

# Changes in Yield and Quality of Agar from the Agarophytes, *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* Cultivated in Earthen Ponds

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## ABSTRACT

*Gracilaria fisheri* and *G. tenuistipitata* var. *liui* cultured in earthen ponds containing shrimp farm effluent (P1) and ambient seawater (P2), were collected from October 1998 to March 1999 for agar extraction. Yield and quality of agar extracted from both *Gracilaria* species were determined. Agar yields with highest value were obtained from *G. fisheri* grown in P2 (43.48±1.00%) and P1 (40.07±2.06%) while *G. tenuistipitata* var. *liui* yielded only 35.75±0.42% and 39.28±0.50% agar in P2 and P1, respectively. Yields of agar from each species were not significantly different when grown in P1 and P2 ( $p>0.01$ ). Gel strength of agar from *G. fisheri* showed a maximum value of 623 g/cm<sup>2</sup> in P1 and 658.6 g/cm<sup>2</sup> in P2 while *G. tenuistipitata* var. *liui* gave a maximum value of 565.8 g/cm<sup>2</sup> in P1 and 611 g/cm<sup>2</sup> in P2 during low seawater temperature of 28.5°C in December 1998. Agar gel strength from *G. fisheri* was inversely related to total dissolved inorganic nitrogen ( $r = -0.54$  in P1;  $r = -0.77$  in P2,  $p<0.01$ ,  $n = 12$ ) while *G. tenuistipitata* var. *liui* showed a positive correlation ( $r = 0.41$  in P1;  $r = 0.88$  in P2,  $p<0.01$ ,  $n = 12$ ). Increasing in agar gel strength of *G. tenuistipitata* var. *liui* was observed during decreasing in alkalinity in seawater. Agars extracted from both *G. fisheri* and *G. tenuistipitata* var. *liui* gave rather high gelling temperature during dry season. The agar quality of both cultivars showed relation with nutrient and environmental factors of the seawater.

**Key words:** agar quality, earthen pond, *Gracilaria*

## INTRODUCTION

In Thailand, there are several agar producing red seaweeds such as *Gelidium pulchellum*, *Gelidiella acerosa* and *Gracilaria* spp. growing along the coast of the Gulf of Thailand and Andaman Sea. The majority of utilized agarophyte belong to the genera *Gracilaria* in which 18 species have been recognized (Lewmanomont, 1994; Chirapart and Ruangcha, 1999; Lewmanomont and Chirapart, 2004). They are found in several parts of the country, particularly in Pattani, Songkhla and Trat provinces. Among these, *Gracilaria fisheri* and *G. tenuistipitata* are commonly harvested in large quantity as a commercial source of agar and abalone feed (Lewmanomont, 1998).

Several studies have shown that changes in agar yield and property depend upon species (Craigie *et al.*, 1984), season (Chirapart and Ohno,

1999; Lewmanomont and Chirapart, 2004). They are found in several parts of the country, particularly in Pattani, Songkhla and Trat provinces. Among these, *Gracilaria fisheri* and *G. tenuistipitata* are commonly harvested in large quantity as a commercial source of agar and abalone feed (Lewmanomont, 1998).

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1993; Villanueva et al., 1999; Marinho-Soriano et al., 2001) and environmental parameters (Martinez and Buschmann, 1996; Phang et al., 1996; Wakibia et al., 2001). Gel properties of agar can also vary according to stage of the life cycle (Craigie et al., 1984; McLachlan and Bird, 1986). The yield and physical properties of agar, such as gel strength and gelling temperature as well as chemical properties, determine its commercial value. In Thailand two commercial agarophyte species, *G. fisheri* and *G. tenuistipitata* are generally harvested from natural stock for agar extraction. These two species have long been studied on their taxonomy and cultivation. However, there have been no published studies on seasonal change in the yield and quality of agars of these seaweeds.

This study was done to investigate seasonal variation in yield, physical and chemical properties of agar extracted from two agarophyte species, *Gracilaria fisheri* and *G. tenuistipitata* var. *liui*, grown in earthen ponds using shrimp farm effluent and ambient seawater. Environmental parameters effect on agar were also determined.

## MATERIALS AND METHODS

### Sampling

*Gracilaria fisheri* (Xia et Abbott) Abbott, Zhang et Xia and *G. tenuistipitata* var. *liui* Chang et Xia were collected in earthen ponds at the Phetchaburi Coastal Aquaculture Station, Phetchaburi province (lat 100° 05' 15" E, long 13° 02' 30" N), Thailand. The *Gracilaria* species was previously cultured in the earthen ponds (Chirapart and Lewmanomont, 2004) using extensive shrimp farm effluents (P1) and ambient seawater (P2). Agar yield and quality were determined from the cultivars that could be harvested during growing period. The harvested materials of *G. fisheri* were determined from October 1998 to March 1999, while those of *G. tenuistipitata* var. *liui* were determined from October 1998 to January 1999

since the harvested algal materials were not enough for determination. The harvested algal materials were washed in fresh seawater to remove epiphytes and mud, then dried and kept in freezer at -20°C. The frozen samples were soaked and washed in running tap water for at least 2 h to remove remaining salt, cut into small pieces (10 – 20 mm), and then soaked in acetone overnight to remove pigment and remain contaminant, washed again with fresh acetone (99% v/v) until the supernatant solution was colorless, and dried at room temperature (26 – 30 °C). The dried colorless *Gracilaria* was kept in dry glass bottle.

During the sampling period, determination of surface seawater temperature, salinity, turbidity, alkalinity, phosphate and total dissolved inorganic nitrogen (DIN = ammonia + nitrite + nitrate) were done every sampling day as one point measurements. Water temperature and salinity were measured using an alcohol thermometer, and refracto-salinometer, respectively. Turbidity of the seawater was determined at 420 nm with a spectrophotometer (Shimadzu UV-1601). Alkalinity was determined according to APHA, AWWA and WPCF (1980) and phosphate phosphorous (PO<sub>4</sub><sup>-3</sup>-P), ammonia nitrogen (NH<sub>3</sub>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) and nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N) according to Strickland and Parsons (1972). Triplicate samples were analyzed for each parameter. The data analyzed was averaged per month (n = 12 to 15). The differences in yield and properties of agar between species and culture ponds were analyzed utilizing an ANOVA (analysis of variance).

### Agar extraction

The agar extraction method was performed as described by Chirapart et al. (1995). The dried colorless algal sample (30 g) was boiled for 2 h in 900 ml distilled water, and used for non alkali treatment of crude extracted agar. Another 30 g of sample was incubated in 1,200 ml of 5% NaOH solution at 80°C for 2 h and washed in tap

water for 30 min., then neutralized with 2% H<sub>2</sub>SO<sub>4</sub> for 1 h and washed in tap water for 2 h. The agar samples were extracted by boiling for 2 h in 900 ml distilled water and used for alkali treatment of crude extracted agar. The agar solution was filtered through muslin cloth. The extract was allowed to gel at room temperature and then placed in a freezer at -20°C for 24 – 48 h. The frozen gel was thawed in tap water. The agar was dehydrated using acetone (99% v/v) and dried at room temperature for determination of agar yield. The yield was expressed as the percentage of agar obtained from dry weight sample of each species of *Gracilaria*.

#### Measurement of agar quality

The gel strength of 1.5% agar solution (3 replicates per sample) was determined at 20°C using Texture Analyzer (TA.XT 2, England). Viscosity of this solution at 80°C was determined using a Brookfield viscometer (SYNCHRO•LECTRIC, USA).

Dynamic gelling temperature was determined using test tubes (15 mm × 120 mm) containing 10 ml of 1.5% agar solution. The test tubes were inclined slowly at 20°C and temperatures were recorded when the surface of the agar solution became semi-solid. Melting temperature was determined from 5 ml of 1.5% agar in a glass test tube (10 mm × 100 mm). After holding at 20°C for 24 h, the test tube was turned over and placed in a water bath, which was heated to boiling point at the rate of 0.5 – 1.0 °C rise per minute. The temperature at which the gel in the test tube suddenly dropped was recorded as the melting temperature.

Total carbohydrate content of the agar extract was determined using the phenol-sulfuric acid method (Dubois *et al.*, 1956) having galactose as the standard. 3, 6-anhydrogalactose content (3, 6-AG) was determined by the resorcinol-acetal method (Yaphe and Arsenault, 1965). Sulfate content was determined turbidimetrically with

BaCl<sub>2</sub> after HCl hydrolysis (Craigie *et al.*, 1984).

The extracted agar quality was compared to commercial-grade agar. The laboratory grade agar (Difco Bacto-Agar, USA) was purchased from Arsom Co. LTD. Tissue culture industry grade agar (PAC agar-agar powder) was obtained from Pure Agar Co. LTD., Thailand. The food grade agar powder was purchased from a supermarket.

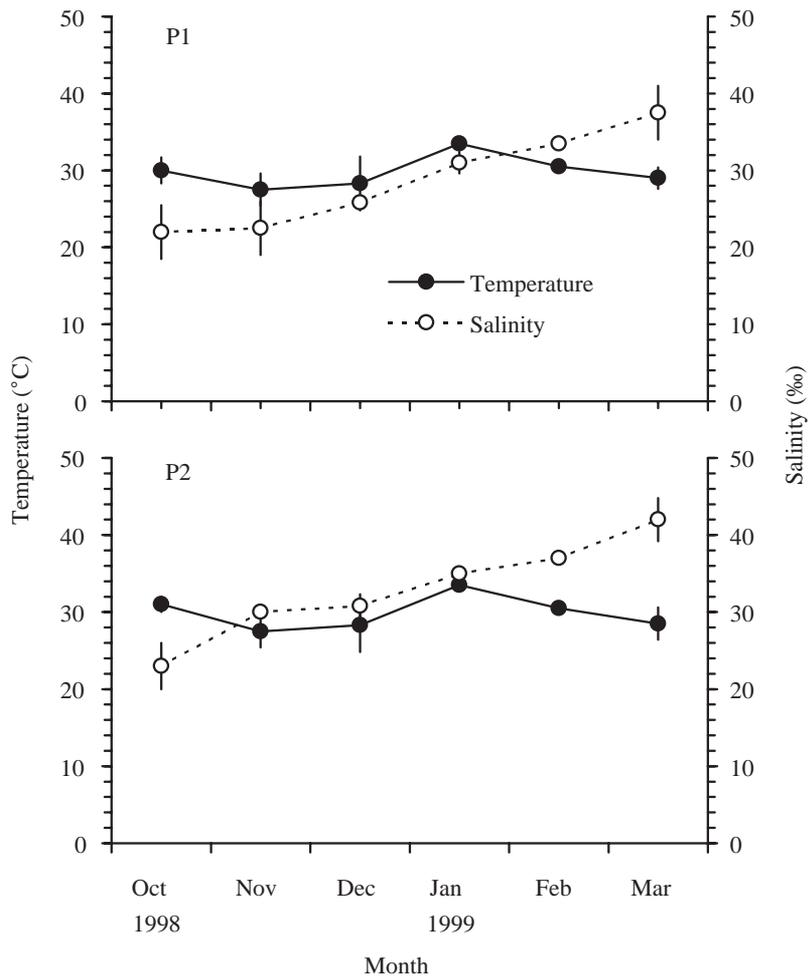
## RESULTS

### Environmental parameters

Changes in physical and chemical characteristics of surface seawater in earthen ponds from October 1998 to March 1999 are shown in Figures 1 and 2, respectively. The characteristics of surface seawater both in P1 and P2, temperature ranged from 27.5±2.1°C in November 1998 to 33.5±0.7°C in January 1999. Salinity fluctuated from 22.0±3.5 in October 1998 to 42.0±2.8‰ in March 1999 (Figure 1). The content of dissolved inorganic nitrogen (DIN) in P1 varied from 0.12±0.02 mg/l in December 1998 to 1.35±1.70 mg/l in January 1999. The DIN in P2 ranged from 0.12±0.01 mg/l in November 1998 to 0.59±0.31 mg/l in March 1999. The PO<sub>4</sub><sup>-3</sup>-P content in P1 varied from 0.00±0.00 mg/l (trace) in February and March 1999 to 0.02±0.02 mg/l in October 1998. Content of the PO<sub>4</sub><sup>-3</sup>-P in P2 varied from 0.00±0.00 mg/l (trace) in March 1999 to 0.20±0.28 mg/l in February 1999. Seawater turbidity ranged from 20.21±7.93 mg/l in November 1998 to 119.77±13.3 mg/l in March 1999 in P1 and from 30.79±12.69 in October 1998 to 77.03±5.95 mg/l in February 1999 in P2. Seawater alkalinity in P1 varied from 84.25±26.17 mg/l in December 1998 to 120.5±45.96 mg/l in January 1999 and in P2 varied from 76.00±33.94 mg/l in March 1999 to 117.00±33.94 mg/l in November 1998 (Figure 2).

### Agar yield

The highest agar yields were obtained

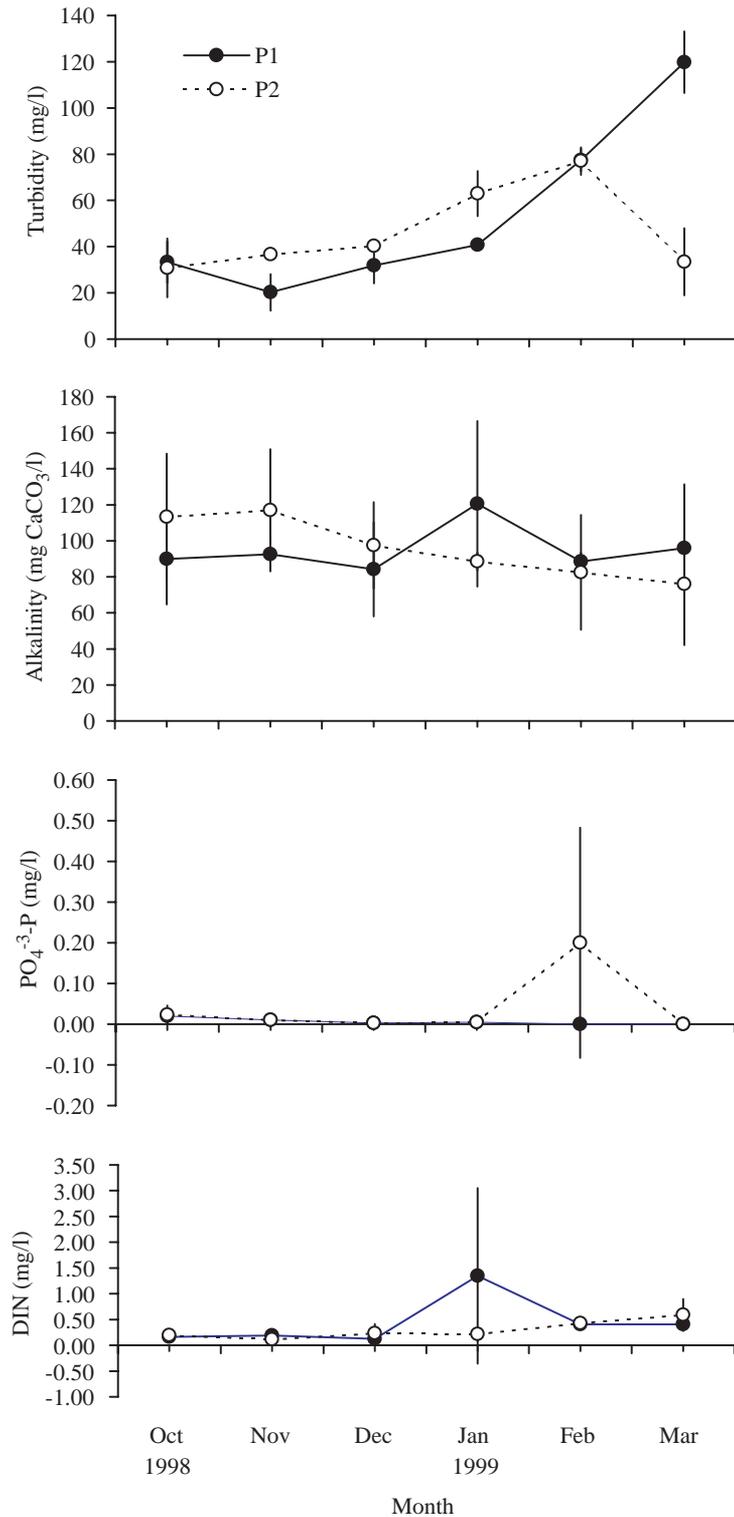


**Figure 1** Changes in temperature and salinity of surface seawater in earthen ponds from October 1998 to March 1999. P1 = culture pond using shrimp farm effluent seawater, P2 = culture pond using ambient seawater.

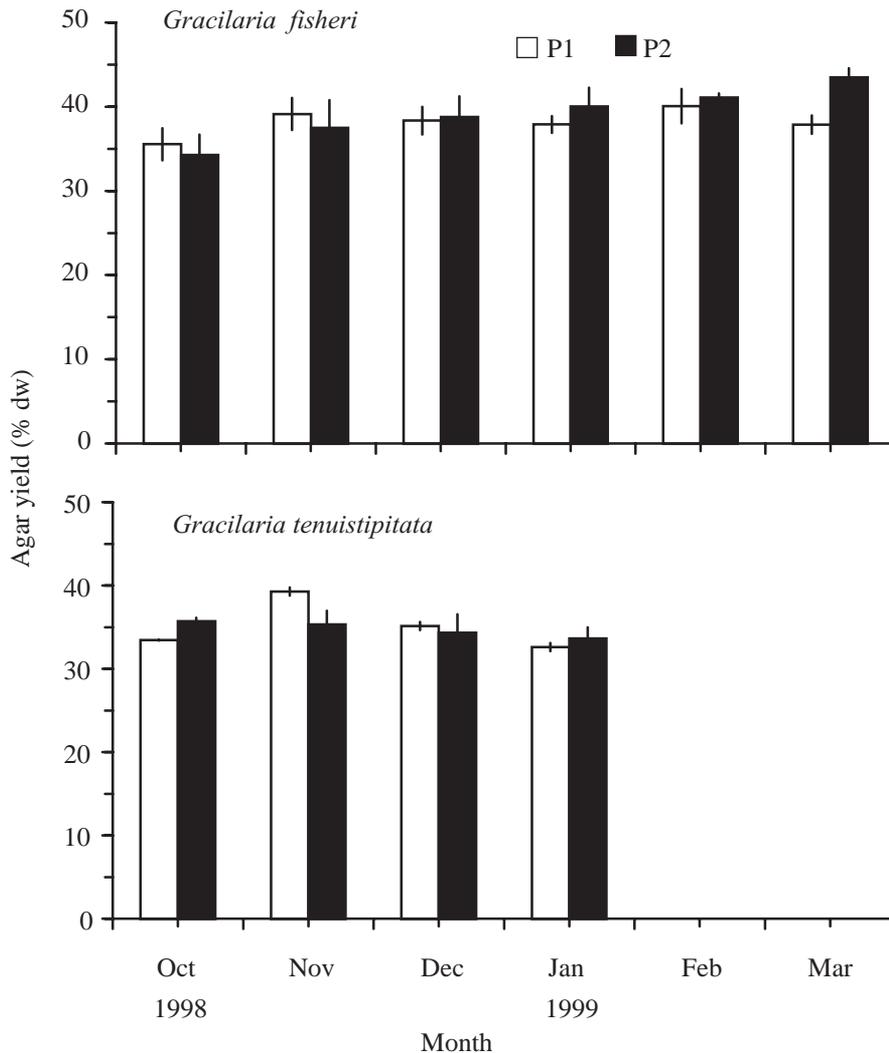
from *G. fisheri* grown in P2 ( $43.48 \pm 1.00\%$ ) in March 1999 and P1 ( $40.07 \pm 2.06\%$ ) in February 1999. *G. tenuistipitata* var. *liui* gave the highest agar yield of  $35.71 \pm 0.42\%$  in October 1998 and  $39.28 \pm 0.50\%$  in November in P2 and P1, respectively. There was slightly change in the yield of agar during the growing period (Figure 3). However, each species grown in the same condition was not significantly different in agar yield ( $p > 0.01$ ). Non significant differences in agar yields were also observed from the same species grown under different conditions in P1 and P2

( $p > 0.05$ ).

Positive correlations were observed between agar yield from *G. fisheri* and total DIN ( $r = 0.03$ ,  $p < 0.01$  in P1,  $r = 0.81$ ,  $p < 0.01$  in P2,  $n = 12$ ) as well as turbidity ( $r = 0.16$ ,  $p < 0.01$  in P1,  $r = 0.38$ ,  $p < 0.01$  in P2,  $n = 12$ ) and salinity ( $r = 0.35$ ,  $p < 0.01$  in P1,  $r = 0.99$ ,  $p < 0.01$  in P2,  $n = 12$ ). In contrast, *G. tenuistipitata* var. *liui* showed inverse correlation between agar yield and DIN in P1 ( $r = -0.54$ ,  $p < 0.01$ ,  $n = 12$ ) and in P2 ( $r = -0.56$ ,  $p < 0.01$ ,  $n = 12$ ).



**Figure 2** Physical and chemical characteristics of surface seawater in earthen ponds from October 1998 to March 1999.



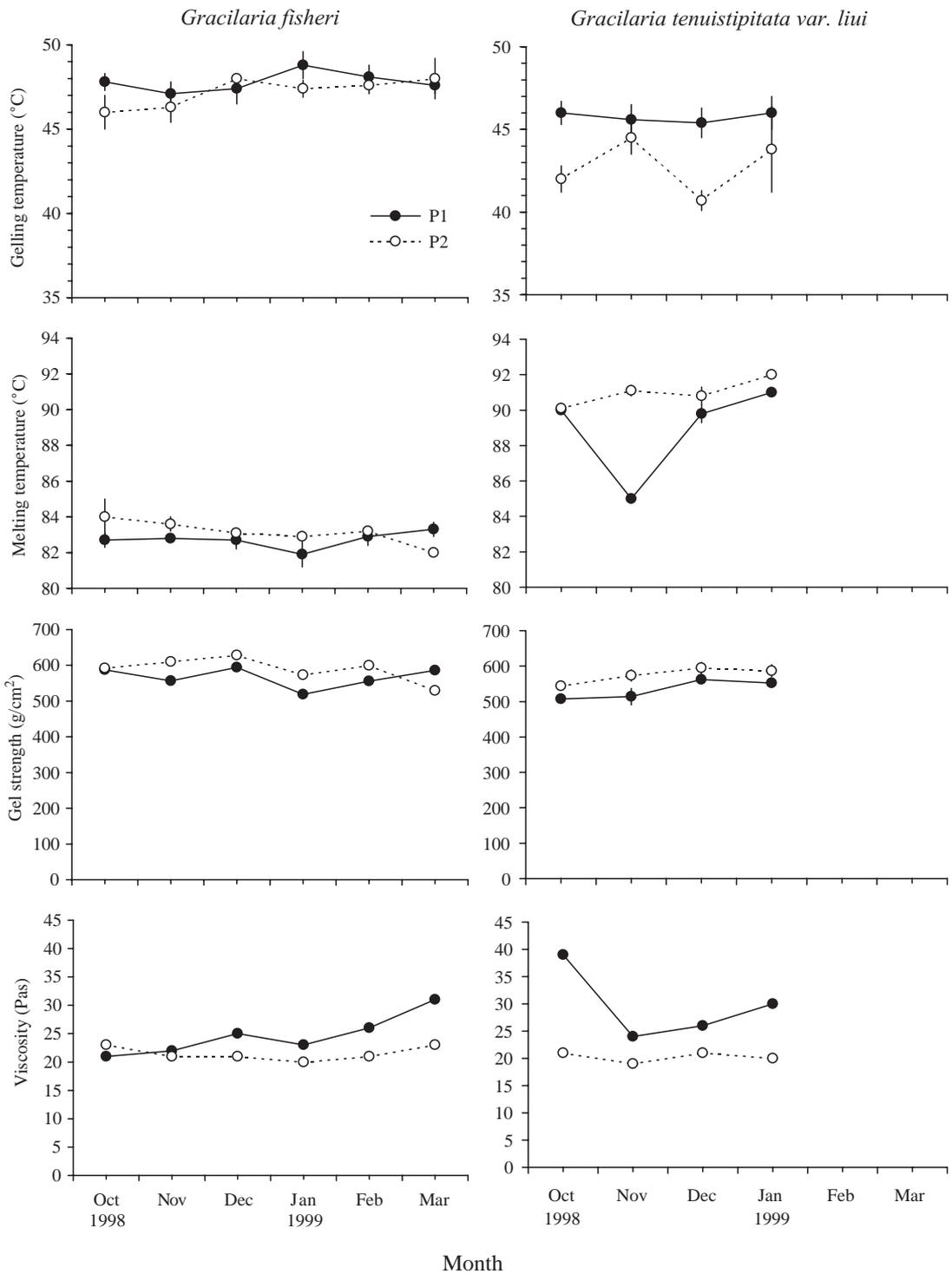
**Figure 3** Yield of agar extracted from *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* grown in earthen ponds using shrimp farm effluent seawater (P1) and ambient seawater (P2) from October 1998 to March 1999.

### Physical properties of agar

The agars from *G. fisheri* had significant higher gel strength than that of *G. tenuistipitata* var. *liui* ( $p < 0.05$ ) (Figure 4). Agar gel strength from *G. fisheri* reached a maximum value of 623 g/cm<sup>2</sup> for P1 (mean = 594.1 ± 28.9 g/cm<sup>2</sup>) and 658.6 g/cm<sup>2</sup> for P2 (mean = 628.4 ± 30.2 g/cm<sup>2</sup>). Gel strength of agar from *G. tenuistipitata* var. *liui* had maximum values of 565.8 g/cm<sup>2</sup> for P1 (mean = 562.4 ± 3.4 g/cm<sup>2</sup>) and 611 g/cm<sup>2</sup> for P2 (mean =

594.7 ± 16.3 g/cm<sup>2</sup>) during low seawater temperature of 28.5°C in December 1998.

Change in agar gel strength from *G. fisheri* was inversely related to total DIN both in P1 ( $r = -0.54$ ,  $p < 0.01$ ,  $n = 12$ ) and P2 ( $r = -0.77$ ,  $p < 0.01$ ,  $n = 12$ ) while *G. tenuistipitata* var. *liui* showed a positive correlation in P1 ( $r = 0.41$ ,  $p < 0.01$ ,  $n = 12$ ) and P2 ( $r = 0.29$ ,  $p < 0.01$ ,  $n = 12$ ). The positive relation was also observed between agar gel strength from *G. tenuistipitata* var. *liui*



**Figure 4** Physical properties of agar extracted from *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* grown in earthen ponds using shrimp farm effluent (P1) and ambient seawater (P2) from October 1998 to March 1999.

and salinity in P1 ( $r = 0.77$ ,  $p < 0.01$ ,  $n = 12$ ) and in P2 ( $r = 0.88$ ,  $p < 0.01$ ,  $n = 12$ ). Agar gel strength from *G. tenuistipitata* var. *liui* had positive correlation with alkalinity in P1 ( $r = 0.26$ ,  $p < 0.01$ ,  $n = 12$ ) but showed negative correlation in P2 ( $r = -0.69$ ,  $p < 0.01$ ,  $n = 12$ ). In contrast, agar gel strength from *G. fisheri* showed inverse relationship with alkalinity in P1 ( $r = -0.82$ ,  $p < 0.01$ ,  $n = 12$ ) while had positive correlation in P2 ( $r = 0.60$ ,  $p < 0.01$ ,  $n = 12$ ).

Agar from both cultivars in P1 showed significant higher viscosity ( $p < 0.05$ ) than those in P2 (Figure 6). However, *G. fisheri* in P2 had higher viscosity than those grown in P1, during October 1998. The viscosity of agar from *G. tenuistipitata* var. *liui* gave maximum values of 39 Pas in October 1998 for P1 and 21 Pas in October and December 1998 for P2. *G. fisheri* reached the highest viscosity of 31 Pas in March 1999 for P1 and 23 Pas in October 1998 and March 1999 for P2.

Gelling temperature of the agar from *Gracilaria tenuistipitata* var. *liui* ranged from  $45.4 \pm 0.9$  to  $46.0 \pm 1.0^\circ\text{C}$  in P1 and  $44.5 \pm 1$  to  $49.3 \pm 0.8^\circ\text{C}$  in P2, while that of *G. fisheri* were from  $46.3 \pm 0.7$  to  $48.8 \pm 0.8^\circ\text{C}$  in P1 and  $46.0 \pm 1.0$  to  $48.0 \pm 1.2^\circ\text{C}$  in P2. The gelling point of agar from *G. tenuistipitata* var. *liui* was significantly different ( $p < 0.01$ ) which had a higher value in P2 than in

P1 except for those in November. In contrast, those of *G. fisheri* were not significantly different ( $p > 0.01$ ) when grown in P1 and P2. The gelling temperature changed slightly for each species and season (Figure 4). However, there was significant difference ( $p < 0.01$ ) between algal species at different growing conditions.

Melting temperature of agar from the cultivars was significantly higher in P2 than in P1 ( $p < 0.01$ ). Melting point of agar from *G. tenuistipitata* var. *liui* in P1 ranged from  $85.0 \pm 0.1^\circ\text{C}$  in November 1998 to  $91.0^\circ\text{C}$  in January 1999 and in P2 were from  $90.1 \pm 0.3^\circ\text{C}$  in October 1998 to  $92.0 \pm 0.2^\circ\text{C}$  in January 1999. Under the same culture conditions, the melting point of the agar from *G. fisheri* in P1 varied from  $81.9 \pm 0.7^\circ\text{C}$  in January to  $83.3 \pm 0.4^\circ\text{C}$  in March 1999 and in P2 it were from  $82.0 \pm 0.2^\circ\text{C}$  in March 1999 to  $84.0 \pm 1.0^\circ\text{C}$  in October 1998. Agar extracted from *G. tenuistipitata* var. *liui* had a higher melting point than *G. fisheri* (Figure 4).

#### Chemical properties of agar

Chemical properties of the agar extracted from *G. fisheri* and *G. tenuistipitata* var. *liui* are shown in Table 1. A higher total carbohydrate content was obtained from *G. fisheri* than from *G. tenuistipitata* var. *liui*. The total carbohydrate content of *G. fisheri* reached the highest value of

**Table 1** Chemical properties of agar extracted from *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* cultured in earthen ponds using shrimp farm effluent (P1) and ambient seawater (P2).

| Date | <i>Gracilaria tenuistipitata</i> |       |                     |      |             |       | <i>Gracilaria fisheri</i> |       |                     |      |             |       |       |
|------|----------------------------------|-------|---------------------|------|-------------|-------|---------------------------|-------|---------------------|------|-------------|-------|-------|
|      | Carbohydrate content (%)         |       | Sulfate content (%) |      | 3, 6-AG (%) |       | Carbohydrate content (%)  |       | Sulfate content (%) |      | 3, 6-AG (%) |       |       |
|      | P1                               | P2    | P1                  | P2   | P1          | P2    | P1                        | P2    | P1                  | P2   | P1          | P2    |       |
| 1998 | Oct.                             | 37.66 | 38.89               | 0.71 | 0.63        | 19.16 | 21.9                      | 42.07 | 42.64               | 0.85 | 0.76        | 20.94 | 22.15 |
|      | Nov.                             | 41.10 | 39.19               | 0.75 | 0.64        | 22.22 | 20.3                      | 41.37 | 41.07               | 0.79 | 0.77        | 15.87 | 23.54 |
|      | Dec.                             | 39.77 | 39.39               | 0.77 | 0.63        | 19.05 | 19.05                     | 41.88 | 42.40               | 0.88 | 0.75        | 22.07 | 23.97 |
| 1999 | Jan.                             | 37.69 | 40.89               | 0.76 | 0.63        | 15.04 | 17.35                     | 39.65 | 40.61               | 0.80 | 0.76        | 19.83 | 17.43 |
|      | Feb.                             | -     | -                   | -    | -           | -     | -                         | 41.16 | 40.35               | 0.83 | 0.76        | 17.96 | 23.05 |
|      | Mar.                             | -     | -                   | -    | -           | -     | -                         | 42.75 | 41.90               | 0.84 | 0.86        | 23.97 | 26.62 |

Note - : no data

42.75% in March 1999 (P1) and 42.64% (P2) in October 1998 while the agar from *G. tenuistipitata* var. *liui* gave the highest value of 41.1% in November 1998 (P1) and 40.89% in January 1999 (P2).

After alkali treatment, the sulfate content in agar was lower in comparison to non-alkali treated agar (3.36%) of *G. fisheri* (unpublished data). *G. tenuistipitata* var. *liui* ranged from 0.71% to 0.77% in P1 and 0.63% to 0.64% in P2. *G. fisheri* varied from 0.79% to 0.88% in P1 and 0.75% to 0.86% in P2. Sulfate contents of the extracted agar from *G. tenuistipitata* var. *liui* were significantly higher in P1 than in P2 ( $p < 0.01$ ) while those of *G. fisheri* were not significantly different ( $p > 0.01$ ).

3, 6-AG of the alkali treated agar from *G. fisheri* in P1 varied from 15.87% to 23.97% and in P2 from 17.43% to 26.62% which was higher than non-alkali treated agar (14.48%, unpublished data). For *G. tenuistipitata* var. *liui* the 3, 6-AG content varied from 15.04% to 22.22% in P1 and 17.35% to 21.90% in P2. Agar of *G. fisheri* generally had higher 3, 6-AG than those of *G. tenuistipitata* var. *liui* ( $p < 0.01$ ) except for November agar.

Agars extracted from *G. fisheri* and *G. tenuistipitata* var. *liui* had comparable 3, 6-AG to those of commercial food grade agars (28.61%), tissue culture industry grade agar, PAC (30.72 %) and Bacto Agar (25.87 %) as shown in Table 2. Moreover, the sulfate content in the agar showed similar value with that of commercial food grade agars (0.61%), tissue culture industry grade agar, PAC (0.62%), but lower than Bacto Agar (1.54%).

## DISCUSSION

It has recently been demonstrated that *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* can be cultivated in earthen pond with shrimp farm effluents and ambient seawater (Chirapart and Lewmanomont, 2004). In this study, agars extracted from these two cultivars yielded rather high content (~40-44%) in comparison to previously reports of some species of agarophytes (Phang *et al.*, 1996; Martinez and Buschmann, 1996; Wakibia *et al.*, 2001). Yields of agar varied between 6% and 71% where 20% was a common value for agarophytes (McLachlan and Bird, 1986). Agar yields of 35.8% have been reported for *Gracilariopsis lemaneiformis* (Chirapart *et al.*, 1995), 25 % for *Gracilaria changii* (Phang *et al.*, 1996), 22 % for *G. chilensis* (Martinez and Buschmann, 1996), and 39 % for *G. gracilis* (Wakibia *et al.*, 2001). Higher agar yield of up to 43% has also been reported in *G. chilensis* (Matsuhira and Urzua, 1990). In this study, the positive relationship between agar yield from *G. fisheri* and total DIN in water was in agreement with agar yield from *G. gracilis* reported by Marinho-Soriano and Bourret (2003). In contrast, agar yield from *G. tenuistipitata* var. *liui* gave an inverse relationship with nitrogen content which was in agreement with Bird *et al.* (1981) and Chirapart and Ohno (1993). Agar yield of *Gracilaria* was known to decrease with increasing tissue nitrogen concentration (Bird *et al.*, 1981). However, yield of the agar obtained depended not only on the nutrient content of seawater but also on the species of the cultivars (Bird *et al.* 1981;

**Table 2** Physical and chemical properties of some commercial grade agars. (mean  $\pm$  standard deviation).

| Commercial grade agars             | Gel strength<br>(g/cm <sup>2</sup> ) | Viscosity<br>(Pas) | Gelling<br>temperature<br>(°C) | Melting<br>temperature<br>(°C) | Sulfate<br>content (%) | 3,6-AG<br>content (%) |
|------------------------------------|--------------------------------------|--------------------|--------------------------------|--------------------------------|------------------------|-----------------------|
| Laboratory grade agar (Bacto Agar) | 1104.5 $\pm$ 96.7                    | 14                 | 36.3 $\pm$ 1.5                 | 89.8 $\pm$ 0.3                 | 1.54                   | 25.87                 |
| Tissue culture industry grade agar | 900.1 $\pm$ 16.7                     | 26.7               | 46.7 $\pm$ 0.6                 | 89.9 $\pm$ 0.4                 | 0.62                   | 30.72                 |
| Food grade agar                    | 905.0 $\pm$ 49.5                     | 23.3               | 50.8 $\pm$ 1.3                 | 93.7 $\pm$ 0.6                 | 0.61                   | 28.61                 |

Craigie *et al.*, 1984).

Agar qualities depend on culture conditions, particularly on the nutrient enrichment and the age of the cultivated tissues (Craigie *et al.*, 1984). The positive relation observed between agar gel strength from *G. tenuistipitata* var. *liui* and salinity in P1 and in P2 coincided with recently report in *Gracilaria bursa-pastoris* and *G. gracilis* (Marinho-Soriano and Bourret, 2003). Interestingly increase in agar gel strength of *G. tenuistipitata* var. *liui* was observed during decreasing in alkalinity in seawater. Craigie *et al.* (1984) reported that gel strength, gelling and melting points could be increased when the nutrient levels were increased. Higher melting points and greater gel strengths has been reported in agar extracted from *Gracilaria tikvahiae* grown under nitrogen enriched conditions compared to agars extracted from less enriched thalli (Bird *et al.*, 1981). Generally, the melting point of agar increased with increasing agar gel strength (Matsushashi, 1990). In the present study, agars extracted from *G. fisheri* had a significance melting temperature lower than that of agars extracted from *G. tenuistipitata* var. *liui* ( $p > 0.01$ ). Moreover, rather high gelling temperature was observed from dry season agars of both *G. fisheri* and *G. tenuistipitata* var. *liui* as compared to laboratory grade agar. This might be caused by the methoxyl content in the obtained agar as had been reported by Guiseley (1970) that high content of methoxyl in agar corresponded with high gelling temperature.

Content of 3, 6-AG in agar from *G. fisheri* was comparable to laboratory grade agar and tissue culture industrial grade agar. A rather low value of 3, 6-AG was obtained from *G. tenuistipitata* var. *liui*. However, the increase in agar gel strength of the cultivar had no direct relation with high 3, 6-AG and low sulfate content. Yaphe and Duckworth (1972) reported that the 3, 6-AG content had clearly no correlation with gel strength, while sulfate content showed a direct

correlation with gel strength. Moreover, high content of sulfate in agar extracted from thalli in P1 might be interfered by the sulfur content in earthen ponds. This could be shown by dark mud with smell of hydrogen sulfide in the cultured ponds. Changes in the 3, 6-AG content might be caused not only by species of algae and environmental factors of each pond but also the concentration of nutrients in the seawater.

## CONCLUSION

This study indicated variation in agar yield and gel properties from *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* depending on environmental factors in the earthen pond. Agar extracted from both *Gracilaria* species cultured using shrimp farm effluents showed a high agar yield. Yield of agar from *G. fisheri* had positive relationship with total DIN, salinity and turbidity of the seawater, while *G. tenuistipitata* var. *liui* gave an inverse relationship with total DIN. Yield and agar quality also varied depending on season and species of the *Gracilaria*. However, further investigation needs to clarify an environmental factor to be affected agar quality of the species of *Gracilaria* grow in tropical condition.

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## LITERATURE CITED

- American Public Health Association, American Water Works Association & Water Pollution Control Federation (APHA, AWWA & WPCF). 1980. **Standard Method for the Examination of Water and Waste Water**, 15<sup>th</sup> ed. APHA. Washington D.C. 1134 p.
- Bird, K.T., M.D. Hanisak and J. Ryther. 1981. Chemical quality and production of agars extracted from *Gracilaria tikvahiae* grown in different nitrogen enrichment conditions. **Bot. Mar.** 24: 441-444.
- Chirapart, A. and M. Ohno. 1993. Seasonal variation in the physical properties of agar and biomass of *Gracilaria* sp. (*Chorda* type) from Tosa Bay, southern Japan. **Hydrobiologia** 260/261: 541-547.
- Chirapart, A., Y. Katou, H. Ukeda, M. Sawamura and H. Kusunose. 1995. Physical and chemical properties of agar from a new member of *Gracilaria*, *G. lemaneiformis* (Gracilariales, Rhodophyta) in Japan. **Fisheries Science** 61(3): 450-454.
- Chirapart, A. and R. Ruangchauly. 1999. A new record of *Gracilaria rubra* C.F. Chang et B.M. Xia from Thailand, pp. 137-143. In I. A. Abbott (ed.) **Taxonomy of Economic Seaweed with Reference to Some Pacific Species, Vol. VII**. California Sea Grant College Program. California.
- Chirapart, A. and K. Lewmanomont. 2004. Growth and production of Thai agarophytes cultured in natural pond using the effluent seawater from shrimp culture. **Hydrobiologia** 512 (1): 117-126.
- 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.** 27: 55-61.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Robers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. **Anal. Chem.** 28: 350-356.
- Guiseley, K.B. 1970. The relationship between methoxyl content and gelling temperature of agarose. **Carbohydr. Res.** 13: 247-256.
- Lewmanomont, K. 1994. The species of *Gracilaria* from Thailand, pp. 135-148. In I.A. Abbott (ed.), **Taxonomy of Economic Seaweeds with Reference to Some Pacific and Caribbean Species, Vol. IV**. California Sea Grant College Program, La Jolla.
- Lewmanomont, K. 1998. The seaweed resources of Thailand, pp. 70-78. In A. T. Critchley and M. Ohno (eds.), **Seaweed Resources of the World**. Japan International Cooperation Agency.
- Lewmanomont K. and A. Chirapart 2004. Additional records of *Gracilaria* from Thailand, pp. 201-210. In I. A. Abbott and K. McDermid (eds.). **Taxonomy of Economic Seaweeds with Reference to the Pacific and Other Locations, Vol. IX**. Hawaii Sea Grant College Program.
- McLachlan, J. and C.J. Bird. 1986. *Gracilaria* (Gigartinales, Rhodophyta) and productivity. **Aquatic Bot.** 26: 27-49.
- Marinho-Soriano, E., T.S.F. Silva and W.S.C. Moreira. 2001. Seasonal variation in the biomass and agar yield from *Gracilaria cervicornis* and *Hydropuntia cornea* from Brazil. **Bioresource Technology** 77: 115-120.
- Marinho-Soriano, E. and E. Bourret. 2003. Effects of season on the yield and quality of agar from *Gracilaria* species (Gracilariaceae, Rhodophyta). **Bioresource Technology** 90: 329-333.
- Martinez, L.A. and A.H. Buschmann. 1996. Agar yield and quality of *Gracilaria chilensis* (Gigartinales, Rhodophyta) in tank culture using fish effluents. **Hydrobiologia** 326/327: 343-345.
- Matsuhashi, T. 1990. Agar, pp. 1-51. In P. Harris (ed.). **Food Gels**, Elsevier Science Publisher

- Ltd., London.
- Matsuhira, B. and C. Urzùa. 1990. Agar from *Gracilaria chilensis* (Gracilariales). **J. Appl. Phycol.** 2: 273-279.
- Phang, S.M., S. Shaharuddin, H. Noraishah and A. Sasekumar. 1996. Studies on *Gracilaria changii* (Gracilariales, Rhodophyta) from Malaysian mangroves. **Hydrobiologia** 326/327: 347-352.
- Strickland, J. D. H. and T. R. Parsons. 1972. **A Practical Handbook of Seawater Analysis**, 2<sup>nd</sup> ed. Fisheries Research Board of Canada Bulletin No. 167, Ottawa. 310 p.
- Villanueva, R.D., N.E. Montaña, J.B. Romero, A.K.A. Aliganga and E.P. Enriquez. 1999. Seasonal variations in the yield, gelling properties, and chemical composition of agars from *Gracilaria eucheumoides* and *Gelidiella acerosa* (Rhodophyta) from the Philippines. **Bot. Mar.** 42: 175 – 182.
- Wakibia, J.G., R.J. Anderson and D.W. Keats. 2001. Growth rates and agar properties of three gracilarioids in suspended open-water cultivation in St. Helena Bay, South Africa. **J. Appl. Phycol.** 13: 195-207.
- Yaphe, W. and G.P. Arsenault. 1965. Improved resorcinal reagent for the determination of fructose, and 3,6-anhydrogalactose in polysaccharides. **Anal. Biochem.** 13: 143-148.
- Yaphe, W. and M. Duckworth. 1972. The relationship between structures and biological properties of agars, pp. 15-22. *In* N. Nisizawa (ed.). **Proceedings of the Seventh International Seaweed Symposium**. University of Tokyo Press, Tokyo.