	Grafting	Flesh	Relative Change (%)	Peel	Relative Change (%)
CF	CG	4.29 ± 0.15 a	/	5.53 ± 0.09 cd	/
	G1	4.00 ± 0.06 ab	−6.75	6.60 ± 0.15 a	+19.34
	G2	3.85 ± 0.02 bc	−10.25	5.64 ± 0.21 cd	+1.98
NF1	CG	3.57 ± 0.09 c–e	−16.78	5.59 ± 0.09 cd	+1.08
	G1	3.80 ± 0.16 b–d	−11.42	6.83 ± 0.12 a	+23.50
	G2	3.83 ± 0.01 bc	−10.72	5.86 ± 0.10 bc	+5.96
NF2	CG	3.77 ± 0.07 b–d	−12.12	5.76 ± 0.00 bc	+4.15
	G1	3.38 ± 0.16 e	−21.21	5.32 ± 0.05 d	−3.79
	G2	3.52 ± 0.09 de	−17.94	6.14 ± 0.10 b	+11.03

* The different letters denote statistical significance based on the LSD test at $p < 0.05$. The number following the ± sign represents the standard error of the mean. The relative change (%) represents the change compared to CF × CG. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

3.3. Phenolics Content

On the CF plot, the highest phenolic content in the flesh was observed in CF × G2 (186.24 mg GAE/100 g DM), while the lowest was in CF × CG (171.08 mg GAE/100 g DM), and their difference of 8.86% was statistically significant (Table 4). For fertilization with NF1, the highest phenolics content in the flesh was achieved in NF1 × CG (197.86 mg GAE/100 g DM), while the lowest was in NF1 × G2 (172.65 mg GAE/100 g DM), and their difference was significant. On the NF2 plot, the significantly highest phenolics content was recorded in the NF2 × G1 treatment (209.49 mg GAE/100 g DM), while the lowest was in the NF2 × G2 treatment (176.53 mg GAE/100 g DM), and their difference of 32.96 mg GAE/100 g DM was significant.

Table 4. Effect of fertigation and grafting on phenolics content in cucumber flesh and peel (mg GAE/100 g DM) *.

Fertigation	Grafting	Flesh	Relative Change (%)	Peel	Relative Change (%)
CF	CG	171.08 ± 1.93 e	/	269.65 ± 0.96 a	/
	G1	183.48 ± 1.17 cd	+7.24	222.91 ± 1.39 e	−17.33
	G2	186.24 ± 2.43 c	+8.86	245.87 ± 0.83 c	−8.81
NF1	CG	197.86 ± 2.16 b	+15.65	258.83 ± 1.67 b	−4.01
	G1	186.61 ± 3.26 c	+9.07	221.99 ± 0.93 e	−17.67
	G2	172.65 ± 2.81 e	+0.91	246.52 ± 1.38 c	−8.57
NF2	CG	184.56 ± 3.07 c	+7.87	242.29 ± 2.32 c	−10.14
	G1	209.49 ± 2.28 a	+22.45	266.55 ± 0.99 a	−1.14
	G2	176.53 ± 2.19 de	+3.18	237.30 ± 1.89 d	−11.99

* The different letters denote statistical significance based on the LSD test at $p < 0.05$. The number following the ± sign represents the standard error of the mean. The relative change (%) represents the change compared to CF×CG. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

For the CF variant, the highest phenolics content in the peel was achieved in CF × CG (269.65 mg GAE/100 g DM), while the lowest was in CF × G1 (222.91 mg GAE/100 g DM), and their difference was significant. For NF1, the highest phenolics content in the peel was observed in NF1 × CG (258.83 mg GAE/100 g DM), while the lowest was in NF1 × G1 (221.99 mg GAE/100 g DM), and their difference of 36.84 mg GAE/100 g DM was significant. In NF2, the highest phenolics level in the peel was recorded in NF2 × G2 (266.55 mg GAE/100 g DM), while the lowest was in NF2 × G2 (237.30 mg GAE/100 g DM), and their difference was statistically significant.

3.4. Flavonoids Content

Fertigation and grafting significantly influenced the flavonoid content in cucumber flesh (Table 5). The highest flavonoid content in the flesh on the CF plot was achieved with CF × G2 (330.84 mg CE/100 g DM), while the lowest was observed with CF × CG (285.91 mg CE/100 g DM), and their difference of 15.72% is statistically significant. On the NF1 plot, the highest level of flavonoids in the flesh was achieved with NF1×G2 (456.54 mg CE/100 g DM), and the lowest was observed with NF1 × CG (425.22 mg CE/100 g DM), with the difference being significant. On the NF2 plot, the highest flavonoid level was measured in NF2 × G1 (506.92 mg CE/100 g DM), while the lowest was in NF2 × G2 (398.86 mg CE/100 g DM), with their difference being statistically significant.

Table 5. Effect of fertigation and grafting on flavonoids content in cucumber flesh and peel (mg CE/100 g DM) *.

Fertigation	Grafting	Flesh	Relative Change (%)	Peel	Relative Change (%)
CF	CG	285.91 ± 7.60 f	/	1205.87 ± 10.20 cd	/
	G1	298.49 ± 7.06 f	+4.39	1048.35 ±19.61 g	−13.06
	G2	330.84 ± 4.23 e	+15.71	1240.16 ± 7.64 c	+2.84
NF1	CG	425.22 ± 4.56 cd	+48.72	1309.55 ± 14.57 b	+8.59
	G1	455.85 ± 7.43 bc	+59.43	1124.47 ± 2.38 e	−6.75
	G2	456.54 ± 25.90 bc	+59.67	1172.39 ± 17.38 d	−2.77
NF2	CG	480.47 ± 4.32 ab	+68.04	1174.73 ± 17.66 d	−2.58
	G1	506.92 ± 4.81 a	+77.30	1386.44 ± 16.22 a	+14.97
	G2	398.86 ± 9.25 d	+39.50	1118.93 ± 7.96 e	−7.20

* The different letters denote statistical significance based on the LSD test at $p < 0.05$. The number following the ± sign represents the standard error of the mean. Relative change (%) represents the change compared to CF × CG. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

Fertigation and grafting also significantly changed the flavonoid content in cucumber peel (Table 5). On the CF plots, the highest flavonoid content in the peel was measured with CF × G2 (1240.16 mg CE/100 g DM), and the lowest was observed with CF × G1 (1048.35 mg CE/100 g DM), with their difference being statistically significant. On the NF1 plot, the highest flavonoid content was observed with NF1 × CG (1309.55 mg CE/100 g DM), and the lowest was observed with NF1 × G1 (1124.47 mg CE/100 g DM), with the

difference being statistically significant. On the NF2 plot, the highest flavonoid content in the peel was observed with NF2 × G1 (1386.44 mg CE/100 g DM), while the lowest was observed with NF2 × G2 (1118.93 mg CE/100 g DM), and their difference is significant.

3.5. Indicators of Antioxidative Status

Fertigation and grafting significantly influenced the antioxidant activity of cucumber flesh (Table 6). The highest antioxidant capacity measured using the DPPH assay was found in NF2 × G1 (3.11 mg/100 g DM), while the lowest was in CF × CG, and their significant difference was 25.91%. In the case of the FRAP assay, the highest antioxidant activity in the flesh was observed in NF1 × G1 (1.13 mg/100 g DM), and the lowest was in NF1 × CG (0.67 mg/100 g DM), with a statistically significant difference between them. Regarding the ABTS assay, the highest antioxidant content in the flesh was observed in CF × G2 (1.56 mg/100 g DM), which was 40.36% higher compared to CF × CG (1.09 mg/100 g DM), while the lowest antioxidants were measured in NF2 × G1 (1.08 mg/100 g DM), which is −0.91% less than CF × CG. However, the difference between CF × G2 and NF2 × G2 is statistically significant.

Table 6. Effect of fertigation and grafting on antioxidant indicators in cucumber flesh (mg/100 g DM) *.

Fertigation	Grafting	DPPH	Relative Change (%)	FRAP	Relative Change (%)	ABTS	Relative Change (%)
CF	CG	2.47 ± 0.01 d	/	0.71 ± 0.00 ef	/	1.09 ± 0.07 d	/
	G1	2.63 ± 0.03 c	+6.47	0.86 ± 0.01 c	+21.12	1.53 ± 0.01 a	+40.36
	G2	2.58 ± 0.02 bc	+4.45	0.74 ± 0.01 de	+4.22	1.56 ± 0.03 a	+43.11
NF1	CG	2.69 ±0.09 bc	+8.90	0.67 ± 0.01 f	−5.63	1.09 ± 0.07 d	00.00
	G1	2.78 ± 0.03 b	+12.55	1.13 ± 0.00 a	+59.15	1.13 ± 0.01 cd	+3.66
	G2	2.59 ± 0.05 cd	+4.85	0.86 ± 0.00 c	+21.12	1.25 ± 0.08 bc	+14.67
NF2	CG	2.82 ± 0.06 b	+14.17	0.77 ± 0.01 d	+8.45	1.30 ± 0.01 b	+19.26
	G1	3.11 ± 0.02 a	+25.91	0.99 ± 0.02 b	+39.43	1.08 ± 0.08 d	−0.91
	G2	2.81 ± 0.03 b	+13.76	0.85 ± 0.01 c	+17.71	1.35 ± 0.01 b	+23.85

* The different letters denote statistical significance based on the LSD test at $p < 0.05$. The number following the ± sign represents the standard error of the mean. The relative change (%) represents the change compared to CF × CG. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

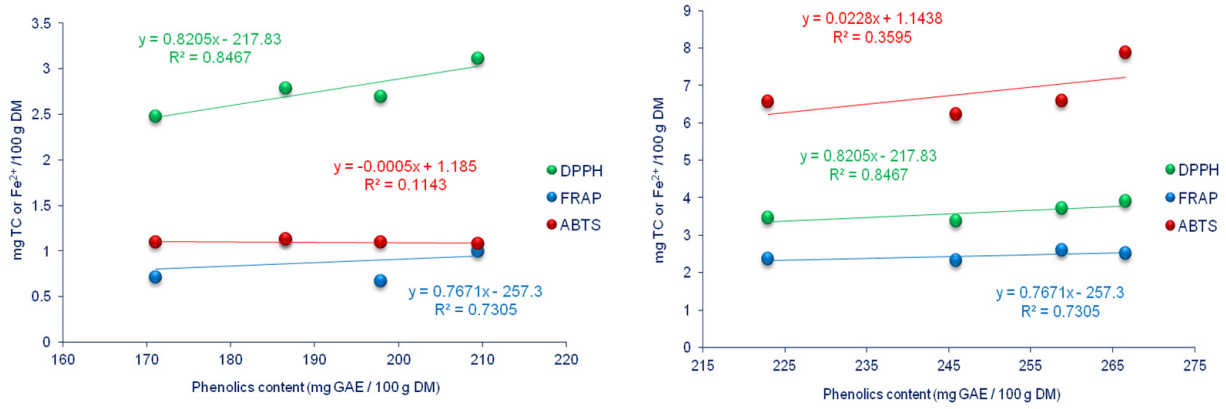
Fertigation and grafting influenced the antioxidant activity in the peel of cucumber (Table 7). The highest antioxidant capacity measured using the DPPH assay was observed in NF2 × G1 (3.90 mg/100 g DM), while the lowest was in CF × CG (3.00 mg/100 g DM), and their difference of 30.00% was statistically significant. Evaluating antioxidants with the FRAP test revealed that the highest antioxidant activity in the peel was found in NF2 × G2 (3.23 mg/100 g DM), and the lowest was in CF × G2 (2.31 mg/100 g DM), with a statistically significant difference between them. In the case of the ABTS assay, the highest antioxidant activity was observed in NF2 × G1 (7.87 mg/100 g DM), while the lowest was in CF × CG (5.07 mg/100 g DM), and their difference of 55.22% was statistically significant.

Table 7. Effect of fertigation and grafting on antioxidant indicators in cucumber peel (mg/100 g DM) *.

Fertigation	Grafting	DPPH	Relative Change (%)	FRAP	Relative Change (%)	ABTS	Relative Change (%)
CF	CG	3.00 ± 0.03 e	/	2.41 ± 0.01 d	/	5.07 ± 0.03 g	/
	G1	3.46 ± 0.02 c	+15.33	2.36 ± 0.00 e	−2.07	6.55 ± 0.05 d	+29.19
	G2	3.37 ± 0.02 cd	+12.33	2.31 ± 0.00 f	−4.14	6.23 ± 0.02 e	+22.87
NF1	CG	3.70 ± 0.04 b	+23.33	2.59 ± 0.00 c	+7.46	6.58 ± 0.03 d	+29.78
	G1	3.80 ± 0.09 ab	+26.66	2.60 ± 0.01 c	+7.88	7.65 ± 0.02 b	+50.88
	G2	3.41 ± 0.01 c	+13.66	2.76 ± 0.01 b	+14.52	6.78 ± 0.05 c	+33.72
NF2	CG	3.26 ± 0.04 d	+8.66	2.43 ± 0.00 e	+0.82	6.22 ± 0.02 f	+22.68
	G1	3.90 ± 0.01 a	+30.00	2.50 ± 0.01 d	+3.73	7.87 ± 0.01 a	+55.22
	G2	3.86 ± 0.01 a	+28.66	3.23 ± 0.01 a	+34.02	7.81 ± 0.08 a	+54.04

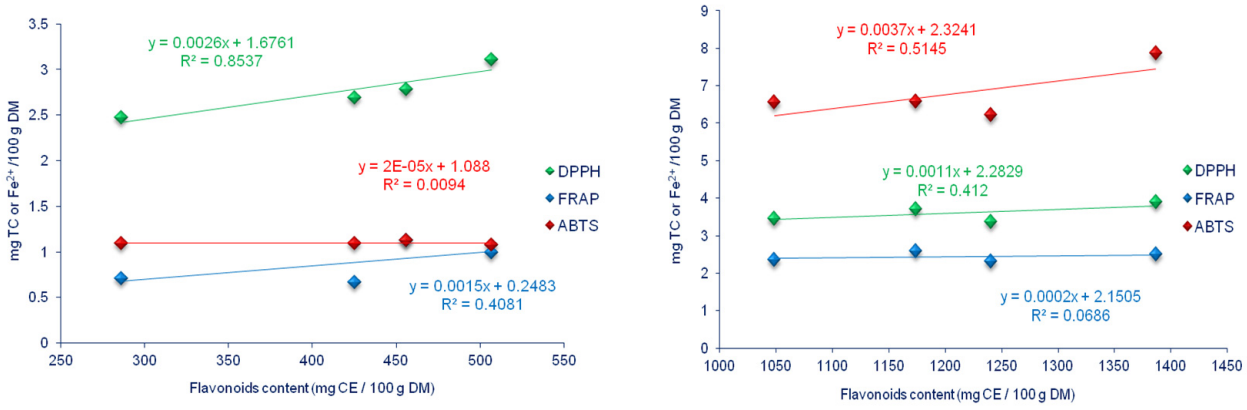
* The different letters denote statistical significance based on the LSD test at $p < 0.05$. The number following the ± sign represents the standard error of the mean. The relative change (%) represents the change compared to CF × CG. CF—standard as a control; NF1 and NF2—two new formulations of nutrient solutions (see Table 1); CG—non-grafted as a control; G1—grafting onto the rootstock *Cucurbita moschata* × *Cucurbita moschata*; G2—grafting onto the rootstock *Lagenaria siceraria*.

Based on Figures 3 and 4, a positive correlation between the total phenolic, flavonoids, and antioxidant indicators in the flesh and peel of cucumber is evident. The linear increase in antioxidants determined through the DPPH, FRAP, and ABTS tests indicates that, as the phenolics and flavonoids content increases, the antioxidant capacity of both the flesh and peel of cucumber also increases.



(**a**) (**b**)

Figure 3. The relationship between total phenolics content and indicators of antioxidant status (DPPH, FRAP, and ABTS) in cucumber flesh (**a**) and peel (**b**).



(**a**) (**b**)

Figure 4. The relationship between flavonoids content and indicators of antioxidant status (DPPH, FRAP, and ABTS) in cucumber flesh (**a**) and peel (**b**).

4. Discussion

In cucumber production, it is essential to align mineral nutrition with the growth and development phase. In this study, the highest yield was recorded for NF1, while an increased fertilizer quantity (NF2) resulted in a decline in yield. Similar results were noted by Cui et al. [33], indicating that, with an increase in N dose, the cucumber yield rises to a specific limit beyond which a further increase in N dose leads to a decrease in yield.

Grafting cucumbers onto rootstocks G1 and G2 significantly increased the fruit yield. This can be explained by the fact that grafted plants possess a stronger root system, enabling a more efficient uptake of water and nutrients and consequently resulting in a larger leaf surface area and enhanced CO_2 assimilation [32]. Similar yield increases in grafted cucumbers on pumpkin (*Cucurbita maxima*) were documented by Amerian et al. [45].

Grafting on the G1 rootstock significantly increased the yield of CF × G1 (↑ 17.4%), NF1 × G1 (↑ 45.3%), and NF2 × G1 (↑ 15.3%) compared to the absolute control, CF × CG. In the case of G2 as the rootstock, on the plots CF × G2 (↑ 17.0%) and NF1 × G2 (↑ 23.2%), the yield increased significantly compared to CF × CG. This can be attributed to the strong vigor of the intraspecies rootstock, promoting the flow of minerals and water from the roots to the fruits [46]. In contrast, in the NF2 × G2 treatment, there was no significant increase in yield compared to CF × CG. This can be explained by the sensitivity of the non-hybrid rootstock to salt conditions.

The highest DM content was found in CF while, with a further increase in the fertilizer dosage (NF1 or NF2), the dry-matter content significantly decreased. This can be explained by the fact that the elevated dose of nutrients, primarily N, affects cell composition, altering the carbon (C) to N ratio. In other words, it reduces the C content in favor of N, implying relatively lower fiber (cellulose) content in the cells and higher water content [2]. A similar conclusion was reached by Vojnović [47] in a study of onion (*Allium cepa*).

Under CF and NF2 fertilization, grafting significantly reduced the DM content in the fruit flesh. Similar results were reported by Khapte et al. [31], indicating that grafting significantly decreased the DM content in cucumbers. Based on this, it was found that, under conditions of insufficient and excessive nutrition, the rootstock absorbs a greater amount of water, which increases its content in the flesh of the fruit. Similarly, Zhang et al. [48] found that grafting mulberry increased root vigor under salt stress and led to higher water content in the plant.

Regardless of the fertigation, grafting significantly increased the DM content in the cucumber peel, which can contribute to better fruit firmness and thus improve storage longevity. Therefore, it is recommended to examine the effect of grafting on post-harvest

cucumbers in future research, as it has been proven that grafting improves storage in watermelons [49].

Phenolics are a group of secondary plant metabolites that significantly influence the taste and biological value of cucumber fruits. Additionally, phenolics possess potent antioxidant properties, making them essential in human nutrition [6,50,51].

Compared to CF × CG, the most significant increase in phenolics in the flesh was observed in NF2 × G1 (↑ 22.4%). This can be related to the fact that the intraspecies rootstock has a greater tolerance to excessive nutrition, and in response to stress, it synthesizes a higher quantity of secondary metabolites, such as phenolics, thereby reducing the adverse effects of radicals. Similarly, in melons (*Cucumis melo*), grafting increased the phenolic and antioxidant capacity measured via DPPH and ABTS tests [52].

In cucumber flesh in this study, fertigation with NF1 × CG increased the phenolics content by 15.65%, while in the NF2 × CG, the increase was 7.87% compared to CF × CG. Similarly, Putra et al. [53] reported that increasing rates of NPK fertilizer increased the phenolics content in purslane (*Portulaca grandiflora*) up to a specific limit beyond which the phenolics level began to decline. Additionally, a similar conclusion in the case of flavonoids was obtained with onions grown under increased N rates [54].

The quality of cucumbers largely depends on the presence of flavonoids in the flesh and peel of the fruit. Flavonoids have strong antioxidant properties, meaning they are highly effective in scavenging free radicals [44,55]. Therefore, consumers are advised to increasingly seek cucumbers with a high content of antioxidant compounds and to increase the share of vegetables in their diet [56].

In this study, the G1 rootstock significantly increased the flavonoid content in the fruit flesh compared to CG. This can be explained by the fact that grafting significantly up-regulated enzymes (e.g., flavanone 3-hydroxylase, dihydroflavonol-4-reductase, cinnamate 4-hydroxylase, chalcone isomerase, and naringenin-chalcone synthase) involved in the biosynthesis of flavonoids [57]. Additionally, Xu et al. [57] noted that flavonoids aid in enhancing callus formation. The increase in flavonoid content in cucumbers grafted on pumpkin (*Cucurbita maxima*) was recorded by Amerian et al. [45]. In the case of tomatoes (*Lycopersicon esculentum*), an increase in flavonoids in grafted plants was observed by [58].

NPK plant nutrition can significantly alter the flavonoid content in plants. In this experiment, it was found that the new NF1 and NF2 nutrition formulas significantly increased the flavonoids in the flesh compared to CF. Previous studies also reported significant changes in flavonoid content after NPK fertilization in soybeans (*Glycine max*), Lanzhou lily (*Lilium davidii* var. *unicolor*), and moringa (*Moringa oleifera*) [59–61].

In this study, grafting increased the antioxidant activity in the cucumber flesh. According to this, there is a known relationship between the production of antioxidant compounds and callus formation [62]. The highest antioxidant activity in the cucumber flesh, measured via the DPPH test, was recorded in the NF2 × G1 treatment, while the FRAP test showed the highest activity in the NF1 × G1 treatment, and the ABTS test indicated the highest activity in the CF × G2 treatment. The discrepancy in the test reactions may be explained by each test responding to different mechanisms of action. For example, Greathouse et al. [63] observed differences between the results of the DPPH and ABTS antioxidant tests, attributing them to variations in the molecular mechanisms of the two antioxidant capacity assays.

In the treatments NF2 × G1 and NF1 × G1, the antioxidant activity in cucumber flesh, measured via the DPPH and FRAP tests, was most significantly increased. This can be explained by the high flavonoid content measured in NF2 × G1, as Pietta [55] stated that flavonoids possess strong antioxidant properties. Additionally, this is consistent with the findings of Vojnović et al. [54], who established a correlation between flavonoids and antioxidant activity measured via the DPPH and FRAP tests in onions.

In cucumber peel, the highest antioxidant capacity was observed in NF2 × G1, as indicated by the DPPH and ABTS tests. However, the FRAP test showed the highest antioxidant activity in NF2 × G2. In this study, a linear correlation was found between

phenol content and antioxidant activity (DPPH, FRAP, and ABTS) in cucumber flesh and peel. Similar correlations were reported by Vojnović et al. [64] for onions grown from direct seed for two years.

5. Conclusions

In this study, the yield of fruit and the content of bioactive compounds in cucumber flesh and peel were significantly influenced by the new nutrient formulation and grafting.

For farmers aiming to achieve high yields of greenhouse cucumbers with high levels of total phenolics and flavonoids, the combination of NF1 × G1 is recommended.

In terms of antioxidant activity, the treatment NF2 × G1 resulted in the most significant increases in DPPH and FRAP levels in the fruit flesh.

Consumers are encouraged to eat cucumbers with the peel, as this study found that the peel contains higher levels of antioxidant compounds compared to the flesh.

Future research should investigate the effects of fertigation and grafting on the post-harvest characteristics of cucumbers, as this study indicated that grafting significantly increases the dry-matter content and antioxidant levels in the peel.

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