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Optimized Alternating Current Electric Field and Light Irradiance for *Caulerpa lentillifera* Biomass Sustainability—An Innovative Approach for Potential Postharvest Applications

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Abstract: Recently published preliminary data proposed alternating current electric field (ACEF) as a promising technique for the postharvest storage of seagrape (*Caulerpa lentillifera*). The current study suggested a combination of storage light irradiance (SLI) and ACEF (intensity and time) to enhance seagrape physicochemical quality (PQ). It utilized Taguchi orthogonal array design (OAD) to optimize the processing conditions. Results showed all the processing parameters had significant ($p < 0.05$) effects on seagrape PQ. This study found that 50 kV/m for 60 min (ACEF) and 9 mol photons m^{-2} s^{-1} performed the best inhibition on seagrape PQ deterioration. It revealed that adjusting the processing parameters in the range explored in this study (50, 125, 200 kV/m of ACEF intensity; 30, 60, 90 min of ACEF treatment time; 2, 9, 16 mol photons m^{-2} s^{-1} of SLI) can reduce up to 60% of total voltage usage compared to the previous study. With a sufficient SLI and an intermediate treatment time, the finest seagrape PQ can be sustained with a lower electric strength. Therefore, this method can benefit seagrape industries and contribute to realizing sustainable development goals by strengthening resource efficiency and lowering energy consumption.

Keywords: alternating current electric field; *Caulerpa lentillifera*; light irradiance; optimization; orthogonal array

1. Introduction

Seagrape (*Caulerpa lentillifera*) biomass has been reported to have potential applications for food, feed, pharmaceuticals, cosmetics, wastewater treatment, biofertilizers, and biopolymers. Seagrape is an edible species of ulvophyte green algae, and its shelf-life and quality are crucial to determining the market price [1,2]. The reduction of shelf-life and quality of food products is influenced by stressed environments or storage conditions, and reactive oxygen species (ROS) play a big role in that mechanism [3]. Yamauchi [3] explained that ROS were extremely reactive substances that, once accumulated, lead to oxidative stress, which directly damages the cell components and causes physiological disorders of fresh produce during the postharvest period. In general, the physicochemical quality (PQ) deterioration of seagrape is led by the desiccation effect due to oxidative stress conditions in their storage environment [1,4]. Additionally, studies also reported that storage light irradiance (SLI) has a significant effect in altering the quality of seagrape chlorophyll pigment, which influences the photosynthesis performance of harvested seagrape [5]. Guo et al. [5] explained that high SLI degraded the chlorophyll content and chlorophyll fluorescence (Fv/Fm) of seagrape. In addition, studies reported that lighting treatment (including UV light irradiation) of food products during postharvest storage is essential for PQ and shelf-life preservation [3,6].

Zhao et al. [7] mentioned that ROS accumulation that caused vital degradations in PQ of food products, such as membrane damage and the loss of chlorophyll, color, and phenols, could be suppressed by exposure to the alternating electric field (ACEF) treatment. ACEF treatment has been reported to elevate the shelf-life and quality of different food products during their postharvest handling stage [8,9]. Sulaimana et al. [1] showed that ACEF treatment could inhibit the deterioration of seagrape PQ, including water loss, malondialdehyde (MDA) production, total phenolic content (TPC) reduction, and chlorophyll degradation during postharvest storage. Accordingly, the ACEF is an alternative method to maintain the quality of seagrape for a longer time since the common industrial methods of seagrape preservation, i.e., drying and brining, are inefficient [1]. Increasing seagrape PQ is expected to reduce food loss and food waste during preparation and postharvest handling up to distribution, in particular, decreasing the negative impact of food production on the environment [10,11].

Punthi et al. [12] described that pulsed electric field (PEF) causes changes in the transmembrane potential, thereby causing structural changes or damage to the membrane. In addition, reversible or irreversible permeabilization of the cell membrane can occur after being subjected to a transmembrane potential that exceeds a certain threshold, thereby causing physiological and biochemical changes in the cell. Furthermore, cell membrane damage was demonstrated by pore and non-pore models that is caused by electrical instability and changes in energy balance induced by a pulsed electric field [12]. Electric field (EF) treatments applied in food industries vary in the current electricity, including static/direct current (DC), alternating current (AC), and pulsed DC/AC. These EF technologies were developed for the inactivation of enzymes and microorganisms, as well as to assist other food processing such as drying, thawing, extraction, etc. [12,13]. Nevertheless, ohmic heating is a process that still uses a thermal process produced by alternating electric current (AC). The heat generated by AC flows into the food components that are part of the electrical circuit. As a result, the heat generated from this process causes uniform heating and minimal deterioration of food quality [14].

In this case, the raw seagrape product is preserved under postharvest storage by initial exposure to the ACEF pretreatment [1] because this form is high-demanded in the market for consumption [4,15,16]. The eventual application aimed to enrich the advancement of macroalgae-based biomass for food and non-food industry applications [17]. To summarize the above phenoms, the ACEF pretreatment and SLI treatment are vital for seagrape PQ and shelf-life preservation. Nevertheless, different treatments of ACEF intensity, ACEF treatment time, and SLI levels have shown different impacts related to their effectiveness

during application [6,8,9]. An effective experimental design is required to establish the best conditions to preserve the seagrape during the postharvest storage handling stage.

Math modeling, such as an orthogonal array design (OAD), is effective for investigating relationships between different factors, along with searching for the best operating conditions due to its ability to test as many factor combinations in food processing technology [18–20]. For process sustainability, OAD reduces the number of options that must be considered and provides a decision tool for selecting a process design [21,22]. The experimental design purpose of an OAD is to seek the optimal configuration of numerous factors aiming for cost reduction, quality improvement, and efficiency processes [23,24]. However, the application of OAD in optimizing the electric field treatment in agricultural product preservation is still rarely found, particularly on algae products. In addition, the OAD approach can address the resource and environmental challenges resulting from the loss of capital for industrial applications due to inappropriate processes [21,25], in particular, for the simultaneous power optimization and efficiency of energy consumption [19]. Using the proper process is expected to achieve sustainable development goals as well as environmental sustainability [22].

This study aims to optimize the ACEF technology under different intensities and treatment times, assisted with different levels of SLI to preserve the seagrape by using OAD L9 (3^3) [26]. It is designed to evaluate the optimization factors (ACEF intensity, ACEF treatment time, and SLI level) affecting the performance principles. It also evaluates which are more influential than others [27] in preserving the raw seagrape product under postharvest storage. As preliminary reported, ACEF treatment at 125 kV/m for 60 min can inhibit the PQ deterioration of seagrape [1]. However, optimization is needed to obtain a more efficient and economical process [28]. Currently, there is a great emphasis on increasing seagrape quality using green technologies, such as ACEF, an interesting study. Therefore, green processing interventions are used in this study to provide cutting-edge knowledge and opportunities for sustainable development.

2. Materials and Methods

2.1. Materials

Freshly harvested seagrape (*C. lentillifera*) was acquired from a seagrape farmer in winter (Heshu Food Company Ltd., Pingtung, Taiwan). The samples were stored in a plastic bag in seawater and packaged inside a polystyrene box. Furthermore, the seagrape will be shipped to the Bioproduct Development Laboratory at National Chung Hsing University (Taichung City, Taiwan) for 24 h (27 ± 2 °C).

2.2. Seagrape Packaging

After arriving, the seagrape was smoothly parched with hygiene absorbent sheets. The parched sample (5 g) was packaged into the modified transparent polystyrene container (Figure 1). Within the container (volume 5 × 5 × 5 cm^3, thickness 0.1 cm) composing a wet absorbent sheet from 100% virgin wood pulp (Cheng Loong Corp., Taipei City, Taiwan) poured with 5 mL seawater and a dry holed polymer pad. The wet absorbent layer aimed to maintain the containers' relative humidity (±RH 90%), and the dry holed layer aimed to keep the sample dry [4]. Furthermore, the container lid was wrapped with duct tape to prevent free air exchange during postharvest storage [29].

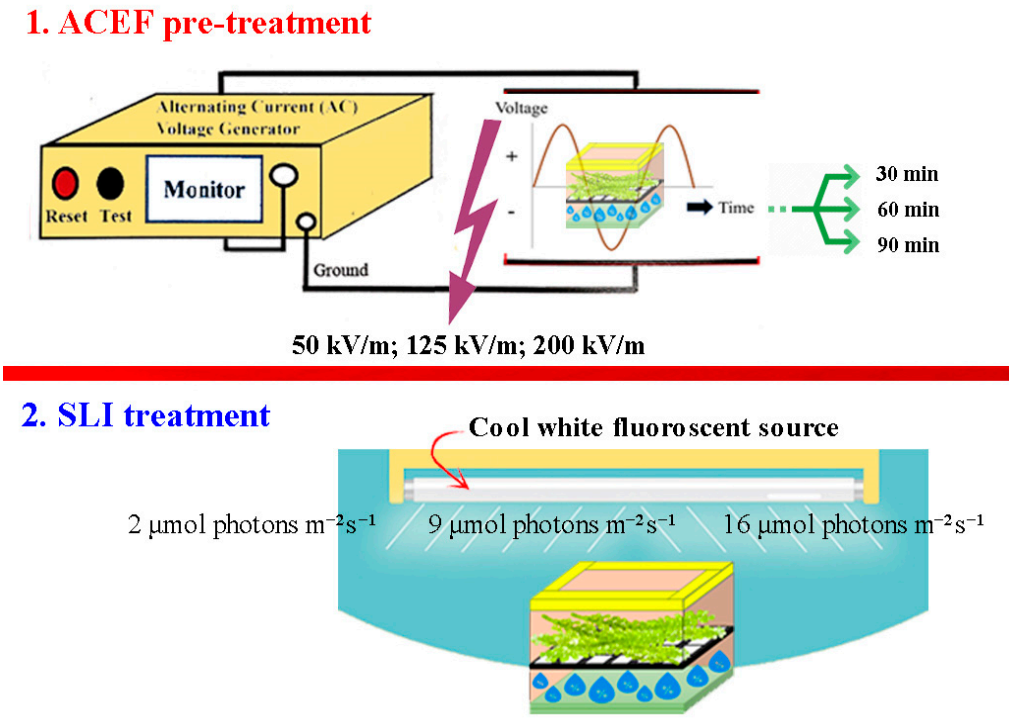

Figure 1. Instrumental devices and pretreatment method of ACEF by using a two-tier parallel board assisted by SLI treatment.

2.3. Taguchi Orthogonal Array Design (OAD)

A standard of OAD L9 (3^3) was performed to investigate the optimum condition of ACEF and SLI treatments in preserving the fresh seagrape [24]. The postharvest storage experiments evaluated 3 factors, each having 3 levels of value as follows; ACEF intensity (50, 125, 200 kV/m), ACEF treatment time (30, 60, 90 min), and SLI level (2, 9, 16 μmol photons m^{-2} s^{-1}). The range of each factor level was based on the results of single-factor tests, which were analyzed to have a significant impact on maintaining the seagrape PQ after 24 h of postharvest storage.

2.4. Postharvest Handling

The postharvest storage treatments on seagrape were conducted in two integrated ways, as seen in Figure 1. The protocol of the ACEF experiment was modified based on Hsieh et al. [8]. The ACEF equipment is configured to achieve an electric current into the two-tier parallel boards within a 6 cm distance. Those shelf-spacing and applied output voltages generate different intensities (50, 125, and 200 kV/m, respectively). The applied ACEF treatment time was 30, 60, and 90 min. The ACEF device (Model 7470, Extech Electronics Co., Ltd., New Taipei, Taiwan) is composed of an EF generator with a maximum output voltage of 20 kV with a frequency of 50 Hz. After being treated with the ACEF, the samples were stored at 25 ± 0.8 °C at different SLI levels (2, 9, 16 μmol photons m^{-2} s^{-1}, respectively) [5,29]. The SLI level was determined by a light meter (Lutron LM-81LX, Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan). The SLI was supplied with a light source of cool white fluorescence (CFL 20D/18, Taipei City, Taiwan), and the irradiance level was achieved through the distance between the sample to the light source [30]. The samples were stored for 24 h to study the effect of ACEF and SLI treatments on chlorophyll content, electrolyte leakage, color change, and firmness of seagrape during postharvest storage.

2.5. Chlorophyll and Carotenoid Content Determination

Chlorophyll content was determined based on Guo et al. [5] with a slight modification. About 0.2 g sample was ground in 3 mL of 80% acetone [PubChem CID: 180] (Choneye,

Taiwan) in the mortar until the sample turned colorless. The supernatant was transferred to an Eppendorf tube (2 mL) and set in the darkness for 15 min. The tubes were centrifuged at 4000× *g* for 15 min (Hettich Zentrifugen Mikro-120, Tuttlingen, Germany). Furthermore, the supernatant (1 mL) was relocated to a flask (10 mL), and 80% of acetone [PubChem CID: 180] was added (9 mL). Eventually, the homogenate sample absorbance was measured at 480, 630, 645, 647, 663, 664, and 665 nm with an ultraviolet-visible (UV-Vis) light spectrophotometer (CT-8600, E-Chrom Tech, Taipei City, Taiwan). The concentration of seagrape chlorophyll pigment was adjusted using the following Equations (1)–(4):

$$\text{Chlorophyll } a \left(\text{mg g}^{-1}\right) = 11.6A_{665} - 1.31A_{645} - 0.14A_{630} \quad (1)$$

$$\text{Chlorophyll } b \left(\text{mg g}^{-1}\right) = 20.7A_{645} - 4.34A_{665} - 4.42A_{630} \quad (2)$$

$$\text{Carotenoid } \left(\text{mg g}^{-1}\right) = 4A_{480} \quad (3)$$

$$\text{Total Chlorophyll } \left(\text{mg g}^{-1}\right) = 8.02A_{663} + 20.21A_{645} \quad (4)$$

2.6. Electrolyte Leakage Determination

The damage to seagrape cellular membranes was measured according to Paull and Chen [29] with a slight modification. Samples (2 g) were cleaned with distilled water and put in a tube with 20 mL of deionized water with constant shaking with an orbital shaker at 25 °C (Lab. Rotator, Digisystem Laboratory Instrument Inc., New Taipei City, Taiwan). Since the seagrape is sensitive to the un-brined solution, the conductivity (C1) was immediately measured by a radiometer (CDM-83 Meter, Copenhagen NV, Denmark). Then, the total conductivity (C2) was measured after boiling the tubes in a water bath for 1 h to release all electrolytes. The percentage of electrolyte leakage was represented based on Equation (5).

$$\text{Electrolyte Leakage (\%)} = \text{C1/C2} \times 100 \quad (5)$$

2.7. Color Change Determination

The color change of the samples was observed with a colorimeter (TC-8600A, Tokyo Denshoku Co., Ltd., Tokyo, Japan). Three measurements were taken at random points on the preserved thallus and matched with the seagrape initial color values (L*, a*, b*) at day 0. The difference in the color index was calculated by using the following Equation (6) [31]:

$$\text{Color difference } (\Delta E) = [(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2]^{1/2} \quad (6)$$

where L_2, a_2, b_2 are the color values of seagrape at the specific storage time (24 h) of postharvest storage, and L_1, a_1, b_1 are the color values of seagrape at day 0.

2.8. Firmness Determination

The physical indicator replicated the protocol from Liu et al. [32], in which the selected uniform-size fresh thallus of each seagrape was sliced into 3 cm. The firmness (N) containing force per square area was recorded using a texture analyzer (Sun Rheo Meter Model Compac-100II, Sun Scientific Co., Ltd., Tokyo, Japan) with a No.14 flat probe, a head speed of 3 mm s^{-1}, and a loading capacity of 30%.

2.9. Statistical Analysis

The data obtained from each measurement was indicated as means ± SD. The standard deviation (SD) was asserted as the error bar. Data processing and the OAD results were statistically analyzed with Minitab 18 software (Minitab, LLC., State College, PA, USA) [19]. All experimental samples were performed in triplicate, and the significant differences were determined at $p < 0.05$.

3. Results and Discussions

3.1. Effect of ACEF Intensity on Postharvest Storage of Seagrape

The effect of ACEF intensity to preserve the seagrape stored at 25 °C has been demonstrated and analyzed with the OAD method in this study. As a result, different applied intensities (50, 125, 200 kV/m) showed significantly different values on physicochemical properties of seagrape (Tables 1 and 2), such as chlorophyll content ($p < 0.01$), electrolyte leakage ($p < 0.05$), color change ($p < 0.05$), and firmness ($p < 0.01$) as can be seen in Table 3. Based on the results in Tables 1 and 2, the higher the applied intensity in treating the seagrape, the lower its physicochemical properties. This study shows that the optimum ACEF intensity to sustain the seagrape PQ is 50 kV/m, followed by 125 and 200 kV/m. High-voltage EF with an AC-source voltage generator had been promoted to preserve the moisture quality of kelp biomass, which shows higher intensity (55 kV/m up to 611 kV/m) generates higher water loss during storage at 15 °C [33]. Fallah-Joshaqani et al. [34] also reported that frozen mushrooms showed better PQ with a lower EF intensity (320, 640, 960 kV/m, respectively) when stored at 4 °C.

Table 1. Orthogonal array design and analysis on phytochemical properties of seagrape.

Sample Runs	Factors			Results of Chlorophyll Content (mg g^{-1})			
	A	B	C	Chlorophyll *a*	Chlorophyll *b*	Carotenoid	Total Chlorophyll
1	1	1	1	1.04 ± 0.03	0.69 ± 0.01	0.31 ± 0.02	1.93 ± 0.07
2	1	2	2	1.13 ± 0.02	0.68 ± 0.01	0.33 ± 0.01	2.06 ± 0.08
3	1	3	3	1.00 ± 0.04	0.68 ± 0.02	0.32 ± 0.01	1.92 ± 0.04
4	2	1	3	1.08 ± 0.05	0.69 ± 0.03	0.33 ± 0.01	2.01 ± 0.05
5	2	2	1	1.05 ± 0.03	0.63 ± 0.01	0.30 ± 0.02	1.94 ± 0.06
6	2	3	2	1.04 ± 0.04	0.62 ± 0.03	0.30 ± 0.01	1.92 ± 0.05
7	3	1	2	0.90 ± 0.07	0.58 ± 0.04	0.27 ± 0.01	1.71 ± 0.02
8	3	2	3	0.91 ± 0.06	0.58 ± 0.01	0.27 ± 0.02	1.72 ± 0.03
9	3	3	1	0.93 ± 0.05	0.57 ± 0.02	0.28 ± 0.02	1.77 ± 0.05
k1	1.971	1.884	1.857				
k2	1.957	1.907	1.945				
k3	1.731	1.868	1.858				
R (delta)	0.240	0.039	0.088				
Rank	1	3	2				

A: ACEF intensity (kV/m); B: ACEF treatment time (min); C: SLI level (μmol photons m^{-2} s^{-1}). The data express means ± SD ($n = 3$). k1, k2, and k3 are means of the total chlorophyll content derived from their respective factor level 1, 2, and 3. R (delta) was a subtraction of the highest and lowest k1, k2, and k3 in each factor, indicating the factors' contribution to the treatment outcome.

Arshad et al. [35] explained that high intensity generates long-lasting damage to the cell membranes, which breaks the cell turgor that affects the mass transfer, viscosity, elasticity, and humidity of the plant tissues. In correspondence, the higher EF intensity (100, 250, 400 V/cm) caused less quantity of water present in the kiwifruit liquid phase to alter its structural quality during postharvest storage at 25 °C [36]. Then it could be assumed that the higher EF intensity might damage the cell membrane and increase the pore circulation in the seagrape biomass that contains excessive tissue made of water, driving the water content loss and resulting in higher physicochemical deterioration during postharvest storage. According to the preliminary studies on packed seagrape for commercial purposes, water loss of seagrape corresponds to the physicochemical deterioration of seagrape during the storage period [1,4].

This study shows more advantages in determining the effect of ACEF intensity in altering the seagrape quality compared to the previous EF studies. As reported, the OAD can reveal other factors that may affect the role of this intensity in interfering with the physicochemical properties of seagrape [27]. For instance, similar intensity (50 kV/m) assisted with different factors showed a significant difference in the electrolyte leakage (7.44%, 5.25%, 7.05%, respectively) on seagrape after 24 h of storage that can be seen in

Table 2. This study concludes that the OAD experiment is an effective method due to its ability to rank the domination of each optimization factor based on their roles during postharvest storage. As a result, the optimization factor of ACEF intensity is ranked first in influencing the overall process in this study. Ghosh et al. [19] have demonstrated an optimization of EF in saving energy during the drying process, which shows that EF intensity plays a critical role in the overall process. EF preservation is a method that uses two neighboring electrodes (cathode and anode) with any configuration equipment surfaces to interfere with the treated samples [37]. The main factor that influences the effectiveness of this treatment is the selection of the applied intensity, which should match with morphology and tissue type of the samples [35].

Table 2. Orthogonal array design and analysis on physicochemical properties of seagrape.

Sample Runs	Factors			Results of Physicochemical Properties		
	A	B	C	Electrolyte Leakage (%)	Color Change (ΔE)	Firmness (N)
1	1	1	1	7.44 ± 0.23	5.67 ± 0.42	0.0397 ± 0.003
2	1	2	2	5.25 ± 0.34	1.78 ± 0.22	0.0519 ± 0.002
3	1	3	3	7.05 ± 0.47	5.70 ± 0.44	0.0386 ± 0.003
4	2	1	3	7.30 ± 0.41	3.49 ± 0.53	0.0448 ± 0.003
5	2	2	1	7.56 ± 0.52	5.08 ± 0.67	0.0447 ± 0.005
6	2	3	2	7.95 ± 0.42	6.65 ± 0.48	0.0316 ± 0.002
7	3	1	2	9.84 ± 0.47	8.56 ± 0.59	0.0276 ± 0.003
8	3	2	3	9.28 ± 0.59	9.81 ± 0.34	0.0256 ± 0.004
9	3	3	1	8.06 ± 0.45	7.72 ± 0.42	0.0286 ± 0.004
k1	6.582	8.195	8.225			
k2	7.604	7.364	6.871			
k3	9.061	7.688	8.151	Electrolyte Leakage (%)		
R (delta)	2.479	0.831	1.354			
Rank	1	3	2			
k1	4.384	5.906	7.374			
k2	5.073	5.556	4.330			
k3	8.695	6.691	6.448	Color Change (ΔE)		
R (delta)	4.312	1.135	3.044			
Rank	1	3	2			
k1	0.043	0.037	0.032			
k2	0.040	0.041	0.042			
k3	0.027	0.033	0.037	Firmness (N)		
R (delta)	0.016	0.008	0.009			
Rank	1	3	2			

A: ACEF Intensity (kV/m); B: ACEF treatment time (min); C: SLI level (μmol photons m^{-2} s^{-1}). The data express means ± SD (n = 3). k1, k2, and k3 are means of the total chlorophyll content derived from their respective factor level 1, 2, and 3. R (delta) was a subtraction of the highest and lowest k1, k2, and k3 in each factor, indicating the factors' contribution to the treatment outcome.

3.2. Effect of ACEF Treatment Time on Postharvest Storage of Seagrape

This study shows various results in the physicochemical properties of seagrape in different treatment times (Tables 1 and 2). Yet, among the observed parameters in this study, ACEF treatment time only showed a significant effect on the seagrape firmness ($p < 0.05$) during postharvest storage (Table 3). This is confirmed by the OAD result that shows the optimization factor of ACEF treatment time is ranked third in affecting the overall process (Tables 1 and 2). Liu et al. [32] reported that persimmons have a better PQ as the longer applied treatment time (60, 90, and 120 min, respectively) of the high voltage electrostatic field with 600 kV/m of intensity. Somehow, this study shows that the firmness of seagrape treated at different times was found to be fluctuating. For instance, the seagrape was treated with EF intensity of 50 kV/m for 30, 60, and 90 min, resulting in firmness of 0.0397, 0.0519, and 0.0387 N, respectively. This phenomenon might be due to the rise time becoming critical for charging and possible electroporation of the cell surface membrane because it is related

to the intracellular membrane charging time and the limit of transmembrane voltage [28,38]. In this case, the seagrape tissue may still endure and reach its peak to interfere with its physicochemical properties from 30 to 60 min of treatment, but it becomes not effective anymore after reaching 90 min of treatment. Wang et al. [9] also explained that the molecular weight of the sample is affected by the EF treatment time, which starts with an increment and is then followed by a decrement at a point due to water electrolysis.

Table 3. Analysis of variance of optimization factors on physicochemical properties of seagrape.

Quality	Factor	DF	Adj SS	Adj MS	F-Value	*p*-Value
Total Chlorophyll Content	A	2	0.108666	0.054333	147.97	0.007
	B	2	0.002348	0.001174	3.2	0.238
	C	2	0.015229	0.007614	20.74	0.046
	Error	2	0.000734	0.000367		
Total	Total	8	0.126977			
Electrolyte Leakage	A	2	9.3122	4.65609	123.28	0.008
	B	2	1.0533	0.52666	13.95	0.067
	C	2	3.4775	1.73874	46.04	0.021
	Error	2	0.0755	0.03777		
Total	Total	8	13.9185			
Color Change	A	2	32.1844	16.0922	44.31	0.022
	B	2	2.0254	1.0127	2.79	0.264
	C	2	14.6101	7.3051	20.11	0.047
	Error	2	0.7263	0.3632		
Total	Total	8	49.5463			
Firmness	A	2	0.000441	0.000221	165.27	0.006
	B	2	0.000092	0.000046	34.41	0.028
	C	2	0.000134	0.000067	50.37	0.019
	Error	2	0.000003	0.000001		
Total	Total	8	0.000670			

A: ACEF intensity (kV/m); B: ACEF treatment time (min); C: SLI level (μmol photons m^{-2} s^{-1}). The data express means ± SD (n = 3).

Sánchez-Vega et al. [39] also mentioned that the treatment time is related to the samples' steady-state, and the type of electric wave configuration has a significant impact in confronting the enzyme activity of the sample. Accordingly, this study indicates that the effect of ACEF treatment time may depend on the durability of the sample to the electric inducement and the equipment configuration during treatment. In this study, the ACEF technology utilizes high voltage generated by an alternating current (AC) electric generator with a two-tier parallel board configuration (Figure 1) to preserve the PQ of algae products. According to OAD, the result shows that the optimum ACEF treatment time is 60 min to conserve the seagrape PQ. The result in this study is also supported by the effect of different microwave treatment times (30, 40, 50 min) in optimizing the extraction process of seagrape by using OAD, which found that 40 min is the optimum condition to yield the greatest antioxidant activity of the seagrape [40]. Eventually, the OAD in this study seems to be a proper method to optimize the EF mechanism in treating the product due to its ability to define the factors' performance in the overall process.

3.3. Effect of Storage Irradiance Level on Postharvest Storage of Seagrape

In this study, the application of different SLI levels (2, 9, 16 μmol photons m^{-2} s^{-1}) derived from CFL lighting that may produce UV radiation yielded a significant difference ($p < 0.05$) in the total chlorophyll content, color change, electrolyte leakage, and firmness of the treated seagrape during postharvest storage (Table 3). As a result, the effect of SLI level is ranked second in affecting postharvest storage, as seen in Tables 1 and 2. It was previously reported that light could trigger the antioxidative value of *C. lentillifera* to

reach high values, similar to anti-oxidant-rich fruits [41]. The result corresponds to the study covered by Kim and Garbary [30], which showed a significant impact of SLI on the photosynthesis performance index [relative electron transport rate (rETR)] and Fv/Fm of green algae. Guo et al. [5] reported the differences in irradiance level cause the difference in photosynthesis performance, resulting in different chlorophyll contents by showing that lower irradiance contains higher chlorophyll. Sommer et al. [41] also stated that producing chemical compounds, including phenolics, in marine organisms is influenced by the environment's irradiance level. Moreover, studies have reported that lighting treatment during the postharvest period is vital for the PQ conservation of food products [6]. Thence, this study concludes that the SLI treatment during storage in this study may have a great influence on interfering with the phytochemical and antioxidant properties of the seagrape during postharvest storage. The findings in this study proposed a proper method to explain the effect of SLI on the postharvest stage of seagrape. Furthermore, the SLI treatment in this study was optimized by the OAD to provide an overview of how the irradiance level may affect the physicochemical properties of seagrape during postharvest storage.

In seagrape, chlorophyll is the abundant pigment that is vital for photosynthesis, which is divided into chlorophyll *a* and chlorophyll *b* [15,38]. Luo et al. [42] stated that chlorophyll *a* represents the content of chlorophyll, while chlorophyll *b* is responsible for the product's color. According to the previous study, photoinhibition in seagrape is led by oxidative stress during postharvest storage that leads to severe seagrape PQ deterioration [1]. Additionally, Paull and Chen [29] also mentioned that SLI had a big impact on influencing the quality of algae products during postharvest storage. In this study, the seagrape was treated with EF intensity of 200 kV/m and stored within the SLI level of 2, 9, and 16 µmol photons m^{-2} s^{-1}, resulting in the color change (ΔE) of 7.72, 8.56, and 9.81, respectively. However, there is fluctuating data in the seagrape PQ as the increment of the SLI level (Tables 1 and 2). This phenomenon might be due to the treatment methodology derived from the OAD, which combined several optimization factors during postharvest that will significantly influence the overall process [18]. As a result, the SLI level at 9 µmol photons m^{-2} s^{-1} is the optimum condition for preserving the seagrape PQ during postharvest storage. The result may correspond to the studies that revealed that UV irradiation could preserve the food PQ [3,41], demonstrated by the lighting treatment in this study. Nevertheless, strong UV radiation as the increment of SLI level during storage may induce more antioxidant defense that lower the seagrape PQ. This phenomenon is related to the light compensation point required for seagrape bioactivities [6].

3.4. Effect of Postharvest Storage Treatments on Physicochemical Properties of Seagrape

According to Sulaimana et al. [1], the ACEF treatment has a significant effect on maintaining the water content, MDA, chlorophyll content, and TPC of the treated seagrape. Furthermore, the shelf-life of seagrape was successfully prolonged after being treated with ACEF for 60 min with 125 kV/m of intensity and 9 µmol photons m^{-2} s^{-1} of SLI level [1]. Therefore, this study deeply examined to find the optimum postharvest condition (higher or lower treatment setting on the applied optimization factors) to extend the shelf-life of seagrape longer with better PQ by applying ACEF technology assisted with the SLI. After being confirmed by the experimental results, the OAD resulted in an optimal combination of process parameters with ACEF for 60 min with 50 kV/m of intensity and 9 µmol photons m^{-2} s^{-1} of SLI level according to the highest S/N ratio and the analysis of variance (ANOVA) [20].

3.4.1. Chlorophyll and Carotenoid Content

According to the OAD, the seagrape that contains the highest total chlorophyll content with 2.06 mg g^{-1} derived from treatment condition no. 2 (Table 1 and Figure 2a). Based on the result in Table 1, the chlorophyll content of the seagrape gradually degraded due to the increase in ACEF intensity. The previous study showed that ACEF treatment for 60 min with an intensity of 125 kV/m has a significant impact on maintaining the chlorophyll

content, which led to the high TPC of seagrape during storage [1]. In this study, the content of chlorophyll *a* and carotenoid of seagrape treated with the intensity of 50 and 125 kV/m are quite similar, but the chlorophyll *b* content is different by showing a higher content at 50 kV/m. However, the total chlorophyll content of seagrape has a significant difference between the applied intensity ($p < 0.01$) by showing higher content at 50 kV/m, followed respectively by 125 kV/m and 200 kV/m (Tables 1 and 3). In this case, the higher intensity may retard the seagrape antioxidant properties that lower the phytochemical properties of seagrape, particularly the chlorophyll and carotenoid compounds.

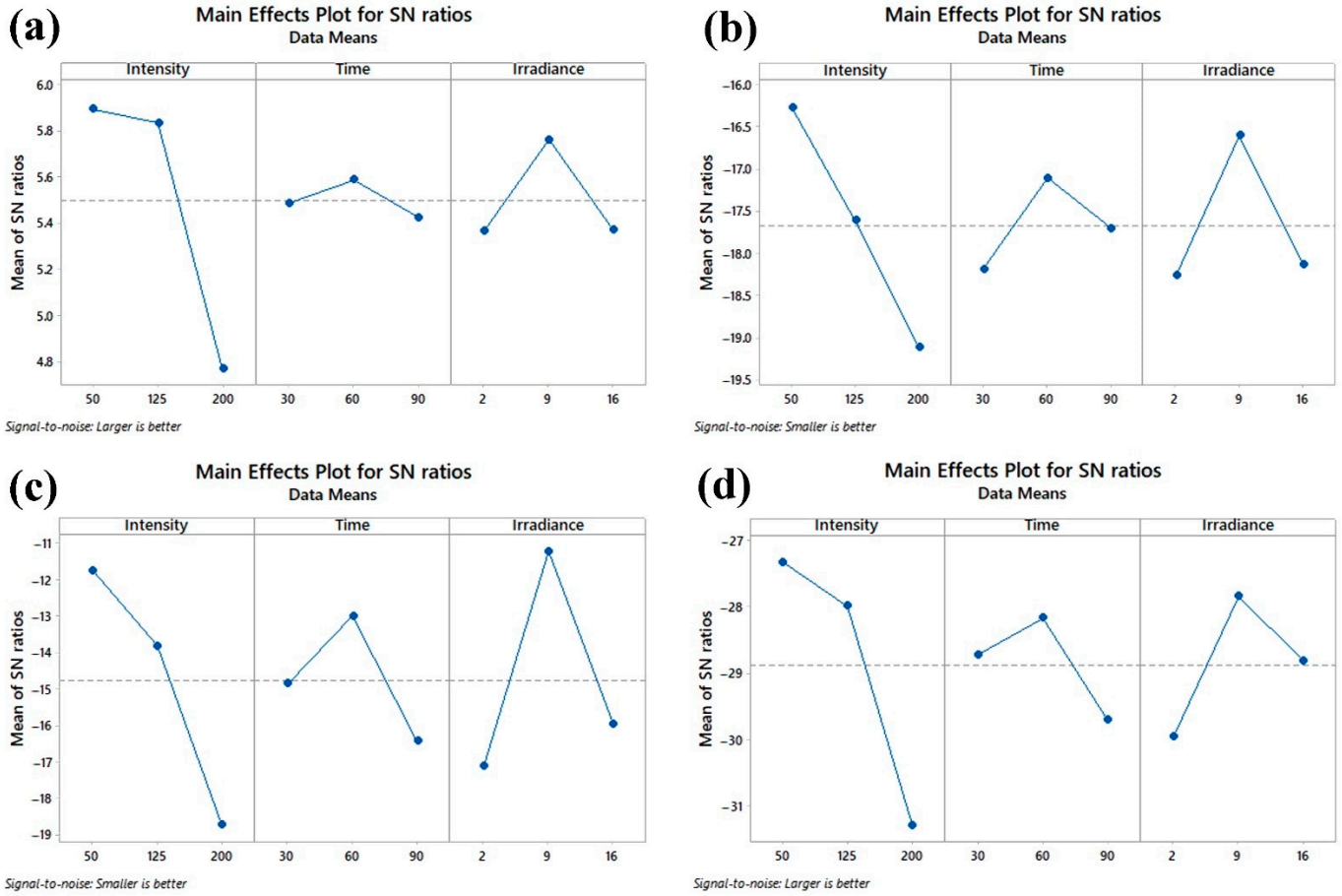

Figure 2. Analysis of Taguchis' OAD in the impact of 3 optimization factors [ACEF intensity (kV/m), ACEF treatment time (min), and SLI level (μmol photons m^{-2} s^{-1})] on seagrape PQ after 24 h of storage. (**a**) total chlorophyll content, (**b**) electrolyte leakage, (**c**) color change, and (**d**) firmness.

Due to the presence of Mg^{2+} in the porphyrin ring and numerous double bonds in their structure, chlorophyll and carotenoids present in seagrape may enable a migration toward the cathode and anode surfaces when subjected to EF conditions [43]. Furthermore, studies have reported that chlorophyll and carotenoid are prone to oxidation, and the activity of enzymes is essential in this role due to postharvest handling processes [39,42]. Yan et al. [44] explained the ability of EF to change the secondary and tertiary structures of mushrooms to reduce their enzyme activities. Based on the result in Table 3, the SLI has a notable effect in interfering with the chlorophyll content of seagrape ($p < 0.05$). The darker irradiance (2 μmol photons m^{-2} s^{-1}) and lighter irradiance (16 μmol photons m^{-2} s^{-1}) show lower chlorophyll content than the medium (9 μmol photons m^{-2} s^{-1}) SLI level. Therefore, this study concludes that medium light exposure is better for maintaining the chlorophyll fluorescence of seagrape during postharvest storage. This result is also confirmed by Stuthmann et al. [45], who reported that gradually elevated light irradiance exposure significantly reduces the chlorophyll quality, however, the seagrape still needs sufficient

exposure to light irradiance to increase its antioxidant quality. In this case, chlorophyll quality plays a vital role in determining the physiological condition of seagrape in response to different stressors during the postharvest period [46].

3.4.2. Electrolyte Leakage

Electrolyte leakage concentration is often linked with the damage degree that reflects the cell membrane permeability in agricultural products [47]. The higher the concentration, the more severe the damage to the cell membranes. In this case, the packaging of fresh seagrape suppresses the photosynthesis activity due to dehydration under limited water conditions that leads to oxidative stress condition [1]. Dehydration of the cellular generates the electrolyte concentration in the cell that changes the membrane structures, especially the thylakoids in green algae [30]. Hence it causes the decrease of rETR and Fv/Fm in seagrape, resulting in a significant photosynthesis depression [45,46]. Consequently, oxidative stress that induces damage to cell membranes in seagrape may increase the electrolyte leakage of seagrape and a loss of the cell's ability to repair themselves during this stressful condition [4].

In this study, seagrape increases its electrolyte leakage after 24 h of postharvest storage. Tables 2 and 3 show the electrolyte leakage concentration of seagrape varies in different ACEF intensities and SLI levels ($p < 0.05$). Nonetheless, seagrape experiences electrolyte leakage below 10% after 24 h of storage when subjected to ACEF and SLI treatments. According to the OAD, the lowest concentration of electrolyte leakage in seagrape was derived from treatment condition no. 2 (Table 2 and Figure 2b), with a concentration of 5.25%. The ability of seagrape to press its electrolyte leakage may be caused by its high desiccation-tolerance traits enhanced by ACEF treatment. The result in this study corresponds to the previous study that reported the ability of ACEF treatment to lower the electrolyte leakage of the sample, leading to a longer shelf-life during postharvest storage [8].

3.4.3. Color Change

Color change (discoloration) on seagrape is a phenomenon of the occurrence of chlorophyll degradation [42]. Several bio-degradations occurred in this phenomenon, such as the activity of enzymes in changing the green color of seagrape [3]. Seagrape is highly sensitive to temperature and osmotic pressure once experiencing the desiccation effect [4]. Since the desiccation effect triggers the enzymatic reactions to cause several decompositions, such as changing the color of the seagrape, the ACEF pretreatment and SLI treatment during storage suppress the degradation. Based on Table 3, the color difference (ΔE) of seagrape has a significant difference in different applied intensities and SLI levels ($p < 0.05$). According to the OAD, the lowest ΔE was derived from treatment condition no. 2 (Table 2 and Figure 2c), which shows a ΔE value of 1.78. Referring to the standard observer seeing the color difference [31], the value of $1 < \Delta E < 2$ is perceptible through close observation that indicates ACEF pressed the desiccation effect to have a small impact in changing the color of seagrape during postharvest storage. Compared to other conditions in Table 2, the value of $2 < \Delta E < 10$ is indicated as perceptible at a glance, $11 < \Delta E < 49$ is indicated as colors are more similar than the opposite, and $50 < \Delta E$ is indicated as colors are the exact opposite, revealing the desiccation effect has an intermediate to massive impact on the color of seagrape.

The effect of the bio-degradation of chlorophyll due to the enzymatic activities will produce various colors like bright green, swamp green, dark green, olive green, or even colorless [42]. The previous study also reported the seagrape thalli experienced severe discoloration during the lengthened period of postharvest storage [1]. In this study, the ACEF technology assisted with sufficient SLI treatment was found to prevent the color change of seagrape during postharvest storage. Recent studies related to SLI effects on seagrape have revealed that high light irradiance (light-stress) damage the seagrape photosynthesis performance, resulting in the thalli bleaching (discoloration) [45,46]. In correspondence

to the previous studies, ACEF and UV irradiation derived from lighting treatment can lower pigment degradation to delay discoloration during postharvest storage [1,3,6,29]. Therefore, this study concludes that the seagrape color tends to be better in dim (not dark and not bright) light exposure conditions during postharvest storage.

3.4.4. Firmness

According to the OAD, the greatest firmness was derived from treatment condition no. 2 (Table 2 and Figure 2d) by showing 0.0591 N of firmness after 24 h of postharvest storage. The firmness of agricultural products is often used to represent the integrity of their cell walls, which is closely related to their water molecule activities. Traffano-Schiffo et al. [36] stated that EF treatment over plant tissue allows electrical energy to be stored and converted into free energy to entice and maintain water molecules on their membrane and cell wall surfaces. Yan et al. [44] reported the EF treatment maintained the integrity that leads to the delayed softening and better textural characteristics of mushrooms during storage. The amount of lipid peroxidation (MDA production) as a marker of oxidative stress (ROS) can accumulate in cell membranes and is considered a measure of its damage [47]. According to Sulaimana et al. [1], a limped thalli on seagrape is caused by the increase in the amount of MDA and water loss during oxidative stress conditions. The prolonged oxidative stress causes damage to cell structures that leads to the loss of its integrity [4,47]. In this study, the ACEF treatment may effectively suppress the oxidative stress condition and maintain the seagrape texture, as indicated by a more compact seagrape thalli.

Studies mentioned the saturated air layer inside the packaging may interfere with the diffusion of water molecules from the water surface to the bulk of airflow [37]. At this stage, applying ACEF with a two-tier parallel board may overcome this boundary layer and reduce water loss. Thus, the ACEF technology was found to be a promising treatment in improving the texture of seagrape during storage by showing a significant difference in firmness occurred in seagrape due to the different intensities ($p < 0.01$) and time ($p < 0.05$) that can be seen in Table 3. The results in this study correspond to the earlier studies that stated that EF is a treatment for physical and structural modification [44]. Nonetheless, different SLI levels also show a significant effect in interfering with seagrape firmness, proving the previous study findings on SLI advancement for food preservation during the postharvest period.

3.5. Determination of Optimum Performance Characteristics

Based on the analysis in this study, the postharvest storage of seagrape is significantly optimized through OAD. The result shows that the optimum level attained in Tables 1 and 2 was ACEF intensity at level 1, ACEF treatment time at level 2, and SLI at level 2. The optimum condition derived from the analysis of OAD shows that the response characteristic for observed parameters corresponds to the confirmed experiment (Figure 2). Furthermore, the OAD also predicted the optimum value of the estimated response characteristic from the selected level of the major process through the following Equation (7) according to D'Souza et al. [26]:

$$\text{R (Predicted)} = \text{A (OP S/N ratio)} + \text{B (OP S/N ratio)} + \text{C (OP S/N ratio)} - 2\text{T} \quad (7)$$

where R is the response characteristic of the observed parameter, A is Factor A, B is Factor B, C is Factor C, OP S/N ratio is the mean at the optimum level of the observed parameter, and T is the overall mean of the observed parameter [26].

The OAD also shows the contribution percentage (C%) of each optimization factor in each observed parameter during postharvest storage, as can be seen in Figure 3 through the following Equation (8) according to Nor Shafizah et al. [27]:

$$\text{Contribution percentage} = \text{SS i/SS total} \times 100 \quad (8)$$

where SS i is the sum of the square of each important factor, and SS total is the total sum of squares derived from the ANOVA table in each observed parameter [27]. The results show that the applied ACEF intensity and SLI level has a crucial role in preserving the seagrape PQ during postharvest storage by reaching 65–86% and 12–30% of the contribution, respectively. The ACEF treatment time also seemed to have a significant effect in preventing the electrolyte leakage and firmness loss of the seagrape by reaching 2–14% of the contribution. Furthermore, the results obtained by the confirmed experiments according to OAD were found to achieve 2.0629 mg g^{-1} of total chlorophyll content, 5.25% of electrolyte leakage, 1.78 ΔE of color change, and 0.0519 N of firmness. These values were inside the confidence interval of the predicted optimum value of total chlorophyll content (2.0572 mg g^{-1}), electrolyte leakage (5.21%), color change (2.17 ΔE), and firmness (0.0519 N) that have been analyzed with Taguchi OAD.

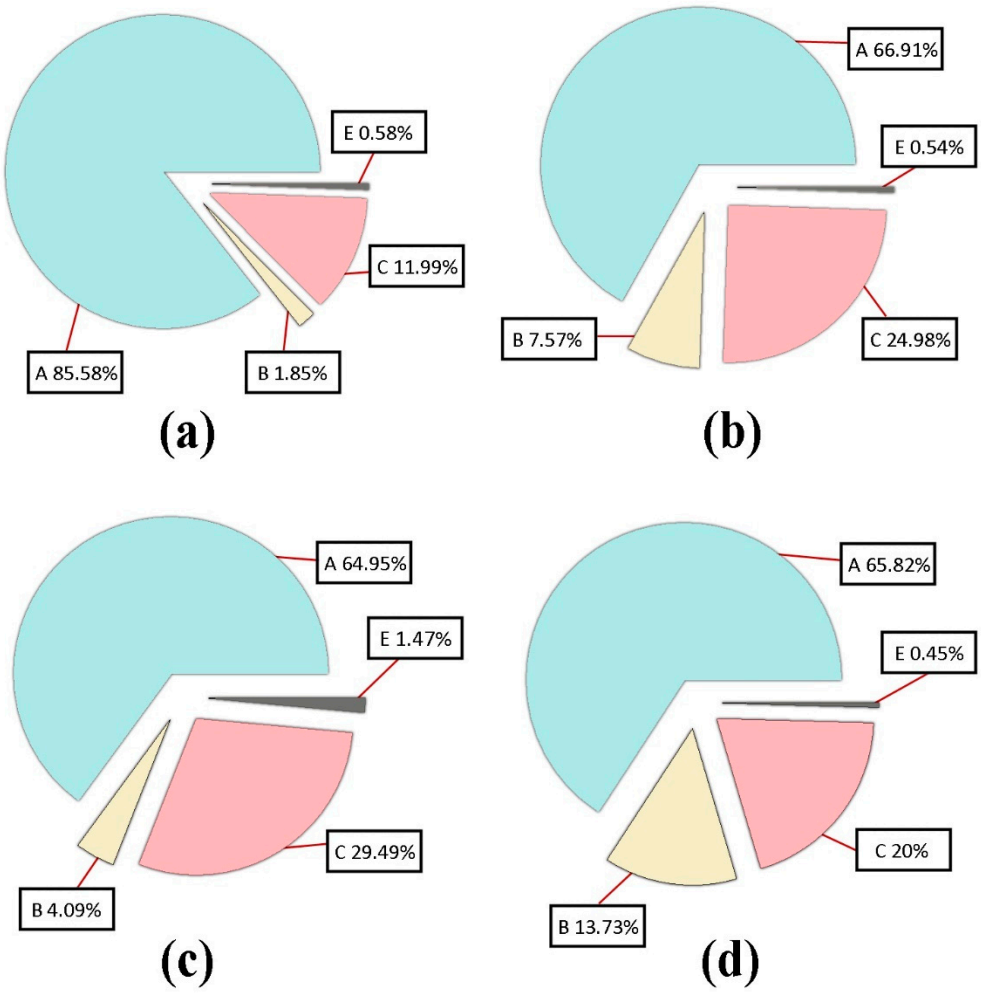

Figure 3. Contribution percentage of 3 optimization factors (A: ACEF intensity, B: ACEF treatment time, C: SLI level, and E: Error) on physicochemical properties of seagrape after 24 h of storage. (**a**) total chlorophyll content, (**b**) electrolyte leakage, (**c**) color change, and (**d**) firmness.

4. Conclusions

According to the above observation, ACEF treatment in a sufficient SLI is a new promising method for preserving seagrape freshness. After Taguchi's OAD analysis, it showed that the most influential factor on PQ of seagrape is ACEF intensity, followed by SLI and ACEF time. The optimum conditions are as follows: 50 kV/m of ACEF intensity, 60 min of ACEF time, and 9 µmol photons m^{-2} s^{-1} of SLI level and lower voltage usage by about 60% compared to the previous study. Therefore, this study endorsed the efficiency of

SLI-ACEF technology for developing the sustainability of seagrape-based functional foods and industrialization.

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