

# Technical Factors Affecting Seagrape (*Caulerpa lentillifera*) Production By Cultivation And Its Stability By Post-Harvest Treatment

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## Abstract.

*Caulerpa lentillifera* (green seagrape) is a grass-green in color, with a soft and succulent texture characterized by thallus consisting of long horizontal stolons with a few rhizoidal branches below, and many erect grapelike branches above. Seagrape is well-known for its rapid spoilage and short shelf life and the quality of seagrapes is often determined by its size with turgor pressure and bright green color. This means that picking methods and post harvest handling and storage conditions may also contribute to the shelf-life. Seagrape is one of species considered as key factor in aquaculture in Central coastal region of Vietnam. As a new cultivating species, there was not much studies mentioned to technical factors affecting to its biomass during cultivation. Moreover, owing to its short shelf life, it's necessary to investigate an appropriate strategy to maintain its quality during storage. Therefore this study focused on parameters influencing to the cultivation (including the effect of salinity and nutrient level) and post-harvest (including the effect of washing, blanching and preserving of seagrape under different conditions). Results revealed that 3.50% of salinity, 0.5 mmol/L of NO<sub>3</sub>-N, 0.1 mmol/L of PO<sub>4</sub>-P were suitable for seagrape growing to get high yield; washing seagrape by 50 ppm Perasan would be appropriated; blanching should be performed at 95°C in 20 seconds; 10% of brine solution was adequate in preservation of seagrape.

**Keywords:** Seagrape, cultivation, salinity, nutrient, post-harvest, washing, blanching, preserving

## I. INTRODUCTION

Productivity of *C. lentillifera* in a 6-week period yielded, on average 2 kg/week. *C. lentillifera* seaweed can be potentially cultured due to its role as biofilter in maintaining water quality in aquaculture activities (Chaitanawisuti et al., 2011; Liu et al., 2016). *C. lentillifera* can survive in salinities ranging from 20 to 50, and can develop at salinities of 30 to 40. Optimal conditions for vegetative reproduction of *C. lentillifera* occurred at a salinity of 35, with a concentration of 0.1 mmol/L of PO<sub>4</sub>-P and 0.5 mmol/L of NO<sub>3</sub>-N (Guo Hui et al., 2015). Meanwhile, Wang (2011) showed that maximum growth of *C. lentillifera* occurred at a salinity of 36.

*C. lentillifera* digestibility showed a good value as a raw material feed tilapia (Nadisa Theresia Putri et al, 2017). Murugaiyan et al. (2012) stated that green algae contain high protein compared to both red algae and brown algae. *C. lentillifera* has a high mineral content as other kind of seaweeds (Kut Guroy, 2007; Matanjan et al., 2009; Natify et al., 2015). According to Saito et al. (2010), it has relatively high polyunsaturated fatty acids including omega 3 fatty acids. It has also high mineral content such as zinc and iron and trace elements including cobalt, selenium and valium that meet daily body requirements (PeñaRodriguez et al., 2011). *C. lentillifera* could be used as a potential antidiabetic agent (Bhesh Raj Sharma, Dong Young Rhyu, 2014). *C. lentillifera* extract produced antibacterial activities against *S. aureus* and *S. mutans* (Faazah Sabirin et al., 2015). *C. lentillifera* extract may have a potential

anticoagulant property due to its component sulfated polysaccharides (Althea R. Arenaj et al., 2017).

Maria Danesa S. Rabia (2015) evaluated the effects of two cultivation methods namely sowing and tray on the growth and biomass production of *C. lentillifera* cultured in brackishwater pond. For the tray method, propagules were clipped in two 0.75 m x 0.75 m tray and were hung in bamboo frame whereas for the sowing method, propagules were planted directly in the pond substrate with an interval of one meter. The weight gain using the sowing method was significantly higher and could be translated to an average of 1 kg every month of new or harvestable biomass. Specific growth rate of *C. lentillifera* grown in the substrate was at 3.85% day<sup>-1</sup> during the first month and at 2.92% day<sup>-1</sup> during the second month and was significantly higher compared to that of stocks grown in trays. High organic load of the soil (substrate) could have improved growth and biomass productivity. The results show that cultivation of *C. lentillifera* using the sowing method is more effective. This system has significantly contributed to increase in biomass yield. Moreover, this method of farming entails lesser capital outlay without any other material requirements such as bamboos and trays. Seagrape (*Caulerpa lentillifera*) is often prepared by marinating in lemon juice, adding grated coconut juice (*lolo*), finely chopped chili and canned fish or fermented coconut. Seagrapes is well-known for its rapid spoilage and short shelf life and the quality of seagrapes is often determined by its size with turgor pressure and bright green color. This means that picking methods, post harvest

handling and storage conditions may also contribute to the shelf-life. Vu Ngoc Boi et al. (2017) examined quality changes of dried seagrape in storage for 12 months. The measurements targeted at the content of chlorophyll, polyphenol, caulerpin and humidity, the index of peroxide, sensory quality and bacteria in the storage time. The results showed that, after 12 months of cold temperature storage, dried seagrape quality still met the trade standards. After 12 months of storage at cold temperatures, dried seagrape contents such as polyphenol, chlorophyll, and caulerpin declined by 23% maximum), sensory quality decreased by 7.8% minimum, humidity content and peroxide index increased by 27.71% and 11.03% respectively, and bacteria content increased 15.5% maximum.

Seagrape is one of species considered as key factor in aquaculture in Central coastal region of Vietnam. As a new cultivating species, there was not much studies mentioned to technical factors affecting to its biomass during cultivation. Moreover, owing to its short shelf life, it's necessary to investigate an appropriate strategy to maintain its quality during storage. Therefore this study focused on parameters influencing to the cultivation (including the effect of salinity and nutrient level) and post-harvest (including the effect of washing, blanching and preserving of seagrape under different conditions).

## II. MATERIALS AND METHOD

### 2.1 Material

We collected seagrape in Coastal central region, Vietnam. They must be cultivated following VietGAP to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments. They was sorted, washed and cleaned in either tap water or 5% brine solution depending on the type of treatment that would be tested. The washing was done using transparent glass bowls to ensure that all debris and sand were completely removed. A second weight was taken after thorough cleaning and removal of excess water. This was done to estimate the percentage recovery of the current handling practices and to assess good quality seagrapes suitable for further processing and preservation. Besides seagrape we also used other materials during the research such as Perasan, NaCl. Lab utensils and equipments included digital weight balance, micrometer, stomacher, vortex, incubator, colony counter, micro-pipette.



Figure 1. Seagrape (*Caulerpa lentillifera*)

### 2.2 Researching procedure

#### 2.2.1 Survey of salinity and nutrient level for seagrape cultivation

A survey of salinity and nutrient level in brine for seagrape cultivation was verified. Salinity of brine for seagrape

cultivation was tested in different positions at different times. Brine for seagrape cultivation was also tested for NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>-</sup> in different places in Central coastal region of Vietnam.

From this survey, the difference between this study and by Wang (2011), Guo Hui et al., (2015) would be discussed.

#### 2.2.2 Post-harvest investigation

##### 2.2.2.1 Effect of washing method

*Treatment 1:* Seagrapes were washed and rinsed in different method (5% brine solution, tap water, 50 ppm Perasan), blanched at 100°C for 15 seconds in 5% brine and stored in sterilized plastic containers containing 5% brine solutions, and refrigerated at 4°C until the weight loss, size and shrinkage, sensory evaluation and microbial test period. These samples were not heat treated but freshly preserved in brine solutions.

##### 2.2.2.2 Effect of blanching temperature and time

*Treatment 2:* Seagrapes were washed and rinsed in the appropriate washing method (as above), blanched by different temperature and time (100°C in 15 seconds; 95°C in 20 seconds and 90°C in 25 seconds) in 5% brine and stored in sterilized plastic containers containing 5% brine solutions and refrigerated at 4°C until the weight loss, size and shrinkage, sensory evaluation and microbial test period. These samples were not heat treated but freshly preserved in brine solutions.

##### 2.2.2.3 Effect of brine solution preservation

*Treatment 3:* Seagrapes were washed and rinsed in the appropriate washing method (as above), blanched by the appropriate temperature and time (as above) in 5% brine solution and stored in sterilized plastic containers containing different brine concentrations i.e. 5%, 10%, 15%, 20% and refrigerated at 4°C until the weight loss, size and shrinkage, sensory evaluation and microbial test period. These samples were not heat treated but freshly preserved in brine solutions.

### 2.3 Physico-chemical and biological analysis

#### 2.3.1 Weight loss determination

Each sample was weighed on a digital scale before and after treatments 1, 2, and 3 as discussed above to determine the percentage of weight loss.

#### 2.3.2 Size and shrinkage determination

After weighing, seagrape sizes were measured using a micrometer to determine the diameter of each vesiculate ramuli. At least 10 measurements were carried out randomly on each fresh variety, from which the average diameters were recorded.

#### 2.3.3 Sensory evaluation

To determine preference and acceptability of the various types of treatment, sensory evaluations were carried out by panelists. They were recruited each day of the sensory evaluation and all panelists had previous experience testing seagrapes or are regular seagrape consumers. Panelists were required to evaluate the odour, colour, taste, sweetness and overall acceptance using the 9-point hedonic scale (1 = dislike extremely, 9 = like extremely).

#### 2.3.4 Microbiological test

A total of 10 preserved seagrapes were tested for microbial contamination to ensure that the treatments applied were

safe for human consumption. *Coliform* was counted by 3M-Petrefilm

### 2.3.5 Salinity and nutrient level

Salinity in brine was measured by salinity meter. Nutrient compositions in brine such as NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>-</sup> were analyzed by the hand-held instrument.

### 2.4 Statistical analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) Statistical analysis was performed by the Startgraphics.

## III. RESULT & DISCUSSION

### 3.1 Survey of salinity and nutrient level for seagrape cultivation

Salinity is one of the most important abiotic environmental factors to influence algal growth and distribution (Lobban and Harrison, 1994). Several studies have documented the effects of salinity on several *Caulerpa* species, including *C. paspaloides* (O'Neal and Prince, 1988) and *C. taxifolia* (Theil et al., 2007; West and West, 2007); *C. lentillifera* (Wang, 2011; Guo Hui et al., 2015).

A survey of salinity and nutrient level in brine for seagrape cultivation was verified. Salinity of brine for seagrape cultivation was tested in different positions at different times. Brine for seagrape cultivation was also tested for NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>-</sup> in different places in Central coastal region of Vietnam. Results showed that 3.50% of salinity, 0.5 mmol/L of NO<sub>3</sub><sup>-</sup>, 0.1 mmol/L of PO<sub>4</sub><sup>-</sup> were suitable for seagrape growing to get high yield.

**Table 1. Salinity and nutrient level for seagrape cultivation**

Sampling site	Salinity (%)	NO <sub>3</sub> <sup>-</sup> (mmol/L)	PO <sub>4</sub> <sup>-</sup> (mmol/L)
Ninh Hai	3.49±0.01	0.52±0.01	0.12±0.01
Cam Ranh	3.50±0.02	0.51±0.00	0.11±0.03
Tuy Hoa	3.50±0.00	0.53±0.03	0.11±0.02
Nha Trang	3.50±0.02	0.49±0.02	0.10±0.00
Ninh Hoa	3.50±0.03	0.48±0.01	0.11±0.02
Lien Chieu	3.50±0.00	0.50±0.00	0.09±0.02
Lang Co	34.96±0.01	0.50±0.02	0.10±0.01

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Wang (2011) showed that maximum growth of *C. lentillifera* occurred at a salinity of 36. Meanwhile, Guo Hui et al., (2015) proved that the maximum specific growth rate for *C. lentillifera* occurred at a salinity of 35. Both chlorophyll content and the ratio of variable to maximum fluorescence (Fv/ Fm) were also at a maximum at salinity of 35. Both the maximum specific growth rate and maximum chlorophyll content were found in algae treated with a concentration of 0.5 mmol/L of NO<sub>3</sub>-N and 0.1 mmol/L of PO<sub>4</sub>-P. The photosynthetic capacity of photosystem II (PSII) was inhibited in cultures of *C. lentillifera* at high nutrient levels. This occurred when NO<sub>3</sub>-N concentrations were greater than 1.0 mmol/L and when PO<sub>4</sub>-P concentrations were at 0.4 mmol/L.

### 3.2 Post-harvest investigation

#### 3.2.1 Effect of washing method

Treatment 1: Seagrapes were washed and rinsed in different method (5% brine solution, tap water, 50 ppm Perasan), blanched at 100°C for 15 seconds in 5% brine and stored in sterilized plastic containers containing 5% brine solutions, and refrigerated at 4°C until the weight loss, size and shrinkage, sensory evaluation and microbial test period. These samples were not heat treated but freshly preserved in brine solutions. Results were elaborated in table 2. Washing seagraped by 50 ppm Perasan would be appropriated.

**Table 2. Effect of washing method**

Testing parameter	5% brine	Tap water	50 ppm Perasan
Weight loss (%)	1.23±0.00 <sup>b</sup>	3.45±0.02 <sup>a</sup>	0.41±0.01 <sup>c</sup>
Size and shrinkage (mm)	0.22±0.01 <sup>b</sup>	0.37±0.01 <sup>a</sup>	0.11±0.00 <sup>c</sup>
Sensory score	6.23±0.00 <sup>b</sup>	5.41±0.01 <sup>c</sup>	7.40±0.03 <sup>a</sup>
<i>Coliform</i> (cfu/g)	0.6 x 10 <sup>1</sup>	1.1 x 10 <sup>1</sup>	0

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Juan E. Álvaro et al., (2009) indicated that the peracetic acid mix is better for washing fruit and improving postharvest life as it is better for the environment (due to low toxicity) and for health safety and does not affect the taste characteristics of the fruit.

#### 3.2.2 Effect of blanching temperature and time

Treatment 2: Seagrapes were washed and rinsed in the appropriate washing method (as above), blanched by different temperature and time (100°C in 15 seconds; 95°C in 20 seconds and 90°C in 25 seconds) in 5% brine and stored in sterilized plastic containers containing 5% brine solutions and refrigerated at 4°C until the weight loss, size and shrinkage, sensory evaluation and microbial test period. These samples were not heat treated but freshly preserved in brine solutions. Results were elaborated in table 3. It's clearly that blanching should be performed at 95°C in 20 seconds.

**Table 3. Effect of blanching time and temperature**

Testing parameter	100°C, 15 seconds	95°C, 20 seconds	90°C, 25 seconds
Weight loss (%)	1.12±0.01 <sup>b</sup>	1.18±0.01 <sup>b</sup>	4.37±0.02 <sup>a</sup>
Size and shrinkage (mm)	0.28±0.02 <sup>b</sup>	0.30±0.02 <sup>b</sup>	0.54±0.01 <sup>a</sup>
Sensory score	6.69±0.01 <sup>b</sup>	7.32±0.00 <sup>a</sup>	6.13±0.02 <sup>c</sup>
<i>Coliform</i> (cfu/g)	0	0	0

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

The way to preserve and process of seaweeds are blanching, boiling, steaming and sterilizing. Eko Susanto et al., (2017) showed a useful proved in the design of brown seaweeds processing which minimize fucoxanthin, antioxidant activity and colour changes.

#### 3.2.3 Effect of brine solution preservation

**Treatment 3:** Seagrapes were washed and rinsed in the appropriate washing method (as above), blanched by the appropriate temperature and time (as above) in 5% brine solution and stored in sterilized plastic containers containing different brine concentrations i.e. 5%, 10%, 15%, 20% and refrigerated at 4°C until the weight loss, size and shrinkage, sensory evaluation and microbial test period. These samples were not heat treated but freshly preserved in brine solutions. Results were elaborated in table 4. Results found that 10% of brine solution was adequate in preservation of seagrape.

**Table 4. Effect of brine solution preservation**

Testing parameter	5% brine	10% brine	15% brine	20% brine
Weight loss (%)	0.49±0.01 <sup>c</sup>	1.13±0.03 <sup>b</sup>	1.97±0.00 <sup>ab</sup>	2.85±0.02 <sup>a</sup>
Size and shrinkage (mm)	0.23±0.03 <sup>b</sup>	0.96±0.00 <sup>ab</sup>	1.45±0.03 <sup>ab</sup>	1.79±0.02 <sup>a</sup>
Sensory score	6.95±0.01 <sup>bc</sup>	8.40±0.00 <sup>a</sup>	7.13±0.02 <sup>b</sup>	6.27±0.03 <sup>c</sup>
Coliform (cfu/g)	0	0	0	0

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ( $\alpha = 5\%$ ).

The most common method of storing harvested *Caulerpa* is to put it in potato or sugar sacks with or without leaves in a cool place. Post-harvest storage ranges from one to three days, depending on distance and method of transport to the market. The introduction of improved post-harvest treatment by the use of proper wound-healing technology would prolong the life of the crop from harvester to consumer.

**IV. CONCLUSION**

Salinity and nutrient composition in brine are the most important abiotic environmental factors to influence algal growth and distribution. It is important to note that good post-harvest handling, pre-preparation, and processing activities are crucial in maintaining high quality and longer shelf-life of the preserved seagrapes. By this research, aquacultural farmers have better information to grow this species in the suitable condition to get the highest production. Moreover, stability of preserved seagrapes could be extended by the effect of washing, blanching and preserving in optimal parameters.

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