AN ABSTRACT OF THE THESIS OF Susanne de Crinis Wilkins for the Master of Science in Biology presented June 30, 1986.

Title: Effects of Post-Harvest Holding Conditions on the Quality of Agar Extracted from Two Species of Gracilaria (Rhodophyta) from Guam.

Approved: 

Stephen G. Nelson, Chairman, Thesis Committee

Post-harvest holding conditions significantly altered the quality of agar extracted from Gracilaria thalli. Thalli held in darkness produced gels with greater strengths than those exposed to light. However, in unenriched systems there was no improvement in gel strengths of agar extracts in relation to those from recently harvested thalli. The most dramatic improvements in gel strength were produced with weekly 1-h exposure to nitrogen enrichment. Post-harvest enrichment of thalli with 750 uM nitrogen resulted in extracts with greatest gel strength. There was a significant correlation between gel strength and thallus nitrogen levels for thalli in the

enriched post-harvest treatment. Additional physical characteristics of agar (agar yield, dynamic gelling temperature, relative viscosity, and ash content) were not correlated with gel strength.

TO THE OFFICE OF GRADUATE SCHOOL AND RESEARCH

The members of the Committee approve the thesis of Susanne de Crinis Wilkins presented June 30, 1986.

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Date

EFFECTS OF POST-HARVEST HOLDING CONDITIONS ON THE QUALITY OF AGAR EXTRACTED FROM TWO SPECIES OF GRACILARIA (RHODOPHYTA) FROM GUAM

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

University of Guam

ACKNOWLEDGEMENTS

I would like to thank the faculty and staff of the Marine Laboratory for their help and support. This study was supported, in part, by grants from the Office of Sea Grant (UG/R-1) and the USDA HATCH program (GUO32) to S. G. Nelson, University of Guam.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The rapid expansion of the agar industry in recent years has resulted in the increasingly limited availability of phycocolloid-bearing seaweeds for this industry, and overharvesting of some species. This has stimulated world-wide attempts to find additional species with extracts suitable for industrial use (Matsuhashi and Hayashi, 1972; Doty and Alvarez, 1973; Oza, 1978; Pringle, 1979; Abbott, 1980; Whyte and Englar, 1980; Whyte et al., 1981; Nelson et al., 1983). Species of the algal genus Gracilaria have received considerable attention, since many have been shown to contain high grade agar and are abundant enough in some areas to be harvested. In addition, several Gracilaria species have been cultivated on a commercial scale, most notably in southern Taiwan (Chen, 1976; Wang and Yang, 1980; Yang et al., 1981; Yang, 1982).

A number of Gracilaria species occur in Micronesia (Tsuda, 1984), and agar extracts from a few of these have been examined. Nelson et al. (1983) reported that suitable yields of agar extracts could be obtained from Guam species of Gracilaria, but that these had relatively low gel strengths (100-240 g cm^{-2}). If the gel strengths of these

extracts could be improved, then the potential for a Gracilaria-based agar industry in Micronesia would be enhanced.

Agar quality is influenced by a variety of factors including the environmental conditions under which the thalli are grown. Seasonality, for example, has been reported to affect both the yield and gel strength of agar from natural seaweed populations (DeBoer and Ryther, 1977; Oza, 1978; Hoyle, 1978; Whyte et al., 1981). Similar patterns of seasonality have been reported for thalli cultured in ponds (Wang and Yang, 1980; Yang et al., 1981; Yang, 1982). In general, agar gel strength is inversely correlated with the length of the photoperiod (Bird et al., 1978; Wang and Yang, 1980; Yang, 1982) and inversely related to thallus growth rate (Hoyle, 1978).

The nutrient concentrations in which the thalli are grown also affect agar quality. Gracilaria thalli grown under controlled conditions and exposed to high levels of ammonium nitrogen fertilization produce agar with relatively high gel strengths (DeBoer, 1978; and Bird et al., 1981).

Although in many cases it may not be feasible to control environmental conditions during culture, the agar quality could possibly be improved by manipulation of environmental conditions in post-harvest treatments. Fuller and Mathieson (1972) and Mathieson (1975) reported

that the agar yields from Chondrus crispus were improved by holding the thalli in tanks for short periods after harvest, and it seems likely that agar extract quality from Gracilaria thalli also could be improved by postharvest treatments which limit growth and/or increase available nitrogen.

This study was designed to examine the effects of post-harvest holding regimes on the yield and quality of agar from wild-harvested thalli of two Guam species of Gracilaria. Post-harvest comparisons of agar gel strength and other properties were made for thalli which were: 1) maintained either under a natural photoperiod or in complete darkness, and 2) exposed to varying degrees of fertilization by periodic, short-term exposures to dissolved ammonium.

MATERIALS AND METHODS

Collection and Maintenance of Algae

The seaweeds used in the post-harvest treatments were collected from various areas around Guam. The species of Gracilaria used during this study are currently undergoing taxonomic revisions and are therefore referred to as Gracilaria salicornia and Gracilaria sp. Thalli of Gracilaria salicornia were harvested from the Piti Channel area of Apra Harbor, while those of Gracilaria sp. were harvested from Sella Bay and Cetti Bay on the southwestern coast. The freshly collected thalli were brought to the University of Guam Marine Laboratory and rinsed with seawater to remove sediment, epiphytes and visible epizooans.

Two sets of experiments were conducted. For each set, 1,000 g (wet weight) of thalli were distributed into each of a series of outdoor flow-through tanks (80x50x40 cm). Seawater flow in the tanks varied from 200 to 360 l h^{-1} depending on the tide level and on the condition of the laboratory seawater pump.

Salinity and water temperature were measured daily. Salinity was measured with a refractometer and ranged from 33 to 36‰; water temperature varied from 27 to 31° C.

Ambient light intensities were determined as photon flux density ($\mu E\ m^{-2}\ s^{-1}$) with a Licor quantum meter with underwater sensors. Measurements were taken at noon and ranged from 150-450 $\mu E\ m^{-2}\ s^{-1}$ in the exposed tanks.

Light vs. Dark Treatment Protocol

The first set of experiments was designed to determine whether light conditions in the post-harvest holding conditions influence the quality of agar extracts. Thalli were exposed in unenriched seawater either to a natural, 12-h, photoperiod or to complete darkness. Two consecutive two-week trials were conducted with Gracilaria salicornia and three with Gracilaria sp.. In each trial, after the first week 500 g (wet weight) of the thalli were removed from the holding tanks, and the remaining thalli were collected at the end of the second week. After they were removed from the tanks, the thalli were washed, sun-dried to remove most of the moisture, and oven-dried to constant weight. Agar was extracted from the thalli and analysed as described below.

Gracilaria salicornia was very difficult to extract and was therefore not used during the second part of the experiment.

Nutrient Enrichment Protocol

The second set of experiments was designed to determine the effect of nitrogen fertilization on the

quality of agar extracts. Only thalli of Gracilaria sp. were used in these trials. Each of the three trials in this set consisted of a series of two weekly exposures of the thalli to one of five concentrations of dissolved ammonium (control, 325, 750, 1500, or 2000 μM NH_4-N). At the beginning of each week, thalli were removed from the tanks and briefly rinsed in filtered seawater, then placed into plastic 80-l aquaria and exposed to the appropriate concentration of dissolved ammonium under gentle aeration for 1 h. The thalli were then returned to the holding tanks. As in the first set of experiments, 500 g (wet weight) of the thalli were harvested after the first week. The experiment was terminated at the end of the second week, and the rest of the seaweed was collected. The thalli were then dried and weighed as described above. Agar extraction and analyses were conducted as described below.

Agar Extraction and Analysis

Replicate agar extractions were made from thalli from each of the treatments and of the pretreated seaweeds. The extraction methods were similar to those used in commercial agar production and by other investigators (Kim, 1970; Wang and Yang, 1980; Durairatnam and Santos, 1980). Samples of clean seaweed, dried (5°C) to constant weight, were treated for 15 min in a preheated 2% aqueous solution of NaOH at 94°C (1:15, w/v). The duration and strength of the alkaline pretreatment chosen for the two species had

been determined from preliminary trials. The seaweeds were then rinsed overnight (12 h) with running tap water. The suspension in the beakers containing the thalli was brought to its original volume and the pH was adjusted to 6.5-7.0 with a 0.03% acetic acid solution. The thalli were then gently boiled for 2 h. The resultant solution was strained through an 8-ply cheese cloth in a stainless steel pressure filter and filtered again through an 8-ply muslin filter on a 20-cm Buchner funnel to remove any remaining algal fragments. The filtered extracts were then allowed to gel overnight in a refrigerator at 4^{0}C, and the gels were sliced and frozen at -5^{0}C for 24 h. The frozen agar was thawed at room temperature over nylon screen and oven-dried at 50^{0}C to constant weight. The samples were then cooled to room tempurature (24^{0}C) over silica gel in a desiccator and weighed. The agar content, expressed as clean anhydrous yield (Santos, 1980), was determined gravimetrically and expressed as a percentage of thallus dry weight.

The gel strengths of the extracts were determined with a gelometer developed by the Japan Agar Industry (Matsuhashi and Hayashi, 1972) and described in detail by Kim (1970). The gel strengths of replicate samples of 1.5% agar gels were determined in 50-ml beakers. Gel strength was calculated as the pressure, in grams on a 1-cm diameter plunger, which the gel could resist for 20 seconds at 20^{0}C

(see Nelson et al., 1983). Two measurements of gel strength were taken for each of the extraction replicates. Measurements taken at slightly higher temperatures as a result of fluctuations in room temperature were adjusted according to the tables provided by Kim (1970).

An Ostwald viscometer was used to measure the viscosity of 0.06% agar solutions at 65^{0}C (Kim, 1970; Whyte and Englar, 1980) with deionized reagent grade water as a reference.

Stoppered 12-cm test tubes (I.D. 18 mm) containing 10 ml of a 1% agar solution were used to measure the dynamic gelling temperature (gel point) of the agar extracts (Yang, 1982). A thermometer was inserted through the stopper into the center of the tube. While the samples were cooled in a water bath at a rate of 2^{0}C min^{-1}, a 3-mm diameter glass ball was placed on the surface of the agar at every 1^{0}C change in temperature of the solution. The temperature at which the surface of the agar solution could support the glass ball was recorded as the dynamic gelling temperature (Yang, 1982).

The nitrogen content of subsamples of thalli within each experimental treatment was determined in duplicate after micro-kjeldahl digestion (Gallaher et al., 1976). The thalli were digested in sulfuric acid with a mixture of sodium thiosulfate and cupric sulfate (100:1, w:w) added as

a catalyst. The nitrogen concentration of the digestate was determined with an Orion 95-10 gas-sensing ammonia probe attached to an Orion model 901 ionalyzer (Gallaher et al., 1976). An amino acid, L-phenylalanine, was used as a standard.

Statistical analyses, including one way analyses of variance (anova) and the calculation of correlation coefficients, were performed with the use of the Systat statistical program; Systat, Inc. Leland Wilkinson 603 Main St. Evanson, Il. 60202, on an IBM - PC computer.

RESULTS

Light vs.Dark

The results of post-harvest treatment of thalli in unenriched systems exposed to either light or complete darkness were ambiguous since the two algal species reacted differently to the treatments. As the results in Table 1 show, the trials with Gracilaria salicornia demonstrated no significant differences in gel strength between treatments in the open versus closed tanks (F_s=2.84, $p>0.05$). However, the gel strength following the dark post-harvest holding treatment was slightly higher (195.5 g cm^{-2}) than the initial gel strength (181.5 g cm^{-2}) by the end of the second week (Figure 1). As shown in Table 2, analysis of the trials involving Gracilaria sp. indicated significant differences in gel strength between the holding conditions (F_s=13.46, $p<0.05$). Agar samples from thalli held in closed tanks had higher gel strengths than those from thalli held in open tanks for both weeks after harvest (Figure 2). However, the gel strengths resulting from the post-harvest treatments were not greater than those of the initial samples.

Additional physical characteristics of the agar, such as agar yield, dynamic gelling temperature, relative

Table 1. Anova results comparing gel strengths of agar extracted from Gracilaria salicornia over a 2-week post-harvest holding period in open and closed tanks.

source	sum-of-squares	df	mean-square	F-ratio	P
(1) week	370.562	1	370.562	.332	.575
(2) treatment	3164.063	1	3164.063	2.836	.118
(1)*(2)	3335.063	1	3335.063	2.990	.109
error	13386.650	12	1115.554		

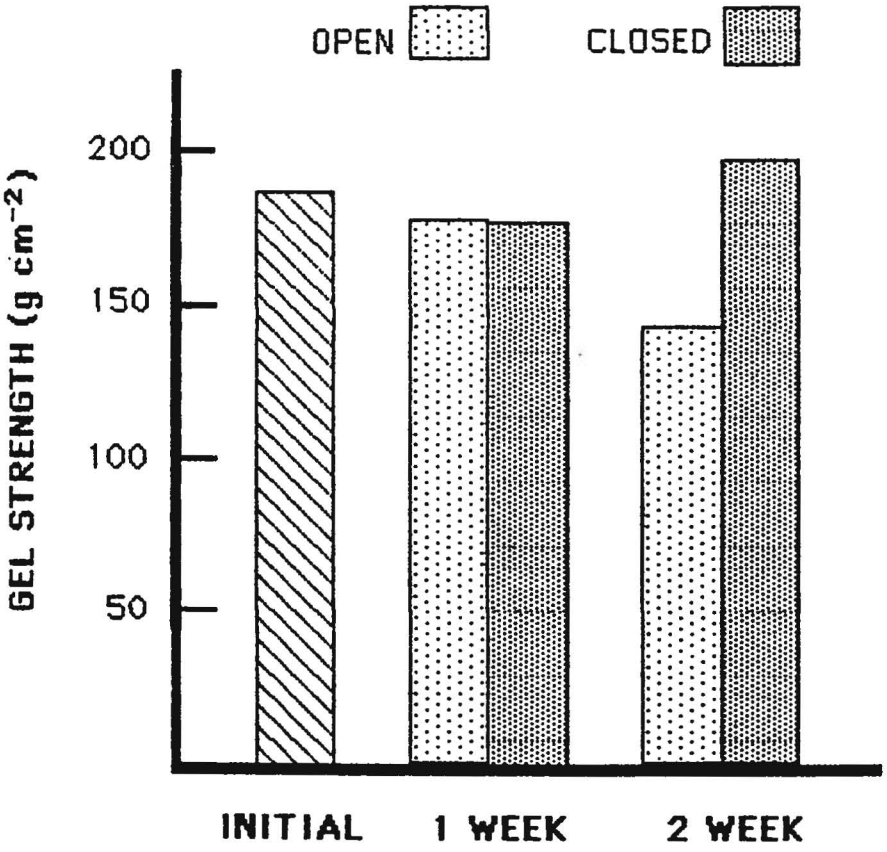

Figure 1. Gel strengths of agar extracted from recently harvested thalli (initial) of Gracilaria salicornia and of thalli held over a 2-week period in open and closed tanks.

Table 2. Anova results comparing gel strengths of agar extracted from Gracilaria sp. over a 2-week post-harvest holding period in open and closed tanks.

source	sum-of-square	df	mean-square	F-ratio	P
(1) week	6.000	1	6.000	.040	.843
(2) treatment	2016.667	1	2016.667	13.457	.002
(1)*(2)	42.667	1	42.667	.285	.600
error	2997.167	20	149.858		

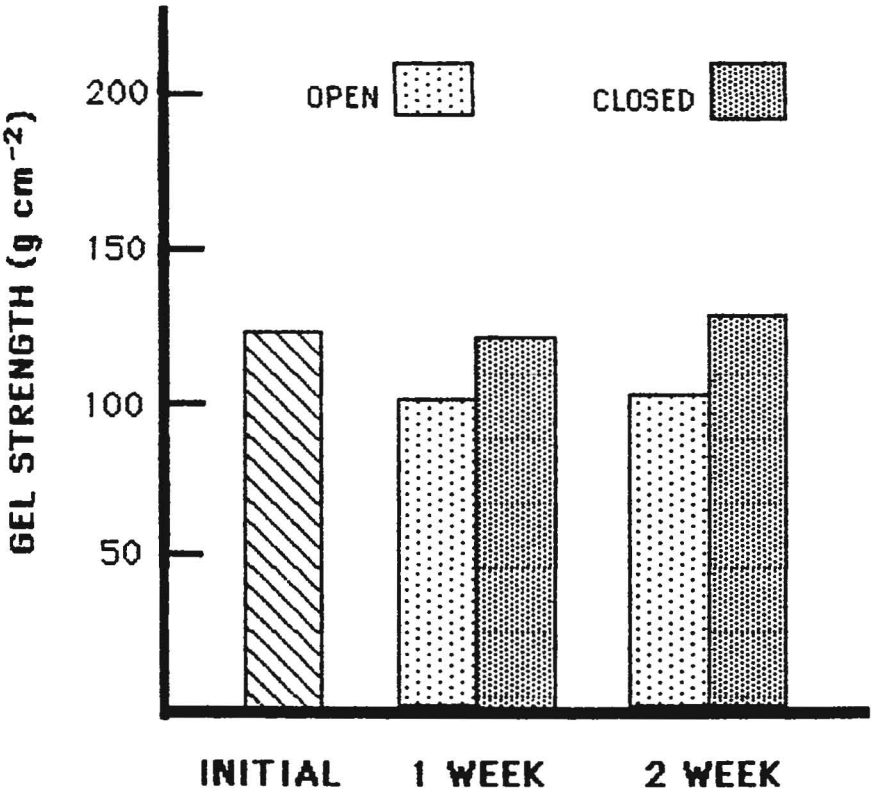

Figure 2. Gel strengths of agar extracted from recently harvested thalli (initial) of Gracilaria sp. and of thalli held over a 2-week period in open and closed tanks.

viscosity, and ash content were examined, but no apparent pattern in relation to treatment emerged. Tables 3 and 4 provide a summary of these properties.

Nutrient Erichment

As shown in Table 5, post-harvest holding of thalli of Gracilaria sp. in nitrogen-enriched systems resulted in significant differences in gel strengths between enrichment levels ($F_s=5.47$, $p<0.05$). Thalli grown at the 750 μM enrichment level produced agar with higher gel strength (145.8 g cm^{-2}) than either the other treatment groups or the pretreatment group (Figure 3). The agar samples from this enrichment level also increased in gel strength each week of the post-harvest treatment. Also, combining the data from all of the enrichment treatments demonstrates (Figure 4) that the gel strengths were significantly correlated with post-harvest thallus nitrogen content ($r=0.512$, $p<0.05$).

Again, other physical characteristics of the agar were examined; however there were no apparent patterns in relation to post-harvest treatment. These properties are summarized in Table 6.

Table 3. Summary of effects of 2-week holding periods in open and closed tanks on thallus nitrogen content (%), and on agar yield (%), dynamic gelling temperature (^{0}C), relative viscosity (ηrel), ash content(%) of extracts of Gracilaria salicornia. Values represent the mean of 2 replicates and standard deviation.

replicates	post-harvest treatment	thallus nitrogen (%)	agar yield (%)	dynamic gelling temperature (^{0}C)	relative viscosity (ηrel)	ash (%)
1 initial		2.9±0.0	22.0±1.4	42.6±0.1	1.4±0.0	3.9±0.4
week 1	open	2.8±0.2	19.0±1.8	43.0±0.0	1.3±0.2	4.9±0.1
	closed	2.4±0.3	20.0±0.0	44.6±0.1	1.4±0.1	4.0±0.1
week 2	open	2.8±0.1	20.0±2.1	42.8±0.2	1.1±0.0	4.4±0.6
	closed	3.1±0.4	17.0±1.5	44.5±0.2	1.2±0.0	3.6±0.1
2 initial		2.4±0.2	21.0±1.8	44.0±0.1	1.4±0.3	4.0±0.1
week 1	open	2.1±0.2	27.0±1.4	45.0±2.8	1.2±0.0	4.6±0.4
	closed	1.8±0.4	24.0±3.5	43.8±2.8	1.4±0.6	5.4±0.7
week 2	open	2.1±0.3	30.0±2.8	45.0±0.0	1.2±0.5	3.5±0.1
	closed	2.6±0.4	22.0±1.4	43.8±0.2	1.3±0.1	4.7±0.4

1. gel-strength measured with 1.5% agar at 20^{0}C 2. dynamic gelling temperature measured with 1% agar 3. viscosity measured with 0.06% agar at 40^{0}C

Table 4. Summary of effects of 2-week holding periods in open and closed tanks on thallus nitrogen content (%), and on agar yield (%), dynamic gelling temperature (^{0}C), relative viscosity (ηrel), ash content (%) of extracts of Gracilaria sp. Values represent the mean of 2 replicates and standard deviation.

replicates	post-harvest treatment		thallus nitrogen (%)	agar yield (%)	dynamic gelling temperature (^{0}C)	relative viscosity (ηrel)	ash (%)
1	initial		2.0±0.6	40.0±3.5	42.0±0.1	1.4±0.0	6.2±0.1
	week 1	open	1.3±0.6	36.0±6.9	41.2±0.1	1.5±0.0	6.6±0.1
		closed	2.0±0.3	25.0±5.6	41.0±1.4	1.3±0.2	6.3±0.1
	week 2	open	2.8±0.3	29.0±2.8	41.5±0.6	1.7±0.3	6.3±0.4
		closed	2.8±0.1	28.0±2.0	40.5±0.7	1.5±0.1	7.5±0.3
2	initial		2.4±0.3	51.0±1.4	40.5±0.2	1.5±0.3	6.8±0.7
	week 1	open	1.8±0.0	50.0±4.9	41.2±0.4	1.5±0.4	7.2±0.3
		closed	2.1±0.5	44.0±3.5	40.8±0.4	1.3±0.1	7.6±0.8
	week 2	open	2.0±0.1	26.0±2.1	40.0±0.0	1.4±0.1	6.9±0.3
		closed	2.8±0.3	22.0±1.6	39.0±0.0	1.2±0.1	6.6±0.4
3	initial		3.1±0.1	20.0±2.8	38.5±0.7	1.2±0.1	6.6±0.1
	week 1	open	2.0±0.3	20.0±0.7	39.0±0.5	1.4±0.1	5.8±0.6
		closed	2.4±0.1	20.0±0.7	39.8±0.4	1.3±0.0	6.5±0.3
	week 2	open	3.0±0.1	20.0±0.0	39.5±0.4	1.4±0.1	7.4±0.7
		closed	2.8±0.3	19.0±0.7	39.4±0.1	1.2±0.3	6.2±0.4

1. gel-strength measured with 1.5% agar at 20^{0}C 2. dynamic gelling temperature measured with 1% agar 3. relative viscosity measured with 0.06% agar at 40^{0}C

Table 5. Anova results comparing gel strengths of agar extracted from Gracilaria sp. over a 2-week post-harvest holding period with different levels of nitrogen enrichment.

source	sum-of-squares	df	mean-square	F-ratio	P
(1) week	330.031	1	330.031	1.394	.244
(2) treatment	5175.865	4	1293.966	5.467	.001
(1)*(2)	562.500	4	140.625	.594	.669
error	9940.251	42	236.673		

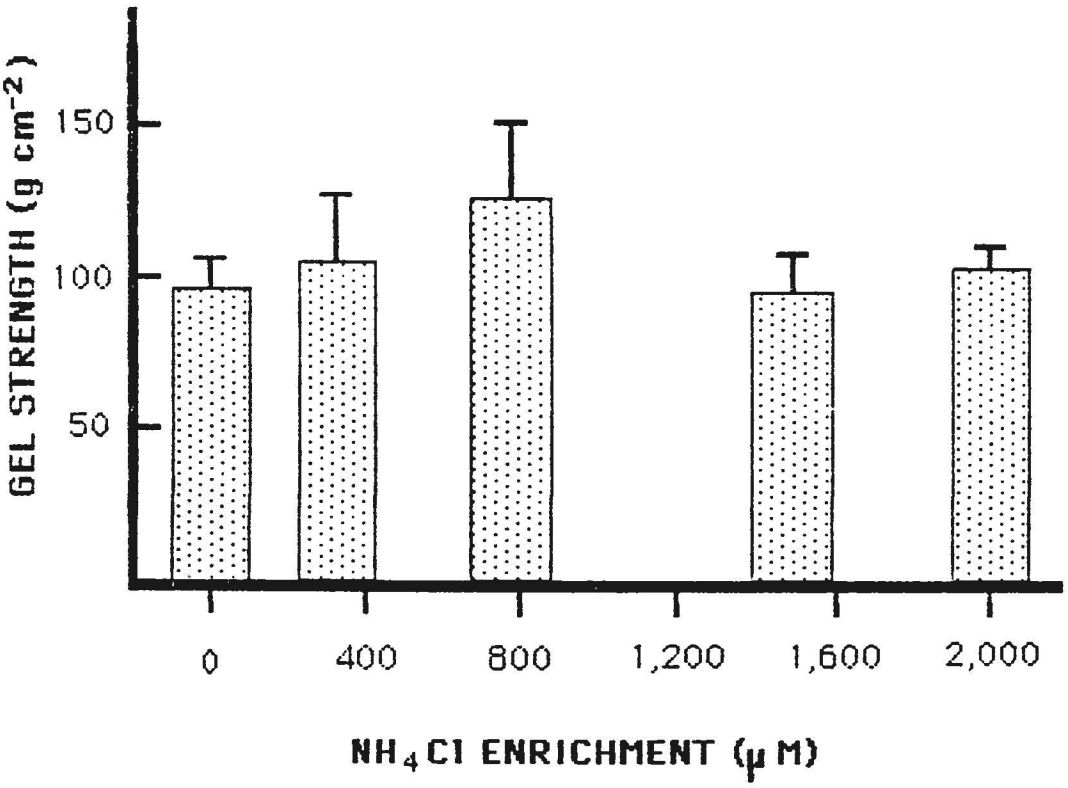

Figure 3. Gel strengths of agar extracted from Gracilaria sp. over a 2-week post-harvest holding period with different levels of nitrogen enrichment and standard deviation.

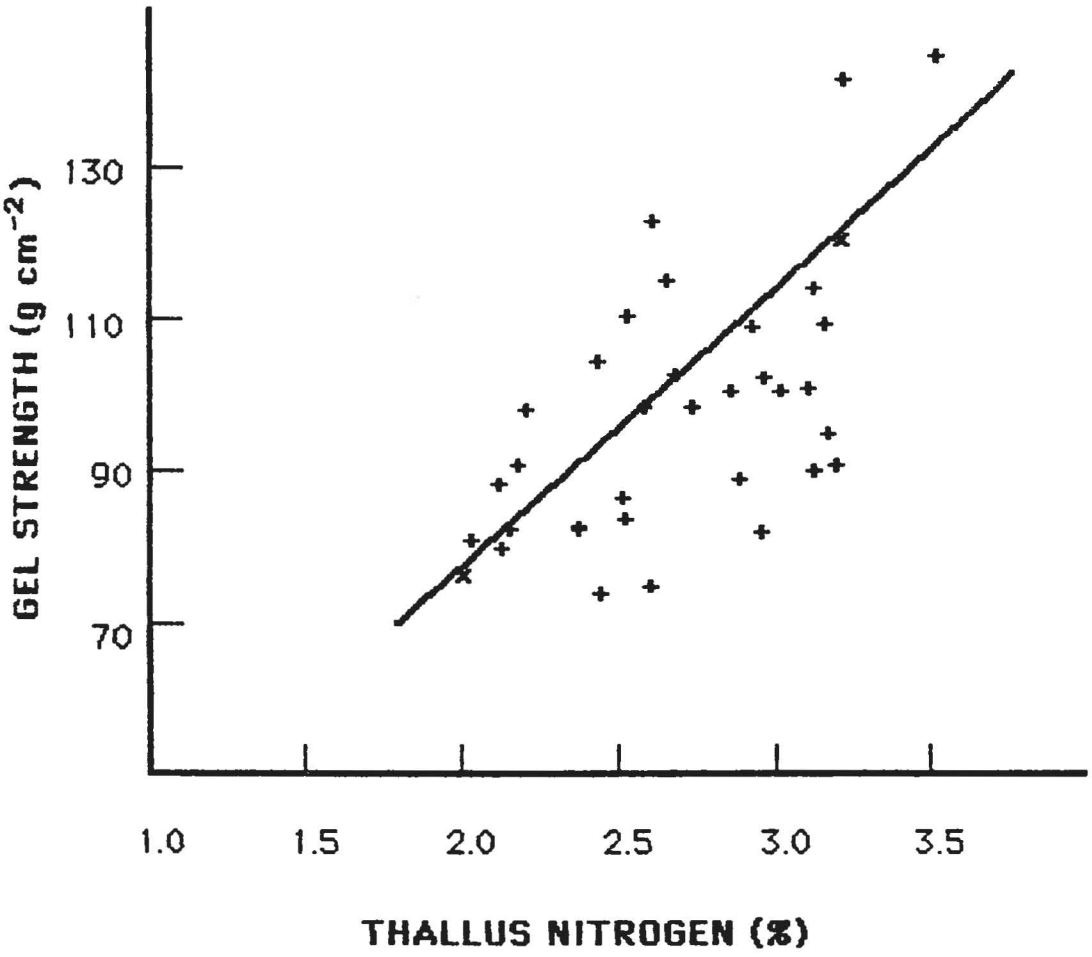

Figure 4. Plot of the gel strengths and the thallus nitrogen content for Gracilaria sp. held over 2-week period with different levels of nitrogen enrichment. The line was fitted by the least squares method.

Table 6. Summary of effects of nitrogen fertilization (NH_4Cl) during a 1- to 2-week post-harvest holding period on thallus nitrogen content (%), and on agar yield (%), dynamic gelling temperature (°C), relative viscosity (η_{rel}), ash content (%) of extracts of Gracilaria sp. Values represent the mean of 2 replicates and standard deviation.

replicates	post harvest treatment	thallus nitrogen (%)	agar yield (%)	dynamic gelling temperature (°C)	relative viscosity (η_{rel})	ash (%)
1 initial		2.1±0.3	18.0±2.1	40.0±0.4	1.4±0.1	6.3±0.5
week 1	ambient seawater	2.8±0.1	15.0±0.0	40.8±0.3	1.3±0.0	6.5±0.3
	325 µM	2.5±0.1	18.0±0.0	40.0±0.0	1.4±0.0	6.5±0.2
	750 µM	3.3±0.1	15.0±0.7	41.2±2.8	1.4±0.2	5.6±0.3
	1500 µM	2.5±0.0	21.0±0.6	41.0±0.7	1.3±0.1	6.4±0.3
week 2	ambient seawater	2.7±0.3	25.0±2.0	40.6±0.4	1.6±0.2	6.0±0.0
	325 µM	2.6±0.1	18.0±0.7	41.4±0.2	1.2±0.0	5.8±0.3
	750 µM	3.5±0.4	20.0±0.7	41.1±0.1	1.4±0.1	5.8±0.4
	1500 µM	3.1±0.3	18.0±0.7	41.0±0.0	1.3±0.0	5.9±0.1
2 initial		1.8±0.4	19.0±0.0	39.5±0.4	1.2±0.1	6.1±0.7
week 1	ambient seawater	2.0±0.4	16.0±0.3	39.8±0.8	1.4±0.0	6.6±0.7
	325 µM	2.4±0.3	17.0±0.1	39.0±0.7	1.2±0.0	6.0±0.2
	750 µM	2.2±0.6	14.0±0.3	39.8±0.2	1.3±0.1	7.2±0.3
	1500 µM	2.6±0.3	11.0±0.0	39.0±0.8	1.3±0.2	5.8±0.1
	2000 µM	2.3±0.3	10.0±0.5	40.0±0.6	1.3±0.0	5.9±0.2
week 2	ambient seawater	2.1±0.0	22.0±2.1	38.1±0.0	1.2±0.1	6.2±0.4
	325 µM	2.8±0.1	18.0±1.4	39.3±1.1	1.3±0.3	6.0±0.6
	750 µM	2.6±0.1	22.0±0.7	40.4±0.6	1.4±0.0	7.1±0.5
	1500 µM	2.7±0.2	15.0±0.0	40.0±0.4	1.2±0.1	6.6±0.3
	2000 µM	2.4±0.1	16.0±1.4	40.6±0.3	1.3±0.0	6.0±0.3
3 initial		2.4±0.3	12.7±0.7	38.5±0.6	1.2±0.1	8.1±0.5
week 1	ambient seawater	3.2±0.4	16.0±0.6	37.9±0.3	1.3±0.0	7.2±0.3
	750 µM	3.2±0.1	22.0±0.0	38.8±0.4	1.3±0.3	6.2±0.3
	1500 µM	2.9±0.2	15.0±0.3	38.5±0.7	1.3±0.1	7.9±0.1
	2000 µM	2.3±0.1	14.0±0.3	39.5±0.6	1.0±0.0	6.6±0.3
week 2	ambient seawater	2.5±0.3	23.0±1.4	38.6±0.6	1.3±0.0	5.9±0.3
	750 µM	3.2±0.3	21.0±2.8	39.1±0.4	1.4±0.1	7.2±0.3
	1500 µM	3.0±0.1	21.0±1.4	40.0±0.3	1.3±0.0	6.3±0.4
	2000 µM	3.3±0.3	21.0±1.4	39.8±0.0	1.2±0.1	5.9±0.3

1. gel-strength measured with 1.5% agar at 20°C 2. gelling temperature measured with 1% agar 3. viscosity measured with 0.06% agar at 40°C

DISCUSSION

The results of this study demonstrate that the gel strength of Gracilaria agar can be altered by post-harvest holding treatments. Post-harvest conditions of both sets of experiments, i.e., the controlling of light and the degree of nitrogen enrichment, altered agar gel strength. The most dramatic improvements in gel stength, however, were obtained when the post-harvest thalli received nitrogen fertilization. This study demonstrates that thalli held under moderate conditions of nitrogen enrichment produce agars with improved gel strength by comparison with recently harvested algae. In addition, significant relations were demonstrated between gel strength and post-harvest thallus nitrogen content. This relationship between gel strength and thallus nitrogen content was also noted and explained, in part, by Cragie et al. (1984) who showed that nitrogen deficient thalli produced extracts with higher proportions of galactose and lower amounts of anhydrogalactose than thalli which were not nitrogen limited. Similar relations between gel strength and thallus nitrogen content for Gracilaria were reported by Patwary and van der Meer (1983) and by Bird (1982).

The optimum level of nitrogen enrichment in respect to gel quality was 750 µM. The decline in gel strength of extracts from thalli held at higher ammonium enrichment

levels may be related to the toxicity of ammonia at high concentrations. Lapointe and Ryther (1979) recorded reduced growth when thalli were exposed to large daily enrichment loads, and a significant portion of the assimilated NH_4^+ was then stored in the inorganic form. In addition, Nelson (1985) reported that photosynthetic rates of Gracilaria thalli were inhibited at ammonium concentrations greater than 100 μM.

Although relations between the physical charcteristics of agar extracts have been studied, the physiological mechanisms whereby thallus nitrogen affects the gel strength are not fully understood. Lapointe and Ryther (1979) reported that availability of nitrogen during growth affects the ratio of protein to carbohydrate in the thalli. Bird (1982) reported that the thallus nitrogen pools of G. tikvahiae consisted mainly of amino acids and proteins; forms of inorganic nitrogen such as NO_3^- and NH_4^+ were found in much smaller quantities. He also showed that when thalli are nitrogen limited, carbohydrate synthesis predominates, agar yield increases, and gel strength decreases.

Although additional physical charcteristics measured to determine the gel quality during this study fell within the ranges reported by other investigators (DeBoer and Ryther, 1977; Oza, 1978; Hoyle, 1978; Whyte et al., 1981;

Nelson et al., 1983), no apparent relation between agar yield, gelling temperature, relative viscosity, and ash content were found. Although it was shown that post-harvest holding conditions used in this study significantly affected gel strength, the improvement in agar quality over the pre-holding levels was not dramatic and further refinement of the technique is needed.

LITERATURE CITED

Abbott, I. A. 1980. Some field and laboratory studies on colloidal-producing red algae in central California. Aquat. Bot. 8:255-66.

Bird, N. L. 1982. Nitrogen allocation and storage pattern in Gracilaria tikvahiae (Rhodophyta). J. Phycol. 18:344-348.

Bird, N. L., L. C.-M. Chen, and J. McLachlan. 1978. Effects of temperature, light and salinity on growth in culture of Chondrus crispus, Furcellaria lumbricalis, Gracilaria tikvahiae (Gigartinales, Rhodophyta), and Fucus serratus (Fucales, Phaeophyta). Bot. Mar. 12:521-527.

Bird, K. T., M. D. Hanisak, and J. Ryther. 1981. Chemical quality and production of agar extracted from Gracilaria tikvahiae grown in different nitrogen enrichment conditions. Bot. Mar. 24:441-444.

Chen, T. P. 1976. Aquaculture production in Taiwan. Fishing News Books Ltd., England. 161 p.

Craigie, J. S., Z. C. Wen, and J. P. van der Meer. 1984. Interspecific, intraspecific and nutritionally-determined variations in the composition of agars from Gracilaria spp. Bot. Mar. 17:55-61.

DeBoer, J. A. 1978. Effects of nitrogen enrichment on growth rate and phycocolloid content in Gracilaria

foliifera and Neogardhiella baileyi (Florideophceae). Proc. Ninth Internat. Seaweed Symp. 9:263-271.

DeBoer, J. A., and J. H. Ryther. 1977. Potential yields from a waste recycling algae mariculture system. In R. W. Krauss (ed.), The marine plant biomass of the Pacific northwest coast. Corvallis, Oregon. p. 231-249.

Doty, M. S., and V. B. Alvarez. 1973. Seaweed farms: A new approach for U.S industry. Mar. Tech. Soc. Annual Conf. Proc. 9:701-708.

Durairatnam, M., and N. Santos. 1980. Agar from Gracilaria verrucosa (Hudson) Papenfuss and Gracilaria sjoestedtii Kylin from northeast Brasil. In T. Levering (ed.), Proc. Internat. Seaweed Symp. 10:669-674.

Fuller, S. W., and A. C. Mathieson. 1972. Ecological studies of economic red algae IV. Variations of carrageenan concentrations and properties in Chondrus crispus Stackhouse. J. Exp. Mar. Biol. Ecol. 10:49-58.

Gallaher, R. N., C. O. Weldon, and F. C. Boswell. 1976. A semiautomated procedure for determining total nitrogen in plant and soil samples. Soil Sci. Soc. Amer. J. 40:887-889.

Hoyle, M. D. 1978. Agar studies in two Gracilaria species G. bursapastoris (Gmelin) Silva and G. coronopifolia

J. Ag. from Hawaii. 1. Yield and gel strength in the gametophyte and tetrasporophyte generation. Bot. Mar. 21:343-345.

Kim, D. H. 1970. Economically important seaweeds in Chile. 1. Gracilaria. Bot. Mar. 13:140-162.

Lapointe, B. E., and J. H. Ryther. 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of Gracilaria foliifera var. augustissima in mass outdoor cultures. Bot. Mar. 22:529-537.

Mathieson, A. C. 1975. Seaweed aquaculture. Mar. Fish. Rev. 37:2-14.

Matsuhashi, T., and K. Hayashi. 1972. Agar produced from Gracilaria foliifera of Florida. Ag. Biol. Chem. 36:1543-1552.

Nelson, S. G., S.-S. Yang, C.-Y. Wang, and Y.-M. Chang. 1983. Yield and quality of agar from species of Gracilaria (Rhodophyta) collected from Taiwan and Micronesia. Bot. Mar. 26:361-366.

Nelson, S. G. 1985. Immediate enhacement of photo-synthesis by coral reef macrophytes in response to ammonia enrichment. Proc. Fifth Internat. Coral Reef Sympos., Tahiti. 5:65-70.

Oza, R. M. 1978. Studies on Indian Gracilaria. IV. Seasonal variation in agar gel strength of Gracilaria cortica J. Ag. occuring on the coast of Varaval.

Bot. Mar. 21:165-167.

Patwary, M. U., and J. P. van der Meer. 1983. Genetics of Gracilaria tikvahiae (Rhodophyceae) IX: Some properties of agars extracted from morphological mutants. Bot. Mar. 26:295-299.

Pringle, J. D. 1979. Aspects of the ecological impact of Chondrus crispus (Florideophyceae) harvesting in Eastern Canada. In A. Jensen and J. R. Stein (eds.), Proc. Internat. Seaweed Symp. 9:225-232.

Santos, G. A. 1980. Quality of carrageenan agar. In I. A. Abbott, M. S. Foster and L. F. Eklund (eds.), Pacific seaweed aquaculture, proceedings of a symposium on useful algae. Calif. Sea Grant College Program, Institute of Marine Resources, Univ. Calif., La Jolla. p. 123-129.

Tsuda, R. T. 1984. Gracilaria from Micronesia: key, list and distribution of the species. In I. A. Abbott, and J. N. Noris (eds.) Taxonomy of economic seaweeds. Calif. Sea Grant College Program, Institute of Marine Resources, Univ. Calif., La Jolla. p. 91-92.

Wang, C.-Y., and S.-S. Yang. 1980. Seasonal variation of agar-agar produced in Taiwan. Proc. National Res. Council, Republic of China 4:78-86.

Whyte, J. C., and J. R. Englar. 1980. Agar from an intertidal population of Gracilaria sp. In T. Levering

(ed.), Proc. Internat. Seaweed Symp. 10:537- 742.

Whyte, J. M. C., J. R. Englar, R. G. Saunders, and J. C. Lindsey. 1981. Seasonal variation in the biomass, quantity and quality of agar from the reproductive and vegetative stages of Gracilaria (verrucosa type). Bot. Mar. 24:493-501.

Yang,S.-S. 1982. Seasonal variation of the quality of agar-agar produced in Taiwan. In R. T. Tsuda and Y.-M. Chiang (eds.), Proc. of Republic of China-U.S. cooperative science seminar on cultivation and utilzation of economic algae. Univ. Guam, Mar. Lab. p. 68-80.

Yang, S.-S., C.-Y. Wang, and H.-H. Wang. 1981. Seasonal variation of agar-agar produced in the Taiwan area. In T. Levering (ed.), Proc. Internat. Seaweed Symp. 10: 737-742.