Contrary to Red, Blue Monochromatic Light Improves the Bioactive Compound Content in Broccoli Sprouts

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Abstract: Broccoli sprouts are rich in health-promoting bioactive compounds. Their content depends on both cultivation light quality and temperature. However, these effects have been previously addressed in isolation. Here, the dual inputs of cultivation light quality [blue (B), red (R), mixture of R and B (R+B), mixture of R and UVA (R+UVA)] and air temperature (15, 19, and 23 °C) on determining growth, external quality, and the cotyledon and hypocotyl content of five major bioactive compounds were investigated. The carbohydrate status at harvest and postharvest ratio of variable to maximum fluorescence ($F_v/F_m$) were also assessed. Hypocotyl length was generally enhanced under monochromatic light (R or B) and elevated temperature. Total phenolic, total flavonoid, and glucoraphanin contents were generally higher in cotyledon as compared to hypocotyl. Hypocotyl anthocyanin, total phenolic, total flavonoid, and ascorbic acid contents were generally enhanced by R+B, and were decreased by R. Cotyledon content in these metabolites was generally stimulated by B, and reduced under R or R+UVA. Temperature affected metabolite content depending on the metabolite, organ, and light quality. Lower temperatures, R (23 °C) or R+UVA (15, 19, and 23 °C) were associated with decreased postharvest $F_v/F_m$. In conclusion, low cultivation temperature (<23 °C), as well as R or R+UVA ought to be avoided. Instead, B and R+B are suitable, with B being preferable, owing to better external quality and enhanced metabolite content in cotyledon which generally holds higher content than hypocotyl.

Keywords: anthocyanins; ascorbic acid; Brassica oleracea; flavonoids; glucoraphanin; polyphenols

1. Introduction

Following seed germination, edible sprouts are typically harvested before the development of true leaves [1,2]. In this perspective, they are immature seedlings composed of two cotyledons, hypocotyl and radicle. Sprouts typically exhibit nutritional superiority as compared to seeds, and enhanced bioactive compound content as compared to their mature counterparts [2–4]. In recent years, their consumption has been steadily increasing in the context of rising consumer awareness for healthy diet benefits [2,5]. A typical example is broccoli (Brassica oleracea L.) sprouts, which are recognized as a functional food, owing to their preventive action against several chronic diseases [4,6].

Broccoli sprouts are an excellent source of a wide range of bioactive compounds including glucosinolates, anthocyanins, polyphenols, flavonoids, and vitamins, especially ascorbic acid (vitamin C) [2,6]. Glucosinolates are well-documented health promoters, with glucoraphanin being the most abundant one (>50%) in broccoli [7]. In a wide range...
of clinical studies, the above-mentioned secondary metabolites have been repeatedly associated with anti-diabetic, anti-cancer, anti-inflammatory, and antioxidant activities [5,6]. On this basis, enhancing the bioactive compound content is a direct means of improving produce quality, and health-promoting potential [8,9].

Sprout characteristics are set by the growth environment, with light and temperature arguably being the two most important conditions. While sprouts are generally cultivated in darkness [10], it was found that light exerts a promotive effect on nutritional and bioactive compound profiles depending on its quality (spectral distribution) [1,2,4]. For instance, blue (B) light exerted a promotive effect on the content of some bioactive compounds in kale [11] and broccoli [12] sprouts, and in this way has been suggested as a suitable growth spectrum [13,14]. Accordingly, a stimulatory effect of red (R) light and UVA (320–400 nm) on the content of some secondary metabolites has also been suggested [15,16]. Instead, far-red light has been associated with adverse effects on metabolite content [17]. Given the rising application of light-emitting diodes (LEDs) in horticultural practice [6,18–20], the interest in manipulating light quality to improve both productivity and quality is currently expanding [19,20].

Light quality can be combined with other environmental conditions to optimize its impact on plants [21]. For instance, sprout characteristics depend on growth temperature [7]. However, the effect of either light quality or temperature has been previously addressed in isolation. It, therefore, remains an open question whether or not these two factors interact to determine sprout growth and productivity in general, as well as specific traits in particular. Taking such a perspective into consideration is vital for optimizing sprout quality in horticultural applications. This type of optimization is eco-friendly, since it does not rely on the addition of chemicals [22].

Short-term storage temperature does not generally change the content of the bioactive compounds [23]. Although postharvest UV-B radiation has been shown to exert a stimulatory effect on bioactive compound content [24], broccoli sprouts are conventionally kept under either darkness or low light intensity along the postharvest chain [6]. Advanced carbohydrate status has been associated with prolonged postharvest longevity [25]. This status depends on both the carbohydrates available at harvest, and the postharvest photosynthetic activity [18]. Therefore, maintaining photosynthetic activity following harvest appears advantageous in occasions where the produce is exposed to light (e.g., at display) [25].

The objective of this study was to evaluate the dual inputs of cultivation light quality and temperature on determining growth, external quality, along with the (cotyledons and hypocotyl) content of five major bioactive compounds in broccoli sprouts. Carbohydrate status at harvest and photosynthetic activity following harvest were also assessed.

2. Materials and Methods
2.1. Plant Material and Growth Conditions

Broccoli (B. oleracea var. ZHONGQING No.16) seeds were sanitized (15 min) in sodium hypochlorite solution (1%, v/v), and then rinsed (2 min) with distilled water. Following pre-germination, seeds were put on moist vermiculite into petri dishes (9 cm diameter × 1 cm height; 35 seeds per petri dish), in darkness (23 °C) for 36 h. Seedlings were then transferred to four environmentally controlled growth chambers (l × w × h = 1.3 × 0.7 × 0.6 m) for realizing the treatments.

In the growth chambers, light was provided using LED modules (iGrowLite Co., Ltd., Guangzhou, China), and included B (peak at 450 nm), R (peak at 660 nm), a mixture of R and B (R+B), as well as a mixture of R and UVA (R+UVA, for UVA peak at 365 nm) (Figure 1). In all four light qualities, photon flux density was set at 50 µmol m⁻² s⁻¹ for 16 h d⁻¹ (09:00 to 01:00 h) at the sprout level. The employed light intensity has been reported as optimal for sprout growth [2]. Photon flux density and spectra were routinely monitored by using a spectroradiometer (Avaspec-2048CL, Avates, Apeldoorn, The Netherlands). For each light quality, three air temperature treatments (15, 19, and 23 °C) were employed. This
resulted in twelve treatments (four light qualities \times three air temperatures) in total. In all treatments, relative air humidity was maintained at 77 ± 1%.

During the experiment, five batches of sprouts (each containing five petri dishes) were grown for each treatment. Treatment position was randomly switched, whenever a new cultivation was initiated.

Sprouts were destructively harvested 5 d following treatment. The time between sampling and the start of the evaluation did not exceed 15 min. For growth and external quality determination, 10 sprouts were randomly taken from one petri dish, which were considered as one sample. For metabolite and biochemical assays, cotyledon and hypocotyl were separately sampled, placed in vials, flash-frozen in liquid nitrogen, and transferred to a freezer (−80 °C) for storage. For these assays, each sample was taken by powdering 20 sprouts from one petri dish. For the above-mentioned measurements, five samples were collected per treatment per batch cultivation. The destructively harvested samples from the first three cultivation batches were considered as replicates.

Intact sprouts from the latter two batches (i.e., two replicates) were stored at 4 °C and 50% relative air humidity under darkness. These conditions are commonly employed during transportation, storage, and commercial handling practices [6].

2.2. Growth and External Quality

Hypocotyl length (from the radicle-to-hypocotyl junction to the apical end) was determined. Following removal of the substrate from the radicle via gentle washing, sprout fresh and dry masses were recorded (±0.001 g; BASA124S-CW, Sartorius, Goettingen, Germany). For measuring dry weight, samples were placed in a forced-air drying oven for 72 h at 70 °C. Water content was calculated according to [26].

2.3. Cotyledon and Hypocotyl Contents of the Major Bioactive Compounds

In cotyledons and hypocotyl, the content of the major bioactive compounds was quantified. These included anthocyanins, total phenols, total flavonoids, ascorbic acid, and glucoraphanin (the most abundant glucosinolate in broccoli, [7]). All these secondary metabolites are highly beneficial for both sprout stress response and human health [5,6]. These measurements were conducted on three replicates per treatment, each replicate representing the mean of five samples from five petri dishes, respectively.
2.3.1. Anthocyanin Content

Samples (0.1 g) were incubated with 600 µL of 1% hydrogen chloride–methanol solution and stored in darkness (4 °C for 24 h). Distilled water and chloroform were then added to the samples. These were then homogenized (13,000 × g at 4 °C) for 5 min. Anthocyanin content was assayed in the supernatant by measuring the absorbance at 530 and 657 nm, using a UV–vis spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan), according to [27].

2.3.2. Total Phenolic and Total Flavonoid Contents

Samples (0.1 g) were extracted with 1 mL of 80% aqueous methanol in an ultrasonic bath (10 min), and were then centrifuged (15,000 × g for 10 min). The contents of total phenolic and total flavonoid were determined by using the Folin-Ciocalteu assay and aluminum chloride colorimetric assay, respectively, following [25]. The absorbance against prepared reagent blank was determined using a microplate reader (Infinite 200 PRO, TECAN, Männedorf, Switzerland). For total phenolic content, gallic acid was used as the standard reference and gallic acid equivalent (GAE) was expressed as mg per g fresh mass. For total flavonoid content, rutin was used as the standard reference and rutin equivalent (RUE) was expressed as mg per g fresh mass.

2.3.3. Ascorbic Acid Content

Samples (0.1 g) were dissolved in 1 mL of precooled (4 °C) extraction buffer (3% MPA + 8% acetic acid + 1 mM EDTA solution). After centrifugation (15,000 × g at 4 °C) for 20 min, the supernatant was filtered through a PTFE filter (0.22 µm; Jinteng Co., Ltd., Tianjin, China), and collected. The filtered supernatant was analyzed with UPLC (Acquity H-Class; Waters, Milford, MA, USA) equipped with a HSS T3 column (2.1 × 100 mm, 1.8 µm; Waters, Milford, MA, USA). A standard solution was formulated using L-ascorbic acid. The column was eluted with 0.1% (v/v) formic acid with a flow rate of 0.25 mL min⁻¹.

2.3.4. Glucoraphanin Content

Samples (40 mg) were extracted using 1 mL of 70% methanol, vortexed, and extracted in a water bath (20 min at 70 °C). These were subsequently centrifuged (13,000 × g at 4 °C) for 5 min. The supernatant was collected, and the procedure was repeated three times. The collected supernatant was filtered through a PTFE filter (0.22 µm; Jinteng Co., Ltd., Tianjin, China).

The filtered was analyzed with UPLC (Acquity H-Class; Waters, Milford, MA, USA), equipped with a HSS T3 column (2.1 × 100 mm, 1.8 µm; Waters, Milford, MA, USA). Ultrapure water and 20% acetonitrile were used as mobile phase A and B, respectively. Throughout the gradient, a flow rate was set at 0.2 mL min⁻¹. The injection volume was 0.7 µL, and column temperature was maintained at 30 °C. A standard solution was formulated using glucoraphanin (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China).

2.4. Cotyledon and Hypocotyl Soluble Sugar Content

Samples (0.1 g) were incubated with 1 mL of deionized water in a water bath (100 °C for 30 min). The homogenate was centrifuged (15,000 × g for 15 min) at room temperature (25 °C). Then, 0.1 mL of the solution was mixed with anthranone ethyl acetate and H₂SO₄. Soluble sugar content was assayed in the supernatant by measuring the absorbance at 630 nm, using a UV–vis spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan), according to [28].

2.5. Maximum Quantum Yield of Photosystem II (PSII) during Postharvest Storage

As a sensitive indicator of leaf photosynthetic performance, dark-adapted values of the maximum quantum yield of PSII \( F_v/F_m = (F_m - F_o)/F_m \), i.e., the quantum efficiency of all PSII centers were open] were recorded in intact sprouts [29]. Measurements were performed daily with a fluorescence imaging system—PAM (IMAG-MAXI; Heinz Walz,
Effeltrich, Germany). Prior to measurements, sprouts were dark adapted (≥ 30 min). Then, \( F_v/F_m \) was evaluated by applying a saturated photosynthetic photon flux density of 8000 µmol m\(^{-2}\) s\(^{-1}\). These measurements were conducted on 10 petri dishes (i.e., two batches and each batch contain five petri dishes) per treatment.

2.6. Statistical Analysis

Data analysis was performed using the SPSS software (version 23; SPSS Inc., Chicago, IL, USA). A two-way ANOVA was employed, with light quality as the main factor and air temperature as the split factor. Each independent experiment was considered as a block. Data were firstly tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene’s test). Subsequently, estimated least significant differences (LSD) of treatment effects were determined (\( p = 0.05 \)).

3. Results

3.1. Growth and Visually Perceived Quality

Cultivation temperature generally promoted hypocotyl length (a critical external quality trait) and enhanced light quality-induced differences (Figures 2 and 3A). At the highest cultivation temperature (23 °C), sprouts under either B or R light were the tallest, whereas the ones under R+B were the shortest (Figures 2 and 3A).

Figure 2. Morphology of broccoli sprouts grown for 5 d under different light [blue (B), red (R), mixture of R and B (R+B), as well as mixture of R and UVA (R+UVA)] and air temperature (15, 19, and 23 °C) regimes. The scale bar refers to 1 cm length.
Figure 3. Hypocotyl length (A), fresh weight (B), dry weight (C), and water content (D) of broccoli sprouts grown for 5 d under different light [blue (B), red (R), mixture of R and B (R+B), as well as mixture of R and UVA (R+UVA)] and air temperature (15, 19, and 23 °C) regimes. Error bars indicate SEM (n = 3, each replicate representing the mean of five petri dishes). Different letters show statistically significant differences between treatments (p < 0.05) as established by the l.s.d. test.

Light quality affected sprout dry weight only at the highest cultivation temperature (23 °C), where R+B was associated with the highest sprout dry weight (Figure 3C). Cultivation light quality and temperature generally had a small effect on water content (Figure 3D).

3.2. Cotyledon and Hypocotyl Contents of the Major Bioactive Compounds

Total phenolic (Figure 4C,D) and total flavonoid (Figure 4E,F) contents were generally higher in cotyledon as compared to hypocotyl. The same was noted for glucoraphanin, where cotyledon content was at least three times higher than the one in hypocotyl (Figure 4I,J). On the contrary, anthocyanin (Figure 4A,B) and ascorbic acid (Figure 4G,H) contents were similar between hypocotyl and cotyledon.

Hypocotyl anthocyanin (Figure 4A), total phenolic (Figure 4C), total flavonoid (Figure 4E), and ascorbic acid (Figure 4G) contents were generally enhanced by R+B, whereas these were generally decreased by R. Hypocotyl content in these metabolites generally decreased, as cultivation temperature increased, especially under R+B (Figure 4A,C,E,G). At 15 and 23 °C, hypocotyl glucoraphanin content was lower under R (Figure 4I).

Cotyledon anthocyanin (Figure 4B), total phenolic (Figure 4D), total flavonoid (Figure 4F), and ascorbic acid (Figure 4H) contents were generally stimulated by B. Cotyledon content in these metabolites, was generally lower under either R or R+UVA (Figure 4B,D,F,H). Cotyledon glucoraphanin content was also generally promoted by B, and generally decreased by R or R+UVA (Figure 4J). At 23 °C, cotyledon total flavonoid content was the lowest compared to 15 or 19 °C (Figure 4F), whereas cotyledon glucoraphanin content was the highest (Figure 4J).
Figure 4. Anthocyanin (A,B), total phenolic (C,D), total flavonoid (E,F), ascorbic acid (G,H), and glucoraphanin (I,J) contents of broccoli sprouts grown for 5 d under different light [blue (B), red (R), mixture of R and B (R+B), as well as mixture of R and UVA (R+UVA)] and air temperature (15, 19, and 23 °C) regimes. Left panels refer to hypocotyl, and right panels to cotyledon. The difference in the y-axis scale of glucoraphanin content panels (I,J) ought to be noted. Error bars indicate SEM (n = 3, each replicate representing the mean of five petri dishes). DW, dry weight; FW, fresh weight; GAE, gallic acid equivalent; RUE, rutin equivalent.

3.3. Cotyledon and Hypocotyl Soluble Sugar Content

Soluble sugar content was similar between hypocotyl and cotyledon (Figure 5). Hypocotyl soluble sugar content was generally enhanced by R+B, whereas it was decreased by either B or R+UVA (Figure 5A). Cotyledon soluble sugar content was also generally higher at R+B, and generally lower under R+UVA (Figure 5B). No consistent effect of cultivation temperature on soluble sugar content was apparent.
Figure 5. Soluble sugar content of broccoli sprouts grown for 5 d under different light [blue (B), red (R), mixture of R and B (R+B), as well as mixture of R and UVA (R+UVA)] and air temperature (15, 19, and 23 °C) regimes, and then stored for 5 d under darkness (4 °C and 50% relative air humidity). Error bars indicate SEM (n = 3, each replicate representing the mean of five petri dishes). FW, fresh weight.

3.4. Maximum Quantum Yield of Photosystem II (PSII) during Postharvest Storage

Following cultivation, intact sprouts were stored under darkness (4 °C, 50% relative air humidity), and Fv/Fm was daily determined. Lower Fv/Fm values were generally noted in sprouts cultivated at lower cultivation temperatures (Figure 6). At 23 °C, cultivation under B was associated with the highest Fv/Fm values, whereas cultivation under either R or R+UVA was the lowest. At 15 and 19 °C, cultivation under R+UVA was related to considerably lower Fv/Fm values, while differences among the remaining light quality treatments were minor.

Figure 6. Representative image of the dark-adapted Fv/Fm distribution (A) and extracted Fv/Fm values (B) of intact broccoli sprouts grown for 5 d under different light [blue (B), red (R), mixture of R and B (R+B), as well as mixture of R and UVA (R+UVA)] and air temperature (15, 19, and 23 °C) regimes, and then stored for 5 d under darkness (4 °C and 50% relative air humidity). Error bars indicate SEM (n = 2, each replicate representing the mean of five petri dishes). LSD values (p < 0.05) are indicated per plot.
4. Discussion

4.1. Growth and Visually Perceived Quality

In terms of sprout dry weight, light quality was an influencing factor only at the highest cultivation temperature (23 °C), where R+B was optimum (Figure 3C). Considering hypocotyl length, which is a critical external quality trait, monochromatic light (R or B) and elevated temperature were associated with a more elongated hypocotyl phenotype (Figures 2 and 3A). Similar phenotypes have been earlier reported under monochromatic light [30], which were not apparent when adjusted to bi-chromatic [31]. Castillejo [6] also reported that postharvest storage (5 °C) under monochromatic light (especially R but also B) was related to larger sprouts in comparison to fluorescence light. Moreover, 23 °C is a temperature closer to the optimum for hypocotyl elongation in broccoli sprouts [7]. Instead, cultivation under R+B led to the shortest hypocotyl length (Figures 2 and 3A). Taken together, these results indicate that, for producing high visually perceived-quality sprouts, the highest employed temperature (23 °C) and monochromatic light (R or B) are the preferred options.

4.2. Cotyledon and Hypocotyl Contents of the Major Bioactive Compounds

Manipulation of light quality is a viable and safe means to improve the bioactive compound content [10,20], and thus internal quality of the produce [8,9]. For the first time, this possibility was addressed along with a range of cultivation temperatures. Importantly, monochromatic light (R or B) and UVA (320–400 nm) were also included, which have been tested understated in earlier studies [1,2,4]. Since metabolite content varies among sprout organs [7], hypocotyl and cotyledon were separately addressed. Indeed, a differential regulation between hypocotyl and cotyledon was noted for anthocyanin, total phenolic, total flavonoid, and ascorbic acid contents. These were generally promoted by lower temperature in the former, though not in the latter (Figure 4). In addition, their content was stimulated by R+B in hypocotyl and by B in cotyledon (Figure 4). The promotive effect of B light on metabolite content has also been shown in other species [32]. In terms of light quality, glucoraphanin content followed the same trend as in the above-mentioned metabolites in cotyledon, but not in hypocotyl. Notably, R light was generally associated with reduced content of each metabolite under study (anthocyanin, total phenolic, total flavonoid, ascorbic acid, and glucoraphanin) in both organs. In other species, a positive effect of R light on secondary metabolite content has been suggested [33]. At lower temperatures and solely in cotyledon (but not hypocotyl), R+UVA generally exerted an adverse effect on metabolite content similarly to R. Therefore, regarding the content of health-promoting substances, an optimum cultivation temperature across all metabolites under study is not in existence. Still, given the central importance of glucoraphanin (the most abundant glucosinolate, [7]) as a health-promoting element in broccoli sprouts, the highest cultivation temperature (23 °C) appears as the best option. Similarly, an optimum cultivation light quality (B or R+B) for all five metabolites was not apparent. However, considering that cotyledon has a generally richer content, monochromatic B light stands out as the best choice. Our results, however, strongly suggest that monochromatic R or R+UVA light ought to be avoided, since they are generally associated with decreased sprout (hypocotyl + cotyledon) metabolite content. In mature seedlings, monochromatic R light has also been associated with a wide range of undesirable effects, including downward curling of leaf margins (the so-called leaf epinasty), smaller leaf area, and reduced chlorophyll content [18]. Although UVA (320–400 nm) per se has been related to the activation of secondary metabolism, adverse effects were apparent when the optimum dose was exceeded [25].

4.3. Cotyledon and Hypocotyl Soluble Sugar Content at Harvest, and Maximum Quantum Yield of Photosystem II (PSII) during Postharvest Storage

Increased sugar levels may promote respiratory activity, facilitating maintenance processes, and operate as reactive oxygen species scavenger, protecting membrane integrity [25,34,35]. Hypocotyl and cotyledon sugar content was generally enhanced at R+B,
and decreased at R+UVA. In the former, B was also associated with decreased sugar content. 
In more mature seedlings, instead, the highest soluble carbohydrate content was noted 
under R, and the lowest under B [18]. These differences may be due to the low duration of 
sprouting (5 d), as compared to the time needed to produce more mature seedlings (12 d).

The intact sprouts were stored under darkness, as mostly performed in commercial 
facilities [6]. Storage under darkness has been associated with declining carbohydrate 
levels owing to the absence of photosynthesis [25]. On the contrary, storage under light 
improves carbohydrate status and shelf-life, since it drives photosynthesis and, thus, de novo 
carbohydrate synthesis [36]. We here show that cultivation at lower temperatures, R (23 °C) 
or R+UVA (15, 19 and 23 °C) was associated with reduced PSII activity (Figure 6). Therefore, 
sprouts cultivated under these regimes are expected to be less capable of assimilating light 
in different parts of the supply chain, where the produce is exposed to it.

5. Conclusions

In broccoli sprouts, the combined effect of cultivation light quality [blue (B), red (R), 
mixture of R and B (R+B), as well as mixture of R and UVA (R+UVA)] and air temperature 
(15, 19, and 23 °C) on growth, external quality, as well as the cotyledon and hypocotyl 
content of five major bioactive compounds was analyzed. Carbohydrate status at harvest 
and postharvest photosynthetic activity were also determined. Monochromatic light (R or 
B) and elevated temperature generally stimulated hypocotyl length. At 23 °C, light quality 
significantly affected dry weight, where R+B was optimum for biomass accumulation. For 
metabolite content, optimum light quality (B or R+B) and temperature depended on the 
metabolite and the organ. In most cases, R and R+UVA were associated with reduced 
metabolite content. R+UVA also decreased sugar content. Lower temperatures, as well as R 
or R+UVA were associated with lower ratio of variable to maximum fluorescence (Fv/Fm). 
In conclusion, low temperature (<23 °C; decreased hypocotyl length, glucoraphanin content 
and Fv/Fm), and R or R+UVA (decreased bioactive compound and Fv/Fm) ought to be 
avoided during cultivation. Both B and R+B seem suitable, with the former being preferable 
owing to stimulating cotyledon metabolite content, which is generally richer in metabolites 
than hypocotyl.

Author Contributions: Conceptualization, T.L.; data curation, L.Y., D.F.; formal analysis, L.Y., D.F., 
G.T.; investigation, L.Y.; project administration, T.L.; supervision, T.L.; writing—original draft, L.Y., 
D.F., G.T.; writing—review and editing, L.Y., D.F., G.T., K.L., Q.Y. and T.L. All authors have read and 
agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China (No. 31872955), 
and the Central Public-interest Scientific Institution Basal Research Fund (No. BSRF2019111).

Data Availability Statement: Main data are contained within the article; further data presented in 
this study are available on request from the corresponding author.

Acknowledgments: The authors gratefully acknowledge the laboratory staff for their contributions, 
continued diligence, and dedication to their craft. The valuable comments of the editor and four 
anonymous reviewers are greatly appreciated.

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

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