



## Quality changes in sea grape, *Caulerpa lentillifera* at different brine concentrations


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### Abstract

Sea grapes are among the seaweeds being commercialized in the Philippines. They are sold in fresh state, and are highly perishable. It is necessary to develop preservation techniques in order to lengthen its shelf life. This study aims to assess the physico-chemical, microbial and sensorial quality of sea grape *Caulerpa lentillifera* preserved in 0%, 5%, 10% and 15% brine concentrations. Results revealed that brining effectively extended the shelf life of *C. lentillifera* by inhibiting the growth of bacteria and maintaining the osmotic pressure between the product and the solution. Within the 10 days storage period, 10% and 15% were bacteriologically stable and more acceptable upon rehydration. Treatments 0% and 5% were not acceptable because it exceeded the bacterial limit set for fresh vegetables. The sensorial attributes were compromised and became less acceptable due to degradation through bacterial action. In terms of physico-chemical analysis, high salt concentration (15%) decreased chlorophyll and carotenoid content significantly due to shrinkage and water loss. Overall, this study proved that sea grapes in 10% brine solution can extend its shelf life.

**Keywords:** Brine concentration; quality; shelf life; sea grape; seaweed

### 1 | INTRODUCTION

Seaweeds are one of the major marine resources valuable for human consumption either consumed fresh or further processed into seaweed-derived products. In the Philippines, one of the major commercial seaweeds which contribute significantly to the marine fishery export is the sea grape *Caulerpa lentillifera* (BFAR 2006). It is favored and cultivated among other species from the genus *Caulerpa* because of its grape-like appearance, succulent texture and peppery taste.

In the Philippines, cultivation of *C. lentillifera* started in 1952 in the province of Cebu and it became the primary crop in ponds because of its high production rate (Trono 1988). The crops are cultured for two months and harvested by uprooting it from the pond bottom. Before transport to the market, the seaweeds are soaked in pond

water for 24 hours to remove mud and debris. The buyer then delivers it to the market where vendors would heap the seaweeds or wrap them in leaf packs or plastics (Morris and Bala 2012). However, *C. lentillifera* is consumed fresh in the form of salads because it easily deteriorates and unlike other seaweeds no known processed product was derived from it. In order to extend its shelf-life and maintain its freshness, usual local market practice was soaking or sprinkling of brine solution on the seaweed. With this method, it can last for three days at ambient temperature.

Chamberlain (1998) reported that transport in seawater prevented turgor pressure and physical damage cause by abrasion. According to South (1993), *C. racemosa* spoils very rapidly at temperatures below 15°C and over 30°C and a constant temperature of 20°C is optimum

for good handling. However, studies in Fiji and Samoa have reported that 1°C could prolong shelf life of the product (Lako 2012). Thus, the aim of this experiment is to determine the brine concentration which could best extend the shelf life and maintain quality of fresh *Caulerpa*. Also, no studies have been conducted on the changes on the nutritional aspects during storage. With this, the total chlorophyll and carotenoid content will be investigated in this experiment.

## 2 | METHODOLOGY

### 2.1 Sample Preparation

Approximately 5 kg of fresh sea grape *C. lentillifera* was purchased from Iloilo Supermarket, Iloilo, Philippines and transported to the laboratory and washed thoroughly with freshwater to remove mud and debris. Later, fronds were cut from the stolon and 100 g of it was packed in re-sealable stand-alone pouches containing 90 ml of brine. Four different brine solutions (0%, 5%, 10%, and 15%) were prepared by dissolving iodized salt fortified with 1% potassium iodide in distilled water. The packed samples were then stored at chilled conditions 10 days. Physico-chemical, microbial, and sensorial changes were then monitored every 2 days to determine the quality changes during storage.

The seaweed used in this experiment originated from Cebu, Philippines. Before transport, the seaweeds were conditioned in seawater for 24 hours. It was then transported to Iloilo through boat for 24 hours. Seaweeds were put in sacks and placed in polystyrene foam during transport. In supermarket of Iloilo, Philippines, delivery was per order basis and should be in bulk because demand for *Caulerpa* is low (PDH Tolentino and JC Sorio, personal observations). The seaweeds were re-packed in in polystyrene bags upon arrival in the market. Upon acquisition of the seaweed samples, it can be observed that they were not of high market quality because there was paling of the basal portions of the fronds. The samples could be old which exceeded the usual two months culture wherein they were deprived of sunlight due to over-growth. Market quality *Caulerpa* should be of light grass-green color with soft and succulent texture (Trono 1988).

### 2.2 Proximate composition

Moisture, lipid and ash content were determined on Day 0 and Day 10 of the experiment based on the protocols of AOAC (1987). Moisture was determined using infrared; Bligh and Dyer for lipid content; and furnace for ash. Analysis was done in triplicate.

### 2.3 Estimation of chlorophyll

Five hundred mg of fresh seaweed was grounded using mortar and pestle with 10 ml of 80% acetone. The homogenate was filtered and the supernatant was again extracted with 5 ml of 80% acetone. The amount of chlorophyll present in the seaweed was estimated by the

method of Arnon (1949). Absorbance was measured at 645 nm for chlorophyll-*a* and 663 nm for chlorophyll-*b* in a UV-Vis spectrophotometer (Agilent, USA). Analysis was done in triplicate. Total chlorophyll was determined using the following equation, chlorophyll ( $\mu\text{g ml}^{-1}$ ) = 20.2 (A645) + 8.02 (A663) where A is the absorbance at respective wave length.

### 2.4 Estimation of carotenoid

Using the same supernatant, the amount of carotenoid was estimated by the method of Kirk and Allen (1965). Analysis was done in triplicate. Absorbance was determined at 480 nm and calculated with the formula, carotenoid ( $\mu\text{g / g.fr.wt.}$ ) = A480 + (0.114 × A663) – (0.638 × A645) where A is the absorbance at respective wave length.

### 2.5 Microbial analysis

Total plate counts (TPC) of samples were determined by spread plating the serially diluted 10 g sample (up to 10<sup>6</sup>) onto nutrient agar plates. Colonies were counted after 24 hours incubation and recorded as log CFU ml<sup>-1</sup>. Analysis was done in triplicate.

### 2.6 Sensorial analysis

Changes in the sensorial attributes of the seaweed samples in each variable were determined every two days. Samples which were not rehydrated and samples which were rehydrated for a minute were the variables. Ten semi-trained panellists assessed the attributes and rated the acceptability using a qualitative line bar scale. Analysis was done in triplicate.

### 2.7 Statistical analysis

Statistical significance of the treatments every sampling was analyzed using One way ANOVA followed by Tukey post-hoc test at 5% level of significance. Software used for this purpose was Systat SigmaPlot v11.

## 3 | RESULTS AND DISCUSSION

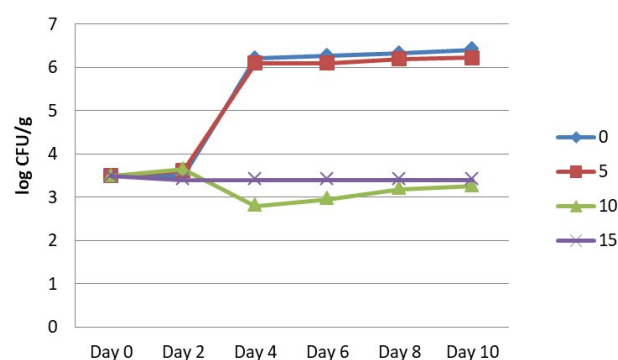
Quality changes were observed as day of storage progressed. Results of proximate composition as shown in Table 1 and Table 2 showed that salting significantly decreased the moisture content due to the action salt which drew out water from the seaweed (Clucas and Ward 1996). When the water was drawn out from the seaweed, the ramuli shrink and the fronds lost its integrity. However, the ash and lipid content significantly increased because these biochemical components concentrated due to decrease in moisture content (Alqarawi *et al.* 2014).

Initial bacterial load of the untreated seaweed sample as shown in Figure 1, it was 3.5 log CFU g<sup>-1</sup>. On the 4th day of storage, significant increase in the bacterial load was observed in 0% and 5% samples. According to FDA

(2013), microbiological standard for ready to eat vegetables with pH > 4.5, coliform test should be negative for *Escherichia coli*. Also, total plate count should not exceed 3 to 6 log CFU g<sup>-1</sup> since beyond this limit can pose imminent health hazard or spoilage. Samples in 10% and 15% brine exhibited no significant increase because of the bacteriostatic action of salt and iodine thus they were microbiologically stable and acceptable. Adding salt to foods can cause microbial cells to undergo osmotic shock, resulting in the loss of water from the cell and thereby causing cell death or retarded growth (Davidson 2001).

The presence of distinctive yellow colonies in all samples throughout the storage period was recorded (Figure 2). Based on Bergey's Manual of Determinative Bacteriology (Bergey and Holt 1994), it was presumed that these colonies were *Staphylococcus aureus* due their circular and yellowish appearance. Also, it is a spoilage organisms associated with improper handling practices (Gutierrez *et al.* 2012). Its prevalence in the brined sea-

weeds can also be attributed to its tolerance to high salt concentrations.



**FIGURE 1** Aerobic plate count of *Caulerpa* samples at different brine concentrations during 10 days storage at chilled conditions.

**TABLE 1** Wet basis proximate composition of *Caulerpa* before packing and on the final days of storage ( $n = 3$ ). Significant differences were compared between results in Day 0 and each brine concentrations in Day 10. Superscripts of the same letters are not significantly different ( $p < 0.001$ ).

Parameters	Day 0	Day 10			
		0%	5%	10%	15%
Moisture	94.26 ± 0.88 <sup>a</sup>	94.17 ± 0.12 <sup>a</sup>	93.13 ± 0.08 <sup>b</sup>	91.06 ± 0.33 <sup>b</sup>	90.43 ± 0.33 <sup>b</sup>
Ash	2.20 ± 0.12 <sup>a</sup>	4.07 ± 0.38 <sup>b</sup>	4.17 ± 0.12 <sup>b</sup>	6.07 ± 0.03 <sup>b</sup>	8.1 ± 0.05 <sup>b</sup>
Lipid	2.10 ± 0.10 <sup>a</sup>	2.17 ± 0.03 <sup>a</sup>	3.13 ± 0.09 <sup>b</sup>	3.13 ± 0.08 <sup>b</sup>	2.03 ± 0.04 <sup>b</sup>

**TABLE 2** Dry basis proximate composition of *Caulerpa* before packing and on the final days of storage ( $n = 3$ ).

Parameters	Day 0	Day 10			
		0%	5%	10%	15%
Ash	38.33 ± 1.5 <sup>a</sup>	69.81 ± 2.1 <sup>b</sup>	60.70 ± 1.3 <sup>c</sup>	67.90 ± 1.0 <sup>b</sup>	84.63 ± 1.2 <sup>d</sup>
Lipid	36.59 ± 1.1 <sup>a</sup>	37.22 ± 1.9 <sup>ab</sup>	45.56 ± 1.0 <sup>c</sup>	35.01 ± 1.2 <sup>ab</sup>	21.21 ± 1.5 <sup>c</sup>



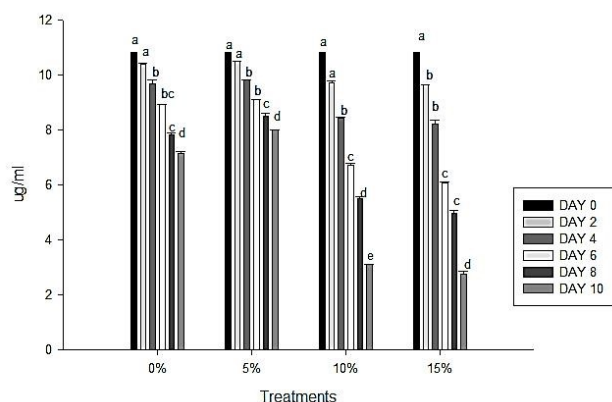
**FIGURE 2** Presumptive *Staphylococcus aureus* growth in nutrient agar characterized by distinctive golden or yellowish appearance of colonies.

Chlorophyll and carotenoid are among the functional ingredients identified from marine seaweeds. These natural pigments are very important since they exhibit

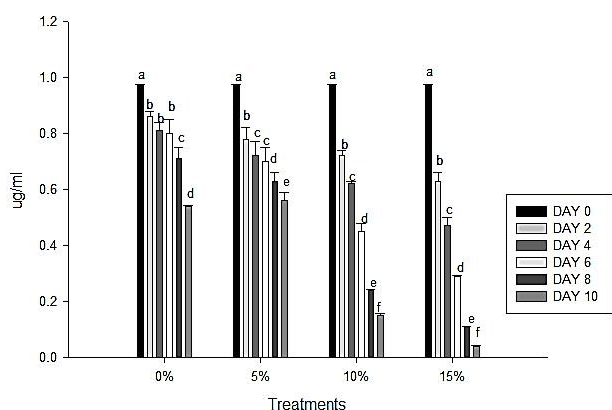
beneficial biological activities (Pangestuti and Kim 2011). Based on the results of the chlorophyll and carotenoid contents in Figure 3 and Figure 4, significant decrease in these natural pigments were observed in 10% and 15% treatments. According to Halket (1913), increase in salinity or concentration of solutes outside the cell will cause water to go out thus shrinkage occurs. Also, in the study of Lako (2012) lesser shrinkage of ramuli was observed since 5% brine is equivalent to 35% brine of sea water in which *Caulerpa* grow best. At this concentration, osmotic pressure within and outside the grapes may be at equilibrium, resulting in the little impact on the shrinkage.

Although decrease was also observed in 0% and 5% samples, this was gradual and not dramatic as compared with 10% and 15%. Decrease became significant on the 8th day to 10th day of storage due to high bacterial load which lead to water loss. *Caulerpa* present nearly ideal conditions for the survival and growth of many types of microorganisms. The internal tissues are nutrient rich have a pH near neutrality. Spoilage microorganisms re-

lease extracellular lytic enzymes that degrade these polymers to release water and the plant's other intracellular constituents for use as nutrients for their growth (Miedes and Lorences 2004). With this, salt and high bacterial load contributed to the decrease on the nutritive value of the seaweed.



**FIGURE 3** Changes in the total chlorophyll content of seaweeds at different brine concentrations ( $n = 3$ ). Values with same superscript have no significant difference at  $p > 0.05$ .



**FIGURE 4** Changes in the total carotenoid content of seaweeds at different brine concentrations ( $n = 3$ ). Values with same superscript have no significant difference at  $p > 0.05$ .

With the changes in microbial load and natural pigments as days of storage progressed in each treatment, the sensorial attributes were also affected. Results in Table 3 are the samples withdrawn from the packaging and not subjected to rehydration. Based on this, attributes of 0% and 5% were initially acceptable because shrinkage was lesser in these two treatments thus it was significantly more acceptable compared to 10% and 15%. However, on the 4th day, acceptability as well as ratings on the attributes significantly decreased until the 10th day. Although 10% and 15% have the greater shrinkage, it became acceptable. Decrease in the ratings of 0% and 5%

was due to emission of strong rotting odor. It can be also be inferred based on the comments from the panelists that a rotting mossy odor was also associated to unacceptable color, texture, ramule, and overall appearance.

Acceptability of 10% and 15% treatments were further confirmed by the results of the rehydrated samples (Table 4). Based on the results, these treatments were more acceptable even as days of storage progressed while 0% and 5% were acceptable up to the 4th day. However, results were contradicting to that of total chlorophyll and carotenoid wherein pigment loss should affect the ratings of the attributes, especially color. According to Cox *et al.* (2012), degree of rehydration depends on the structural integrity of the cell wall and the capacity of the hydrophilic components of the cell. With greater shrinkage in 10% and 15% samples, rehydration should be slower. However, considering the results of the experiment, bacterial action was the primary cause of the degradation and loss of quality on the attributes of the product.

Brining was an effective method in extending the shelf-life of *Caulerpa* by inhibiting the growth of bacteria and maintaining the osmotic pressure between the product and the solution. High salt concentration decreased chlorophyll and carotenoid content significantly due to shrinkage and water loss. Throughout the storage period, 0% and 5% was not acceptable because it exceeded the bacterial limit set for fresh vegetables. Also, the sensorial attributes were compromised and became less acceptable due to degradation through bacterial action. Within the 10 day storage period, 10% and 15% were bacteriologically stable and more acceptable upon rehydration. With the result of this experiment, 10% brine solution was effective in extending the shelf life of *Caulerpa* because it was more economical.

One of the factors that affected the results of this experiment was the handling procedure which welcomed the growth of spoilage organisms. In the future experiments, we suggest that possibility of bacterial contamination should be eliminated to determine the optimum brining solution needed to prolong the shelf life of *Caulerpa* without compromising the nutritive values and sensorial attributes. As recommended by previous studies, use of treated brine or blanching to eliminate bacterial contamination (Lako 2012). Also, color charts should be used to correctly identify and differentiate the colors.

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**TABLE 3** Sensory evaluation of different treatments not subjected to rehydration. Changes were evaluated every 2 days of storage among samples ( $n = 3$ ). Values with same superscript have no significant difference ( $p > 0.05$ ).

Days	% brine	Color	Odor	Texture	Ramule	General accept.
0	0	8.07±0.60 <sup>a</sup>	7.43±0.27 <sup>a</sup>	7.07±0.44 <sup>a</sup>	7.93±0.38 <sup>a</sup>	6.26±0.12 <sup>a</sup>
	5	8.10±0.87 <sup>a</sup>	7.53±0.03 <sup>a</sup>	7.87±0.69 <sup>a</sup>	8.26±0.39 <sup>a</sup>	5.66±0.16 <sup>a</sup>
	10	7.07±0.44 <sup>a</sup>	7.60±0.06 <sup>a</sup>	4.50±0.58 <sup>a</sup>	4.30±0.57 <sup>a</sup>	5.93±0.31 <sup>a</sup>
	15	7.93±0.38 <sup>a</sup>	7.40±0.25 <sup>a</sup>	5.30±0.31 <sup>a</sup>	6.80±0.49 <sup>a</sup>	4.66±0.03 <sup>a</sup>
2	0	8.06±0.60 <sup>a</sup>	7.80±0.40 <sup>a</sup>	5.83±0.33 <sup>a</sup>	7.60±0.05 <sup>a</sup>	7.43±0.12 <sup>a</sup>
	5	7.53±0.33 <sup>a</sup>	6.63±5.93 <sup>a</sup>	6.67±0.61 <sup>ab</sup>	7.20±0.35 <sup>a</sup>	8.30±0.41 <sup>a</sup>
	10	3.70±0.46 <sup>b</sup>	7.83±0.33 <sup>a</sup>	6.46±0.29 <sup>bc</sup>	5.20±0.17 <sup>b</sup>	6.50±0.35 <sup>b</sup>
	15	4.80±0.20 <sup>b</sup>	6.86±0.08 <sup>a</sup>	4.63±0.13 <sup>c</sup>	5.53±0.31 <sup>b</sup>	5.53±0.27 <sup>b</sup>
4	0	4.66±0.44 <sup>a</sup>	5.20±0.35 <sup>a</sup>	4.96±0.29 <sup>a</sup>	5.63±0.13 <sup>a</sup>	7.20±0.35 <sup>a</sup>
	5	4.50±0.57 <sup>a</sup>	5.90±0.40 <sup>ab</sup>	4.63±0.13 <sup>a</sup>	5.63±0.13 <sup>a</sup>	5.93±0.43 <sup>a</sup>
	10	5.63±0.46 <sup>a</sup>	7.16±0.33 <sup>bc</sup>	5.63±0.29 <sup>a</sup>	5.93±0.31 <sup>a</sup>	4.86±0.36 <sup>b</sup>
	15	4.83±0.38 <sup>a</sup>	6.70±0.05 <sup>c</sup>	5.16±0.71 <sup>a</sup>	5.86±0.20 <sup>a</sup>	5.53±0.27 <sup>c</sup>
6	0	4.83±0.33 <sup>a</sup>	4.33±0.16 <sup>a</sup>	5.06±0.21 <sup>a</sup>	4.20±0.15 <sup>a</sup>	4.23±0.37 <sup>a</sup>
	5	4.33±0.16 <sup>ab</sup>	5.16±0.33 <sup>a</sup>	5.00±0.28 <sup>a</sup>	4.20±0.16 <sup>a</sup>	4.16±0.33 <sup>a</sup>
	10	5.53±0.03 <sup>bc</sup>	7.66±0.13 <sup>a</sup>	5.83±0.33 <sup>a</sup>	6.16±0.33 <sup>a</sup>	5.53±0.03 <sup>a</sup>
	15	5.66±0.13 <sup>c</sup>	6.53±0.33 <sup>a</sup>	4.96±0.12 <sup>a</sup>	6.00±0.05 <sup>a</sup>	5.66±0.13 <sup>a</sup>
8	0	3.63±0.43 <sup>a</sup>	3.23±0.14 <sup>a</sup>	4.03±0.24 <sup>a</sup>	3.63±0.41 <sup>a</sup>	3.63±0.43 <sup>a</sup>
	5	2.93±0.41 <sup>a</sup>	4.50±0.57 <sup>b</sup>	2.93±0.06 <sup>b</sup>	3.43±0.14 <sup>a</sup>	3.83±0.33 <sup>a</sup>
	10	6.80±0.10 <sup>b</sup>	7.30±0.20 <sup>c</sup>	8.76±0.13 <sup>c</sup>	5.83±0.16 <sup>b</sup>	5.40±0.10 <sup>b</sup>
	15	6.06±0.21 <sup>b</sup>	6.26±0.29 <sup>d</sup>	5.20±0.20 <sup>d</sup>	6.33±0.06 <sup>b</sup>	5.50±0.20 <sup>b</sup>
10	0	2.56±0.21 <sup>a</sup>	2.66±0.51 <sup>a</sup>	2.72±0.41 <sup>a</sup>	2.50±0.36 <sup>a</sup>	3.56±0.47 <sup>a</sup>
	5	2.03±0.14 <sup>a</sup>	4.50±0.20 <sup>a</sup>	1.93±0.21 <sup>a</sup>	2.40±0.26 <sup>a</sup>	4.33±0.08 <sup>ab</sup>
	10	6.83±0.06 <sup>b</sup>	7.80±0.36 <sup>b</sup>	7.10±0.10 <sup>b</sup>	7.53±0.17 <sup>b</sup>	5.63±0.60 <sup>bc</sup>
	15	6.43±0.36 <sup>b</sup>	5.83±0.41 <sup>b</sup>	5.30±0.05 <sup>b</sup>	5.96±0.31 <sup>b</sup>	5.50±0.20 <sup>c</sup>

**TABLE 4** Sensory evaluation of different treatments subjected to rehydration. Changes were evaluated every 2 days of storage ( $n = 3$ ). Values with same superscript have no significant difference ( $p > 0.05$ ).

Days	% brine	Color	Odor	Texture	Ramule	General accept.
0	0	8.08±0.32 <sup>a</sup>	8.26±0.39 <sup>a</sup>	8.00±0.49 <sup>a</sup>	8.43±0.29 <sup>a</sup>	8.00±0.49 <sup>a</sup>
	5	8.43±0.29 <sup>a</sup>	8.10±0.87 <sup>a</sup>	7.06±0.44 <sup>a</sup>	9.03±0.39 <sup>a</sup>	7.06±0.44 <sup>ab</sup>
	10	8.33±0.27 <sup>a</sup>	7.50±0.57 <sup>a</sup>	7.40±0.26 <sup>a</sup>	7.50±0.57 <sup>a</sup>	7.40±0.26 <sup>b</sup>
	15	9.26±0.39 <sup>a</sup>	7.53±0.03 <sup>a</sup>	7.93±0.38 <sup>a</sup>	7.53±0.03 <sup>a</sup>	7.93±0.38 <sup>c</sup>
2	0	7.53±0.03 <sup>a</sup>	8.06±0.32 <sup>a</sup>	4.80±0.28 <sup>a</sup>	5.66±0.13 <sup>a</sup>	8.08±0.49 <sup>a</sup>
	5	6.66±0.61 <sup>a</sup>	8.06±0.61 <sup>a</sup>	5.33±0.23 <sup>a</sup>	6.00±0.20 <sup>a</sup>	7.06±0.44 <sup>a</sup>
	10	7.53±0.03 <sup>a</sup>	7.80±0.40 <sup>a</sup>	6.86±0.08 <sup>b</sup>	6.53±0.03 <sup>a</sup>	7.40±0.26 <sup>a</sup>
	15	8.43±0.29 <sup>c</sup>	8.10±0.87 <sup>a</sup>	7.86±0.08 <sup>c</sup>	6.70±0.46 <sup>a</sup>	7.93±0.38 <sup>a</sup>
4	0	5.66±0.13 <sup>a</sup>	8.06±0.32 <sup>a</sup>	5.96±0.46 <sup>a</sup>	5.80±0.10 <sup>a</sup>	8.06±0.60 <sup>a</sup>
	5	6.00±0.20 <sup>a</sup>	8.06±0.60 <sup>a</sup>	6.50±0.57 <sup>a</sup>	5.30±0.10 <sup>a</sup>	7.20±0.35 <sup>a</sup>
	10	6.53±0.03 <sup>b</sup>	7.80±0.40 <sup>a</sup>	7.20±0.35 <sup>a</sup>	6.76±0.31 <sup>b</sup>	7.83±0.33 <sup>a</sup>
	15	7.03±0.24 <sup>b</sup>	8.10±0.87 <sup>a</sup>	7.96±0.46 <sup>a</sup>	7.53±0.17 <sup>b</sup>	8.26±0.39 <sup>a</sup>
6	0	5.53±0.03 <sup>a</sup>	5.63±0.46 <sup>a</sup>	5.53±0.03 <sup>a</sup>	4.83±0.38 <sup>a</sup>	5.83±0.33 <sup>a</sup>
	5	6.06±0.43 <sup>a</sup>	6.16±0.66 <sup>a</sup>	6.06±0.43 <sup>ab</sup>	4.66±0.25 <sup>a</sup>	5.20±0.35 <sup>a</sup>
	10	7.30±0.41 <sup>b</sup>	7.16±0.33 <sup>a</sup>	7.30±0.41 <sup>bc</sup>	7.56±0.03 <sup>b</sup>	7.40±0.26 <sup>b</sup>
	15	7.36±0.12 <sup>b</sup>	6.70±0.41 <sup>a</sup>	7.36±0.12 <sup>c</sup>	8.46±0.43 <sup>b</sup>	7.93±0.38 <sup>b</sup>
8	0	2.93±0.41 <sup>a</sup>	5.63±0.46 <sup>a</sup>	6.80±0.10 <sup>a</sup>	2.93±0.41 <sup>a</sup>	2.93±0.41 <sup>a</sup>
	5	3.06±0.41 <sup>ab</sup>	6.16±0.66 <sup>a</sup>	7.23±0.33 <sup>a</sup>	3.06±0.41 <sup>a</sup>	3.06±0.41 <sup>a</sup>
	10	4.50±0.05 <sup>bc</sup>	7.16±0.33 <sup>a</sup>	7.30±0.20 <sup>a</sup>	6.63±0.59 <sup>b</sup>	7.23±0.33 <sup>b</sup>
	15	4.33±0.08 <sup>c</sup>	6.70±0.41 <sup>a</sup>	7.56±0.03 <sup>a</sup>	7.96±0.29 <sup>b</sup>	7.30±0.20 <sup>b</sup>
10	0	2.93±0.41 <sup>a</sup>	5.66±0.13 <sup>a</sup>	4.50±0.05 <sup>a</sup>	2.23±0.46 <sup>a</sup>	2.43±0.28 <sup>a</sup>
	5	3.06±0.41 <sup>a</sup>	6.00±0.20 <sup>a</sup>	4.33±0.08 <sup>a</sup>	2.43±0.28 <sup>a</sup>	2.66±0.51 <sup>a</sup>
	10	6.86±0.68 <sup>b</sup>	6.53±0.03 <sup>a</sup>	7.56±0.03 <sup>b</sup>	8.30±0.41 <sup>b</sup>	7.53±0.17 <sup>b</sup>
	15	8.30±0.41 <sup>b</sup>	6.70±0.46 <sup>a</sup>	8.80±0.10 <sup>b</sup>	6.86±0.68 <sup>b</sup>	7.90±0.46 <sup>b</sup>

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHORS' CONTRIBUTION**

**PDHT** conceptualized the research, conducted the microbial analysis and analysed the data. **JCS** conducted the sensory evaluation and chemical analysis.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available on request from the corresponding author.

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