Journal of Applied Phycology (2019) 31:731–739
https://doi.org/10.1007/s10811-018-1545-8

Contrasting effects of two storage temperatures on the microbial, physicochemical, and sensory properties of two fresh red seaweeds, *Palmaria palmata* and *Gracilaria tikvahiae*

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Received: 8 December 2017 / Revised and accepted: 12 June 2018 /Published online: 23 June 2018

Abstract
The effects of two storage temperatures, 2 and 7 °C, were investigated on the quality changes of fresh red seaweeds, *Palmaria palmata* and *Gracilaria tikvahiae*. Microbial, sensory, and physiochemical properties of the seaweeds were evaluated during 2 weeks of refrigerated storage. The results indicated that the causes and rates of quality loss were species specific, with *P. palmata* deteriorating faster at 7 °C compared to at 2 °C. In contrast, *G. tikvahiae* quality was better maintained at the higher storage temperature. As cellular damage increased in the seaweeds during storage, increased drip loss and the subsequent deterioration in texture and color contributed to quality loss in both seaweed species. Microbial counts in *P. palmata* ranged from 3 to 5 log CFU g^{-1} throughout storage, whereas *G. tikvahiae* microbial counts reached over 7 log CFU g^{-1} by the end of storage. Drip loss, sensory evaluation, and instrumental color results proved to be reliable whereas instrumental texture and soluble protein did not yield consistent, valuable data. Growing interest in minimally processed foods provides an opportunity to promote seaweeds as fresh vegetables. The results of this study provide groundwork to monitor seaweed quality during refrigerated storage and to facilitate marketing and distribution of freshly harvested *P. palmata* and *G. tikvahiae*.

Keywords Rhodophyta · Quality loss · Storage · *Palmaria palmata* · *Gracilaria tikvahiae* · Fresh

Introduction

Edible seaweeds are considered novel foods in mainstream western cuisine and are gaining popularity among health-conscious consumers, owing to their "superfood" status (Tarver 2015; Bever 2016). The richness in antioxidants, vitamins, minerals, and other beneficial compounds present in seaweeds contributes to their bioactive, medicinal, and therapeutic properties (Smit 2004; Wells et al. 2017), adding to their nutrient-dense profile. Although globally seaweeds are predominantly sold in their dried form, increasing consumer interest in fresh or minimally processed health-promoting foods (Raso and Barbosa-Cánovas 2003; Bigliardi and Galati 2013), has opened opportunity in the West to sell fresh seaweeds as vegetables in the produce section of the grocery store.

Palmaria palmata, also known as dulse, is consumed in many European countries including France, Iceland, and Ireland, and in parts of North America including Maine, Hawaii, and Nova Scotia (Mouritsen et al. 2013 2013a, b; Abbott 1978). With flat, leathery, deep red blades, *P. palmata* is commonly found in cold Atlantic waters (Mouritsen et al. 2013a, b) whereas *Gracilaria tikvahiae*, or gracilaria (Fig. 1), is usually found in warmer, tropical waters (Abbott 1978). Since western consumers are already somewhat familiar with *P. palmata* and *G. tikvahiae*, these seaweeds have the potential to be accepted in their fresh form. In Hawaii, *G. tikvahiae* is already sold as a fresh vegetable, which can be pickled or incorporated directly into seafood dishes, soups and salads (Abbott 1978). Several species of seaweeds are currently being tested for their aquaculture potential in North America, with fresh *P. palmata* and gracilaria already making their way in innovative recipes in selected upscale restaurants. However, fresh seaweeds are reported to have a short shelf life of around 3–4 days (Liot et al. 1993; Paull and Chen 2008), albeit storage

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Fig. 1 Fresh *P. palmata* (left) and *G. tikvahiae* (right)

conditions can impact shelf life significantly. Paull and Chen (2008) assessed shelf life of fresh, farm-raised *Gracilaria* spp. under different refrigerated storage conditions and recommended a shelf life of 4 days.

Lack of information on postharvest quality changes and on the effects of storage temperature on shelf life of freshly harvested seaweeds poses a roadblock to their effective processing, marketing, and distribution through multiple channels. To our knowledge, there have been no systematic evaluations of postharvest quality of refrigerated fresh red seaweeds. In the USA, efforts are being made by a number of small businesses to create and sell diverse seaweed food products. The clean Atlantic waters of the state of Maine offer around 250 species of seaweeds (White and Keleshian 1994), giving rise to the potential to incorporate different varieties of seaweeds in the local diet. Select companies in Maine have already started to market fresh or minimally processed seaweeds to gourmet restaurants where chefs innovate dishes with seaweeds for adventurous consumers (Bell 2014; Pols 2015). However, more applied information is needed on shelf life, processing techniques, and distribution methods of fresh seaweeds to facilitate smooth trade between the seaweed producers and restaurant owners. The two species studied in this paper, *P. palmata* and *G. tikvahiae*, belong to different families of red seaweeds, thus being genetically and morphologically different (Abbott 1978; Mouritsen et al. 2013a, b). It was expected that *P. palmata* and *G. tikvahiae* might respond to the storage temperatures distinctively. The primary objective of this research was to evaluate various quality attributes of *P. palmata* and *G. tikvahiae* under two storage temperatures. The lower temperature, 2 °C, is recommended for storage of many fresh vegetables (Gast 2001) whereas the higher temperature, 7 °C, more closely reflects conditions commonly observed in restaurant refrigerators, which are repeatedly opened and closed throughout the day. A second objective was to evaluate the methods used to analyze quality changes in the seaweeds and to recommend appropriate analytical methods for these species.

Materials and methods

Sample preparation

Two separate storage studies were conducted on *P. palmata* (February harvest) and *G. tikvahiae* (September harvest), based on their seasonal availability. Seaweeds were maintained at two refrigeration temperatures, 2 and 7 °C. Microbial, physicochemical, and sensory quality analyses were conducted every 2–3 days, based on the availability of sensory panelists, for up to 2 weeks or until samples were considered inedible. Freshly harvested seaweeds (Maine Fresh Sea Farms, Bristol, ME, USA) were rinsed with seawater and delivered on harvest day (*G. tikvahiae*) or the day after harvest (*P. palmata*). On day 0 of each study, seaweeds were delivered and sorted to remove any damaged parts, and then portioned into 2-gal Ziploc bags with each bag containing 450–500 g sample. The experiment was conducted in triplicate, and sample bags were kept under refrigerated storage and were subsampled each testing day.

Microbial analysis

Seaweeds (15 g) were aseptically placed in a stomacher bag with 0.1% bactopeptone (BD Diagnostics, USA) (1:10 *w*/*v*) and mixed for 2 min using a BAGMixer 400 (Model P, SpiralBiotech, Advanced Instruments, Norwood, MA, USA). Aliquots (1 mL) of varying dilutions (10^{-1}–10^{-7}), depending on the testing day, were plated in duplicate on Petrifilm Aerobic Count Plate (APC) (3 M, St. Paul, MN, USA) and incubated for 48 h at 37 °C after which films with 30–300 colony-forming units (CFUs) were counted for enumeration. Duplicate values for each of the three treatment replicates were averaged, and the data were reported as log CFU g^{-1}. The triplicate values were then averaged to present the data on the graph.

Sensory evaluation

Twelve panelists, between the ages of 18 and 60, who self-identified as seaweed consumers, were recruited for the sensory evaluation of refrigerated seaweeds. Prior to the study, participants were trained for 30 min on each seaweed species to become familiar with their specific quality attributes and to develop descriptors for color, aroma, texture, and overall quality. On each testing day, pooled samples from each treatment replicate were presented on a white ceramic plate under normal white light and evaluated by a minimum of 8 to a maximum of 12 panelists. The evaluation sheet comprised a 15-cm unstructured line scale (Meilgaard et al. 2006) for color, aroma, texture, and overall quality with opposite descriptors (Table 1) on either end of the line scale. On the line scale, 0 represented the poor quality score whereas 15 represented

Table 1 Descriptors used for sensory evaluation of *P. palmata* and *G. tikvahiae*

Sensory attribute	*P. palmata*		*G. tikvahiae*	
	Score 0	Score 15	Score 0	Score 15
Color	Faded plum-red	Dark plum-red	Faded brown-red	Dark brown-red
Aroma	Unpleasant	Pleasant	Unpleasant	Pleasant
Texture	Fragile	Strong	Limp	Firm
Overall quality	Complete loss of freshness	Fresh	Complete loss of freshness	Fresh

an excellent quality score. Approval for research with human subjects was obtained from the Institutional Review Board (IRB) prior to conducting sensory analyses. All panel members were provided with an informed consent document prior to participation.

Colourimetric analyses

Seaweed color during storage was assessed using a colorimeter (LabScan XE, USA). Each treatment replicate was sampled ten times, with three L^*, a^*, b^* values measured for each sample after 120° rotations. Color change (ΔE) over time within treatment was calculated on each test day in comparison to day 1 values using the following formula:

$$\Delta E^*_{ab} = \sqrt{\left(L^*_2 - L^*_1\right)^2 + \left(a^*_2 - a^*_1\right)^2 + \left(b^*_2 - b^*_1\right)^2}$$

where L^*, a^*, and b^* are the lightness, redness, and yellowness values, and 1 stands for day 1 of testing whereas 2 stands for other test days.

Instrumental texture

Different morphologies of *P. palmata* and *G. tikvahiae* (Fig. 1) required different methods for analyzing texture. Texture profile analysis was employed for *P. palmata* due to its thin, flat blades. *P. palmata* samples (80 g) were cut into 3 cm × 3 cm squares using a cutter and then stacked to 0.8 cm in height on the texture analyzer (TA-XTi2, Texture Technologies Inc., Scarsdale, NY, USA) platform. A flat-bottomed cylindrical probe of 5.1-cm diameter was used to compress the samples by 0.3 cm with a 2 mm s^{-1} test speed. Hardness was recorded as force in Newton (N) by the texture analysis software (Exponent 32, version 5.0, 6.0, 2010, Texture Technologies Inc) on eight samples per treatment replicate. *Gracilaria* was evaluated with the Kramer shear method since the thalli branches were firm and stick-like. Samples were packed 3 cm high in a mini Kramer shear cell (TA-XTi2, Texture Technologies Inc) with five flat blades set to travel 2.9 cm in a downward direction at 1 mm s^{-1} to shear the samples. Force (N) required to shear the sample was recorded for a total of 10 samples per treatment replicate.

Drip loss

Profuse drip loss was observed during the *P. palmata* storage study, and hence, drip loss was quantified during the later performed *G. tikvahiae* storage study. Drip loss was defined as cellular fluids lost during storage and was measured in *G. tikvahiae* by decanting the sample bags immediately after taking them out of refrigerated storage for 30 s to remove any pooled liquid. Samples and liquid were weighed (g), and drip loss was calculated as percent fluid lost compared to the initial sample weight using the following formula:

$$\%\text{Drip loss} = \frac{\text{Fluid loss (g)}}{\text{Initial sample weight (g)}} \times 100$$

Soluble protein

Soluble protein was extracted using the methods described by Paull and Chen (2008) with slight modifications. Briefly, chopped sample (8 g) was homogenized (Polytron, Brinkmann Instruments, USA) with 32 mL sodium phosphate buffer (pH 7) for 2 min. Samples were centrifuged (Beckman J-25, Brea, CA) at 14,000×*g* for 15 min, and the supernatant was frozen at − 20 °C until further analyses. Protein analysis of *P. palmata* was performed as described by Lowry et al. (1951), using bovine serum albumin (BSA) as a standard, and absorbance was read at wavelength 700 nm. Since protein precipitation was observed during *G. tikvahiae* analysis using the Lowry method, the Bradford (1976) method was used to assess soluble protein of *G. tikvahiae* and absorbance was read at 595 nm.

Statistical analyses

One-way analysis of variance (ANOVA) was used to test for differences among treatments for each dependent variable on each day. When significant differences were observed, Tukey's honest significant difference (HSD) test was used to determine which treatments were significantly different from one another. Two-way ANOVA was used to assess overall effects of storage time and temperature. Data were analyzed

using JMP 12.2 (SAS Software, USA). A significance level of $p < 0.05$ was chosen for all statistical analyses.

Results and discussion

Microbial analysis

Gracilaria tikvahiae microbial counts increased significantly ($p < 0.05$) for both storage temperatures, reaching 7.5 log CFU g^{-1} by day 12 (Fig. 2) and likely contributed to spoilage during storage. However, Paull and Chen (2008) concluded that microbial growth was not the primary cause of quality loss in *Gracilaria salicornia* during storage. In comparison to *G. tikvahiae*, no significant changes in APC were observed in *P. palmata* over time. Liot et al. (1993) also found no significant change in the microbial load of *P. palmata* washed with seawater and then stored at 4 °C for 2 weeks. Storage temperature did not significantly affect APC of either species. Although it appears that *P. palmata* stored at 2 °C had higher microbial counts on day 1 compared to days 3 and 6, there were no significant differences in microbial activity among these days. The minor decrease in the microbial activity could be attributed to temperature shock due to initial storage at 2 °C. *P. palmata* had lower APC, 3.0–5.2 log CFU g^{-1}, compared to *G. tikvahiae* throughout storage. Differences in APC between species may be attributed to their different growing and harvest seasons: winter for *P. palmata* and late summer for *G. tikvahiae*. The average water temperature was approximately −2 °C during the *P. palmata* harvest and about 15 °C during the *G. tikvahiae* harvest (Perry Phytoplankton & Optics Lab 2010). Moreover, the warmer water temperature in the summer, closer to the optimal growing temperature of 20–45 °C for mesophilic aerobic bacteria, could have contributed to the higher microbial counts for *G. tikvahiae*. However, microbial activity did not appear to be a significant contributor to quality loss for *P. palmata*. Seaweeds are known to contain a variety of antimicrobial compounds, including terpenes, phenols, and tannins, and their extracts have repeatedly shown antimicrobial activity against Gram-positive and Gram-negative bacteria (Cox et al. 2010; Gupta et al. 2010). This could have aided in keeping the microbial counts of *P. palmata* below 6 log CFU g^{-1} over time. Although there is no critical cutoff value for microbial counts in fresh seaweeds, Debevere (1996) recommended an upper microbial limit of 8 log CFU g^{-1} for fresh fruits and vegetables intended for human consumption. Although the aerobic plate counts provided a general assessment of microbial activity in the fresh seaweeds, characterization of specific bacteria present in *P. palmata* and *G. tikvahiae* is necessary to gain a deeper understanding of microbial spoilage during storage.

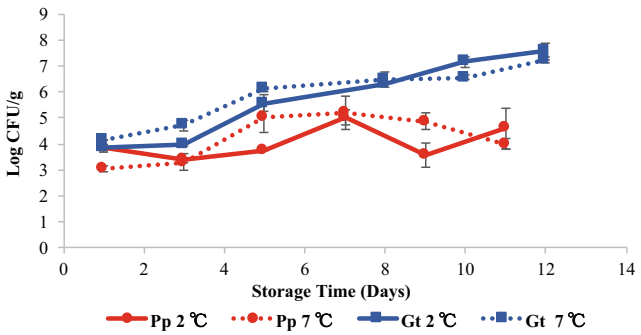

Fig. 2 Aerobic plate counts of *P. palmata* and *G. tikvahiae* during refrigerated storage. Each value represents the mean ± standard error ($n = 3$)

Sensory evaluation

A drop in sensory color scores during storage indicated fading in both seaweed species (Table 2). However, their intrinsic

Table 2 Sensory color, aroma, and texture scores for *P. palmata* and *G. tikvahiae* during refrigerated storage. Each value represents a mean ± standard error, ($n = 8$–12, depending on the testing day). Values not sharing a letter are significantly ($p < 0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test

	P. palmata			*G. tikvahiae*	
Color					
Day	Pp 2 °C	Pp 7 °C	Day	Gt 2 °C	Gt 7 °C
1	12.8 ± 0.5c	13.2 ± 0.2c	1	12.3 ± 0.4c	12.5 ± 0.3c
3	11.2 ± 0.7bc	11.5 ± 0.5c	3	10.9 ± 0.8c	11.8 ± 0.5c
7	9.4 ± 0.7bc	5.6 ± 0.7b	5	10.0 ± 0.8c	10.9 ± 0.5bc
9	9.0 ± 0.9ab	4.2 ± 0.4ab	8	9.6 ± 0.8bc	9.0 ± 0.5ab
11	8.8 ± 1.0abc	3.0 ± 0.5a	10	6.3 ± 0.7ab	9.1 ± 0.7ab
14	5.6 ± 0.9a		12	4.2 ± 1.0a	6.8 ± 1.1a
Aroma					
Day	Pp 2 °C	Pp 7 °C	Day	Gt 2 °C	Gt 7 °C
1	12.9 ± 0.5b	13.2 ± 0.2b	1	12.3 ± 0.4c	12.4 ± 0.5b
3	10.8 ± 0.9b	10.9 ± 1.2b	3	11.4 ± 0.8c	11.9 ± 0.5b
7	9.5 ± 0.9b	6.2 ± 0.8a	5	10.0 ± 0.7bc	9.9 ± 0.7ab
9	9.7 ± 0.9b	5.4 ± 1.3a	8	9.9 ± 1.0bc	7.6 ± 1.0a
11	9.1 ± 0.5ab	4.6 ± 0.8a	10	7.2 ± 1.1ab	7.3 ± 1.2a
14	5.0 ± 1.3a		12	4.6 ± 1.2a	7.2 ± 1.1a
Texture					
Day	Pp 2 °C	Pp 7 °C	Day	Gt 2 °C	Gt 7 °C
1	13.0 ± 0.5b	12.2 ± 0.6c	1	12.9 ± 0.6c	12.8 ± 0.7b
3	12.2 ± 0.6ab	12.2 ± 0.5c	3	11.4 ± 0.4bc	12.2 ± 0.5b
7	11.3 ± 0.3ab	6.7 ± 0.9b	5	10.9 ± 0.3bc	9.6 ± 0.8ab
9	9.4 ± 1.1a	6.6 ± 1.1b	8	9.9 ± 0.8b	9.1 ± 0.9ab
11	9.2 ± 0.5a	1.3 ± 0.3a	10	6.8 ± 0.9a	7.9 ± 1.1a
14	9.3 ± 1.1a		12	4.3 ± 0.9a	7.2 ± 0.9a

colors faded at different rates at 2 and 7 °C, clearly showing a significant effect of storage temperature on color stability. At 7 °C, *P. palmata* color scores plummeted to 5.5 by day 7 compared to an initial score of ~13 on the first day. In contrast, at 2 °C, the scores did not drop significantly until day 14, indicating less fading of the initial plum-red *P. palmata* color at the lower storage temperature. Storage time and temperature both significantly affected sensory color scores for *P. palmata* and *G. tikvahiae*. However, the effects of storage temperature on sensory color scores for *G. tikvahiae* were less pronounced compared to *P. palmata*. For *G. tikvahiae*, the color scores dropped to ~6 on day 10 for samples at 2 °C while samples stored at 7 °C continued to receive higher scores (~10), suggesting that the higher temperature (7 °C) storage maintained *G. tikvahiae* quality better compared to the lower temperature. It was observed that the *P. palmata* did not fade in any particular spatial pattern, with discolored patches randomly appearing on *P. palmata* blades. Gracilaria lost its dark red color starting at the tip, however, within a cluster, random tips faded, with no particular pattern. This lack of uniformity made it difficult to assess seaweed color consistently throughout storage.

Evaluation of aroma clearly indicated distinctly different effects of storage temperature on the two species. For *P. palmata*, the higher temperature accelerated a decrease in sensory aroma scores (Table 2) over time whereas for *G. tikvahiae* stored at 7 °C, the scores were maintained in the middle of the scale. These results indicate that the higher temperature preserved the initial pleasant aroma for longer in *G. tikvahiae*. Time and temperature had a significant effect on aroma of *P. palmata*, but only time affected sensory aroma scores of *G. tikvahiae*. LePape et al. (2002) assessed aroma of *P. palmata* stored in artificial seawater at 4 °C and reported that "fresh" aroma sensory scores dropped during storage. Interestingly, even though seaweeds are not fish, panelists noted the presence of "fishy" aromas in *P. palmata* stored at 7 °C after day 7 and scored the samples on the lower ("unpleasant") end of the scale. Decaying seaweeds produce nitrogenous compounds including ammonia, methylamine, and trimethylamine, which are often responsible for "fishy" aromas (Mouritsen et al. b), and have been reported previously in the seaweed *Fucus vesiculosus* (Smith and Young 1953).

Sensory texture scores decreased significantly for both the species over time (Table 2). However, storage temperature significantly affected only *P. palmata*, with the higher temperature accelerating texture loss. As the samples lost their textural integrity over time, panelists described the *P. palmata* as "wilted" and "mushy." After 11 days of storage, *P. palmata* stored at 2 °C had scores nine times higher compared to at 7 °C, clearly indicating that the lower storage temperature maintained the initial texture better for this species. In contrast, *G. tikvahiae* stored at 7 °C scored approximately 1.5 times higher than samples stored at 2 °C by day 12. Although both species are Rhodophyta, they have very different physical structures and different descriptors were developed to assess their texture. Some panelists noted that toward the end of the study, some *G. tikvahiae* branches were firm whereas some were extremely limp, indicating that texture deteriorated randomly throughout the samples.

For overall quality, storage temperature had a large, significant effect on *P. palmata* scores whereas the effects were minimal for *G. tikvahiae* (Fig. 3), clearly indicating that overall quality was maintained better at lower temperature for *P. palmata*. This is in agreement with the trend seen for all other sensory quality scores for *P. palmata* during storage. Panelists commented that they would not consume *P. palmata* stored at 7 °C but would consume samples stored at 2 °C after 11 days of storage. Differences between storage temperatures appeared toward the end of the study for *G. tikvahiae*, with samples at 7 °C scoring somewhat higher; however, temperature did not have a significant effect on its overall quality scores.

Sensory methods, using trained or untrained panels, have been used widely to measure quality of fresh vegetables, where the advantage over instrumental methods is that the samples are evaluated by the end consumer (Gómez-López et al. 2008; Barrett et al. 2010; Banerjee et al. 2016). In the current study, sensory data were extremely useful in evaluating quality deterioration in fresh *P. palmata* and *G. tikvahiae* and provided valuable results. However, the sensory training provided to panelists was limited to 30 min per species, and some variation in panelist scoring was observed. More comprehensive training following well-established protocols could help to minimize variation in sensory scores in future studies. In addition, it would be beneficial to have freshly harvested seaweed to compare with stored samples each test

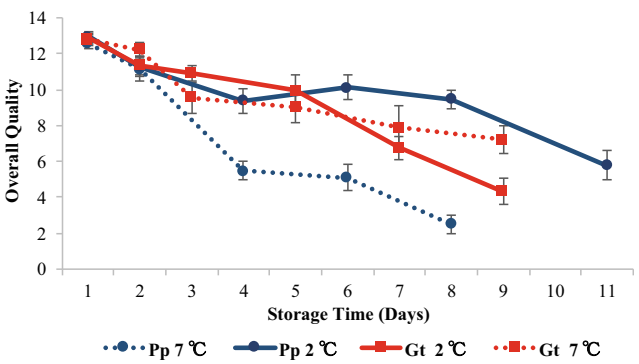

Fig. 3 Sensory overall quality scores for *P. palmata* and *G. tikvahiae* during refrigerated storage. Each value represents the mean ± standard error ($n = 8$–12, depending on the testing day)

day; however, ongoing harvest of seaweeds was not logistically feasible throughout this study. To our knowledge, there are no established quality criteria for fresh seaweeds; thus, panelists' scores and comments provided a deeper insight into sensory quality changes in *P. palmata* and *G. tikvahiae* during refrigerated storage.

Drip loss

Gracilaria stored at 2 °C lost approximately 4% cellular liquid by day 12, twice as much as *G. tikvahiae* stored at 7 °C (Fig. 4), suggesting that cellular integrity of *G. tikvahiae* was maintained better at the higher temperature. These results were consistent with the sensory and instrumental texture data. Low storage temperatures (< 10–12 °C) can cause chilling injury, causing disruption of cellular structure and the subsequent loss of cellular liquid in warm season vegetables (Lyons and Breidenbach 1987; Kader 2002), as seen in *G. tikvahiae*. Similarly, Porter et al. (2003) reported yellowing and wilting due to moisture loss in Chinese cabbage stored at 0 °C and 2 °C. Increased electrolyte leakage and chilling injury deteriorated cellular integrity of *G. salicornia* cultivated in Hawaii and stored at 10–12 °C, compared to at 15–20 °C (Paull and Chen 2008). In contrast, observed (although not measured) drip loss in *P. palmata* was higher at 7 °C than at 2 °C. Drip loss in seaweeds during storage may result in considerable economic and quality loss. A maximum of 3–5% drip loss is permissible beyond which fresh spinach and lettuce are considered unsalable (Robinson et al. 1975). Moreover, Jung et al. (2012) concluded that consumer perception of fresh spinach leaves was strongly and negatively correlated to water loss over time. Drip loss was measured in *G. tikvahiae* because a profuse loss of cellular liquid was observed in *P. palmata* over time. The thin, flat surface of *P. palmata* fronds, similar to that of green leafy vegetables could have accelerated drip loss due to increased rate of respiration (Ben-Yehoshua 1987). In summary, drip loss was a quick, easy, and reliable method to assess quality loss of fresh seaweeds during refrigerated storage.

Colourimetric analyses

P. palmata and *G. tikvahiae* lost their initial dark red color toward the end of storage, which contributed to increasing ΔE values (Fig. 5). Delta E values for *P. palmata* stored at 7 °C increased significantly over time and faster compared to samples stored at the lower temperature, clearly indicating the effect on color due to the storage temperature difference. Increasing a^* and b^* values over time were major contributors to color change in *P. palmata* samples. A rise in a^* values indicates an increase in redness, which did not match the fading of red color that was perceived by the sensory panel. The clumping of *P. palmata* by the end of the study affected the measurement of a^* value, which could be explained as an artifact effect of the wilted *P. palmata*. Contrastingly, for *G. tikvahiae*, the ΔE values were significantly lower in samples stored at 7 °C compared to samples at 2 °C. The significant effects of storage temperature and time on instrumental color agree with the sensory color results. Changes in color may be due to a loss of water-soluble phycobiliproteins, responsible for red color in *P. palmata* and *G. tikvahiae*, with increasing liquid exudation over time in both species (Gantt 1990; Paull and Chen 2008). Color of fruits and vegetables is one of the most important factors in food choice, preference, and acceptability for consumers (Clydesdale 1993; Rico et al. 2007), and deterioration of initial color contributed to the diminished quality of *P. palmata* and *G. tikvahiae* during storage.

Texture analyses

For *P. palmata*, texture profile analysis (TPA) hardness values were significantly affected by time and temperature, with the values decreasing over time and with higher storage temperature. Hardness represents the peak force during the first compression cycle of the TPA. The lower hardness values toward the end of storage indicate decreased resistance of the seaweed to compression (Table 3), which corresponded with the increased wilting that was observed. On day 12, force values

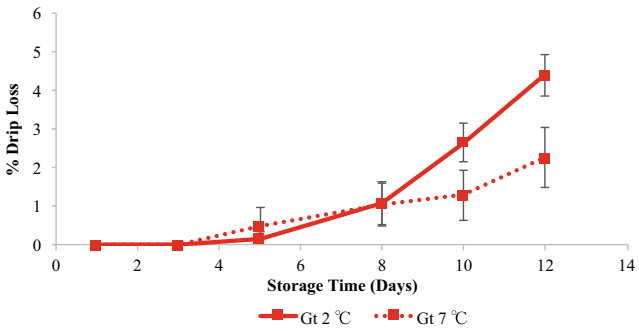

Fig. 4 Drip loss values for *G. tikvahiae* during refrigerated storage. Each value represents the mean ± standard error ($n = 3$)

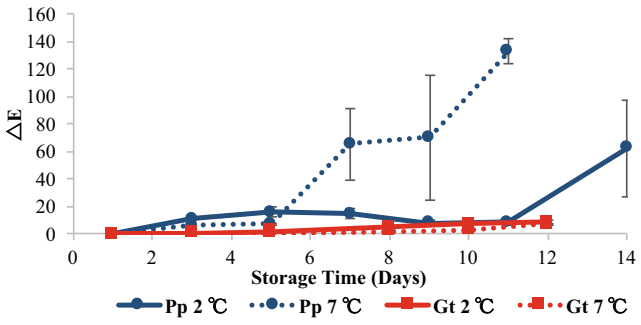

Fig. 5 Instrumental color change (ΔE) values for *P. palmata* and *G. tikvahiae* during refrigerated storage. Each value represents the mean ± standard error ($n = 3$)

Table 3 TPA force and for *P. palmata* and Kramer shear force for *G. tikvahiae* during refrigerated storage. Each value is the mean ± standard error ($n = 3$). Values not sharing a letter are significantly ($p < 0.05$) different within columns, within each treatment, analyzed by ANOVA followed by Tukey's HSD post hoc test

P. palmata			*G. tikvahiae*		
Hardness (N)			Force (N)		
Day	Pp 2 °C	Pp 7 °C	Day	Gt 2 °C	Gt 7 °C
4	162.5 ± 20.4a	121.5 ± 5.7b	2	183.4 ± 8.5a	216.2 ± 23.4a
6	91.4 ± 17.7a	91.9 ± 12.9b	4	180.8 ± 13.6a	221.2 ± 19.5a
8	124.7 ± 26.1a	35.6 ± 16.0a	6	206.8 ± 8.2ab	218.3 ± 32.4a
10	111.8 ± 41.0a	13.3 ± 4.0a	9	243.6 ± 6.0ab	213.6 ± 7.2a
12	55.8 ± 12.7a	10.4 ± 2.2a	11	262.9 ± 27.9b	200.2 ± 16.0a
			13	212.6 ± 4.1ab	184.5 ± 11.4a

for *P. palmata* stored at 2 °C were over 5 times higher than for *P. palmata* at 7 °C, indicating that the *P. palmata* fronds wilted more quickly at the higher storage temperature, which was consistent with the sensory texture values and panelist comments. Similarly, Banerjee et al. (2016) reported that cabbage samples stored at higher temperature lost firmness faster compared to samples stored at lower temperature. In plant cells, turgor pressure is responsible for crispness, and in conjunction with cell wall and adjacent polysaccharides contributes to the overall texture of vegetables (Martens and Baardseth 1987, Waldron et al. 2003). As drip loss in vegetables increases due to transpiration and respiration postharvest, cell polymers, particularly the cell wall polysaccharides agar and carrageenan (in the case of red seaweeds) (Rioux et al. 2017) break down, minimizing cell adhesion and resulting in a soft, wilted texture (Buren 1979; Waldron et al. 1997, 2003; Kader 2002). The excessive water loss observed in *P. palmata*, particularly samples stored at the higher storage temperature, caused the smooth, flat *P. palmata* fronds to shrivel and wilt during storage.

In contrast to instrumental texture for *P. palmata*, shear force values for *G. tikvahiae* were not affected by time or temperature. Shear force values for *G. tikvahiae* at 2 °C increased significantly over time. However, force values for *G. tikvahiae* at 7 °C did not change significantly. Paull and Chen (2008) also assessed *G. tikvahiae* texture during refrigerated storage using a Kramer shear force method and reported that the samples became limp over time; however, the data were highly variable.

Texture for *P. palmata* and *G. tikvahiae* were assessed using two different methods, texture profile analysis using compression and Kramer shear using shear force, respectively. Although a few overall inferences can be made, the data from the two methods cannot be directly compared since there is no established protocol to convert the two data. Texture is a crucial parameter for assessing quality in vegetables since decreased textural quality negatively impacts appearance and sales of produce (Kader 2002). However, the instrumental texture results were highly variable for both methods used. Although texture deteriorated visibly for both the species, the instrumental methods used were not as responsive to change as the sensory texture evaluation results, particularly for *G. tikvahiae*. The lack of uniformity in texture among and within the fronds contributed to the high variability, although sample heights were standardized during texture analysis due to the obvious wilting of the seaweeds over time. However, standardizing the mass may reduce variability. Moreover, alternate instrumental methods other than TPA and Kramer shear could be explored to assess changes in texture over time. In general, TPA results are based on two compressions and assess multiple parameters including hardness, resilience, adhesiveness, gumminess, and chewiness. However, the stacked cutouts of *P. palmata* slid out of the stack post the first compression at multiple occasions, making the data derived from second compression unreliable. Hence, only hardness values, based on the first compression, were reported in this paper.

Soluble protein

The values for soluble protein ranged from 0.1 ± 0.0 to 3.4 ± 0.7 mg g^{-1} for both the seaweeds with no significant differences observed over time in either species regardless of storage temperature (data not shown). In contrast, Paull and Chen (2008) reported losses of as much as 50% soluble protein in fresh *G. salicornia* after 6 days of storage. Differences between studies could be due to different species or to the much higher storage temperatures (16 and 21 °C) used in that study. However, for fresh *P. palmata* and *G. tikvahiae* stored at 2 and 7 °C, soluble protein analysis was not a useful method of tracking quality changes during refrigerated storage. Because the high polysaccharide content in seaweeds can hinder effective protein extraction, pretreatment with osmotic shock as reported by Harnedy and FitzGerald (2013) should be considered to disrupt cells and maximize the extraction of water-soluble proteins.

Conclusions

Palmaria palmata and *G. tikvahiae* followed opposite trends with regard to effects of storage temperature on quality over time. Quality parameters assessed in this study showed that *P. palmata* quality was maintained better at 2 °C, compared to at 7 °C. In contrast, the higher storage temperature preserved quality better in *G. tikvahiae*. Drip loss, wilted texture, and fading of the initial red color were the leading causes of quality loss in fresh *P. palmata* and *G. tikvahiae* during refrigerated storage. Although microbial activity did not increase

consistently in *P. palmata* over time, *G. tikvahiae* microbial counts continued to increase throughout storage. Based on findings of this study, drip loss, sensory evaluation, instrumental color, and aerobic plate counts yielded reliable and useful results, while instrumental texture analyses and soluble protein did not provide consistent data. In summary, this study provides essential information on quality changes of *P. palmata* and *G. tikvahiae* during refrigerated storage, which can support the food industry to promote the distribution and consumption of seaweeds as fresh vegetables. Additionally, the analytical methods used in this study may facilitate the development of established procedures to assess quality and shelf life of fresh seaweeds in future studies.

Acknowledgments This project was funded by the USDA Value Added Producer Grant program GLS494606226. We are thankful to Maine Fresh Sea Farms (MFSF) for providing funds and fresh seaweeds. This project was supported by the USDA National Institute of Food and Agriculture, Hatch Project Number ME0-21410 through the Maine Agricultural & Forest Experiment Station. Maine Agricultural and Forest Experiment Station Publication Number 3611.

Compliance with ethical standards

Approval for research with human subjects was obtained from the Institutional Review Board (IRB) prior to conducting sensory analyses.

Conflict of interest The authors declare that they have no conflict of interest.

References

Abbott IA (1978) The uses of seaweed as food in Hawaii. Econ Bot 32: 409–412

Banerjee A, Chatterjee S, Variyar PS, Sharma A (2016) Shelf life extension of minimally processed ready-to-cook (RTC) cabbage by gamma irradiation. J Food Sci Technol 53:233–244

Barrett DM, Beaulieu JC, Shewfelt R (2010) Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. Crit Rev Food Sci Nutr 50:369–389

Bell T (2014) In Maine, kelp is on the way from Ocean Approved. Portland Press Herald. https://www.pressherald.com/2014/05/01/winning_recipe__frozen_kelp/; searched on 25 April 2018

Ben-Yehoshua S (1987) Transpiration, water stress, and gas exchange. In: Weichmann J (ed) Postharvest physiology of vegetables. Marcel Dekker, New York, pp 113–170

Bever F (2016) Maine seaweed: The next super-food? http://mainepublic.org/post/maine-seaweed-next-super-food; searched on 17 March 2017

Bigliardi B, Galati F (2013) Innovation trends in the food industry: the case of functional foods. Trends Food Sci Technol 31:118–129

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Buren JV (1979) The chemistry of texture in fruits and vegetables. J Texture Stud 10:1–23

Clydesdale FM (1993) Color as a factor in food choice. Crit Rev Food Sci Nutr 33:83–101

Cox S, Abu-Ghannam N, Gupta S (2010) An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J 17:205–220

Debevere J (1996) Criteria en praktische methoden voor de bepaling van de houdbaarheidsdatum in de etikettering. Etikettering, houdbaarheid en bewaring (voedingsmiddelen en recht 2). Die Keure, Brugge p 37–64

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of the red Algae. Cambridge University Press, Cambridge, pp 203–221

Gast KLB (2001) Storage Conditions Fruits & Vegetables. Postharvest Management of Commercial Horticulture Crops. Bulletin no. 4135. The University of Maine, USA

Gómez-López VM, Ragaert P, Jeyachchandran V, Debevere J, Devlieghere F (2008) Shelf-life of minimally processed lettuce and cabbage treated with gaseous chlorine dioxide and cysteine. Int J Food Microbiol 121:74–83

Gupta S, Rajauria G, Abu-Ghannam N (2010) Study of the microbial diversity and antimicrobial properties of Irish edible brown seaweeds. Int J Food Sci Technol 45:482–489

Harnedy PA, FitzGerald RJ (2013) Extraction of protein from the macroalga *Palmaria palmata*. LWT Food Sci Technol 51:375–382

Jung YJ, Padmanabahn A, Hong JH, Lim J, Kim KO (2012) Consumer freshness perception of spinach samples exposed to different storage conditions. Postharvest Biol Technol 73:115–121

Kader AA (2002) Postharvest biology and technology: an overview. In: Kader AA (ed), Postharvest technology of horticultural crops. Oakland, CA: University of California, Division of Agricultural Resources pp 39–48

LePape MA, Grua-Priol J, Demaimay M (2002) Effect of two storage conditions on the odor of an edible seaweed, *Palmaria palmata*, and optimization of an extraction procedure preserving its odor characteristics. J Food Sci 67:3135–3139

Liot F, Colin A, Mabeau S (1993) Microbiology and storage life of fresh edible seaweeds. J Appl Phycol 5:243–247

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Lyons JM, Breidenbach RW (1987) Chilling injury. In: Weichmann J (ed) Postharvest physiology of vegetables. Marcel Dekker, New York, pp 305–326

Martens M, Baardseth P (1987) Sensory quality. In: Weichmann J (ed) Postharvest physiology of vegetables. Marcel Dekker, New York, pp 413–426

Meilgaard MC, Carr TB, Civelle GV (2006) Sensory evaluation techniques. CRC Press, New York

Mouritsen OG, Dawczynski C, Duelund L, Jahreis G, Vetter W, Schröder M (2013a) On the human consumption of the red seaweed dulse (*Palmaria palmata* (L.) Weber & Mohr). J Appl Phycol 25:1777–1791

Mouritsen OG, Johansen M, Mouritsen JD (2013b) Seaweeds: edible, available and sustainable. University of Chicago Press, Chicago

Paull RE, Chen NJ (2008) Postharvest handling and storage of the edible red seaweed *Gracilaria*. Postharvest Biol Technol 48:302–308

Perry Phytoplankton & Optics Lab (2010) Dock sampling. http://perrylab.umeoce.maine.edu/docksampling.php; searched on 10 October 2016

Pols M (2015) With more varieties and growing demand, seaweed is Maine's crop to watch. Portland Press Herald. https://www.pressherald.com/2015/12/06/with-more-varieties-and-growing-demand-seaweed-is-maines-crop-to-watch/; searched on 25 April 2018

Porter KL, Klieber A, Collins G (2003) Chilling injury limits low temperature storage of 'Yuki' Chinese cabbage. Postharvest Biol Technol 28:153–158

Raso J, Barbosa-Cánovas GV (2003) Nonthermal preservation of foods using combined processing techniques. Crit Rev Food Sci Nutr 18: 373–386

Rico D, Martin-Diana AB, Barat JM, Barry-Ryan C (2007) Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci Technol 18:373–386

Rioux LE, Beaulieu L, Turgeon SL (2017) Seaweeds: a traditional ingredients for new gastronomic sensation. Food Hydrocoll 68:255–265

Robinson JE, Browne KM, Burton WG (1975) Storage characteristics of some vegetables and soft fruits. Ann Appl Biol 81:399–408

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol 16:245–262

Smith DG, Young EG (1953) On the nitrogenous constituents of *Fucus vesiculosus*. J Biol Chem 205:849–858

Tarver T (2015) Sea-ing a better way to feed the world. Food Technol 69: 22–29

Waldron KW, Smith AC, Parr AJ, Ng A, Parker ML (1997) New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. Trends Food Sci Technol 8:213–221

Waldron KW, Parker ML, Smith AC (2003) Plant cell walls and food quality. Compr Rev Food Sci Food Saf 2:128–146

Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire ME, Brawley SH (2017) Algae as nutritional and functional food sources: revisiting our understanding. J Appl Phycol 29:949–982

White S, Keleshian M (1994) A field guide to economically important seaweeds of northern New England. University of Maine/University of New Hampshire Sea Grant Marine Advisory Program MSG-E-93-16