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Full Length Research Paper

Effects of season on the yield and quality of agar from *Gelidium sesquipedale* (Rhodophyta) from Mostaganem, Algeria

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The aim of this work was to study the seasonal variations in the yield and physicochemical properties of agar of *Gelidium sesquipedale*. The algae were collected from January 2011 to December 2011, from the coast of Mostaganem, western Algeria. The overall results show that the dry biomass of *Gelidium sesquipedale* was constant throughout the year (22.34 ± 3.18 %), while the agar yield varied seasonally with highest values (*P*<0.05) in spring and summer (32.22 ± 1.7%). Regarding the composition of the agar, total sugars was 87.63% (± 6.9 %), with a maximum in winter (*P*<0.05), the 3,6-anhydrogalactose varied between 30.65 (±2.56%) and 40.40% (±1.34%). The agar of *G. sesquipedale* was slightly substituted with sulphate (2.75 ± 1.37%) and pyruvate (0.32 ± 0.09%) and had a viscosity of 11.11 mPa.s (± 1.50 mPa.s.). In general, the physicochemical characteristics of the agar of *G. sesquipedale* are similar to those of the commercial agar, which leads to the conclusion that this alga can be a source of production of agar.

Key words: *Agarophytes,* seaweed, agar, 3,6-anhydrogalactose, sulphate, pyruvate.

INTRODUCTION

The agar is the main constituent of the cell walls of red algae. It is extracted mostly from the macroalgal orders Gracilariales and Gelidiales (Craigie, 1990). This polysaccharide is characterized by its gelling and stabilizing properties. It has many applications in food processing, pharmaceutics, cosmetics, microbiology, molecular biology and biotechnology (Armisen, 1997). This phycocolloid is constituted by a disaccharidic repetitive unit of D-galactose and 3,6-anhydro-L-galalactose (Imerson, 2011). These units can be substituted by sulfate, methyl, carboxyl or pyruvate groups depending on the alga origin, the season or the extraction method (Nussinovitch, 1997; Armisén et al., 2000; Imerson, 2011). Several studies have shown that the yield and quality of agar are influenced by environmental variations or experimental conditions in cultured species (Craigie, 1990; Hurtado et al., 2010).

Gelidium sesquipedale is among the red seaweeds, the most used for the production of agar. It has a red to reddish brown color with strong aspect and a

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cartilaginous consistency. This algae has a modular type of construction in which erect fronds develop from a basal system of prostrate axes that are attached to the substratum by rhizoids. Fronds are clumped in tufts (Gayral, 1958; Santos, 1994; Mouradi et al., 2007). This red alga is a native species to the northern Atlantic (Riadi, 1998), it is observed along the Atlantic coast from Great Britain to Mauritania. In the Mediterranean Sea, *G. sesquipedale* was reported in Morocco, Corsica, Italy and Algeria (Gayal, 1958; Fischer et al., 1987; Grimes et al., 2003).

In Algeria, the interest to develop marine algae is very new as compared to other countries, and until now, there is a considerable lack of information on the algae in general and red algae in particular. Therefore, there is need to valorize the flora of Algerian coasts. The present paper is one of the first works with interest on the valorization of the red alga and it will contribute to enriching the data on *G. sesquipedale* in the southwestern Mediterranean basin. This work evaluates the variations of yield and physico-chemical properties of agar, extracted from a natural population of *G. sesquipedale*, to determine the period which corresponds to the maximal production and thus to target algae's harvest period, for industrial use.

MATERIALS AND METHODS

Collection of algal material

The thalli of *G. sesquipedale* were collected monthly from January 2011 to December 2011 in the rocky coast of Mostaganem located Northwest of Algeria (35°58N; 0°05E). The collection of the algae was carried out in a water depth ranging from 0.5 to 1 m.

Twenty grams of fresh algae were cleaned thoroughly of epibionts and weighed. The thalli were dried for 48 h at 60 °C and re-weighed to obtain dry weight values:

Dry matter (%) = (Fresh weight alga - Dry weight alga) x 100

Agar extraction

Three grams of dried algae were soaked in a 0.5% (w/v) solution of Na_2CO_3 at 85–90°C for 30 min, prior to extraction. In order to eliminate the excess alkali, the algae were washed with running tap-water for 10 min. Agar extraction was done in triplicate carried out according to Freile-Pelegrín et al. (1995). The agar yield was expressed as percentage of dried algae.

Agar yield (%) = (Dried weight alga - Dried weight agar) x 100

Agar composition

Total carbohydrate was measured by the Anthrone sulphuric acid method (Yaphe, 1960) using galactose as standard. The 3,6-anhydrogalactose content (3,6-AG) was determined by the resorcinol acetal method (Yaphe and Arsenault, 1965), using D-fructose as a standard. The pyruvic acid was measured according to the method of Sloneker and Orentas (1962). The sulfate content was determined by gelatin–$BaCl_2$ method with K_2SO_4 as the standard (Dodgson and Price, 1962). The analyses were carried out in triplicate. The composition results were expressed as percentage of dried agar.

Agar viscosity

The viscosity of 1% agar gel was measured at 60°C by a falling ball viscometer. The dynamic viscosity is given as mPa.s. For the all these analysis, a commercial agar was used as the control.

Statistical analysis

All results were expressed as mean (± standard deviation) and were statistically compared through non parametric analyses of variance, Kruskal-Wallis and Mann- Withney *U*-test.

RESULTS

The results are presented according to the season. Each season was obtained from the average of three months.

Dry biomass and agar yield of *G. sesquipedale*

The average yield of dry matter of *G. sesquipedale* obtained was 22.34% (±3.18%). No significant differences were revealed between seasons ($P \geq 0.05$) (Figure 1). The average yield of agar was 27.96 (±3.5%), and increased significantly from winter (23.70 ± 1.4%) to summer ($P<0.05$) reaching a peak in June (33.22 ± 2.09%). The yield decreased significantly in autumn (September: 23.37 ± 0.8%) ($P<0.05$) (Figure 1).

Total sugars and content of 3,6-anhydrogalactose

Total sugars showed an average of 87.63 (±6.9%). This value varied seasonally, being highest in winter (January: 96.76 ± 2.7%) and lowest in spring (May: 61.85 ± 15%) ($P<0.05$).

The concentration of 3,6-anhydrogalactose was 34.24 (±3.8%). No significant differences ($P> 0.05$) in the content of 3,6-anhydrogalactose were observed between different seasons ($P> 0.05$) (Figure 2). Commercial agar has an average rate of 3,6-anhydrogalactose which is about 49.37 ± 2.94%. This is comparable to the rate measured during the spring (Figure 2).

Sulphate and pyruvate content

The average rate of the sulfate in the agar was 2.75 ± 1.37%. This rate decreased gradually ($P <0.05$) from winter (January: 4.41 ± 0.62%) and reached the minimum in summer (July: 0.77 ± 0.08%), then increased in autumn ($P<0.05$) (Figure 3). The average rate of pyruvate was 0.32 ± 0.09%. It showed no significant difference

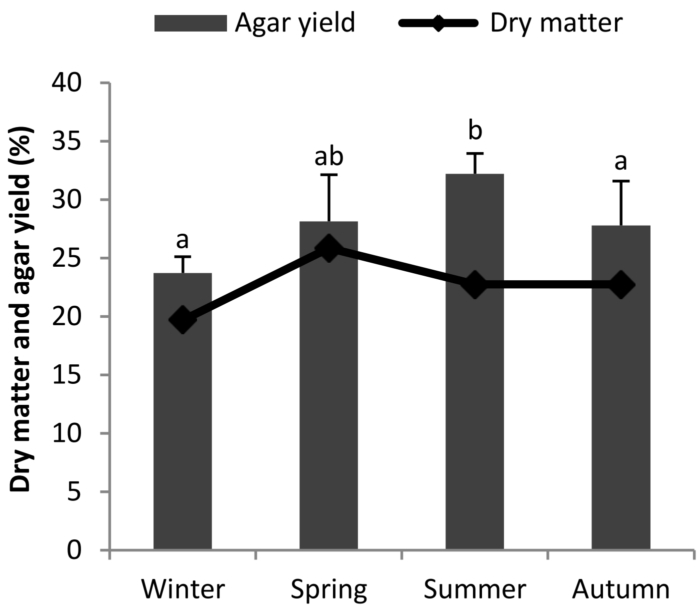

Figure 1. Seasonal variations of dry matter and agar yield of *Gelidium sesquipedale*. Bars with different letters show significant differences.

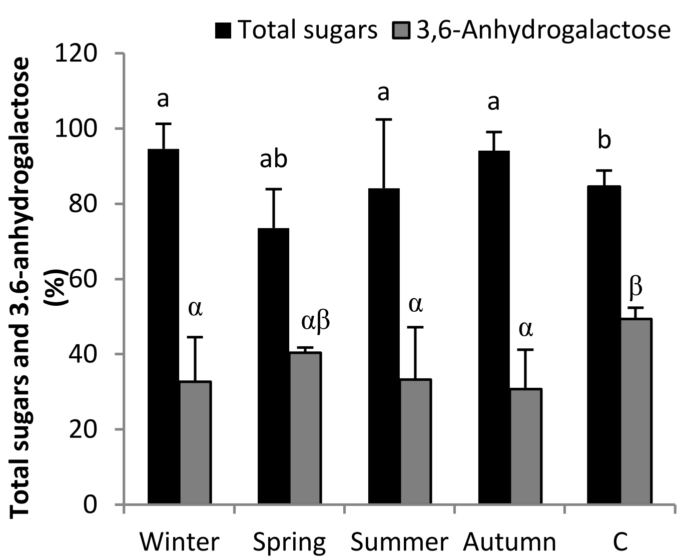

Figure 2. Seasonal variation of total sugars and 3.6-anhydrogalactose, n = 9, C: Commercial agar. Histograms with different subscripts are significantly different (p <0.05).

during the seasons. The sulfate's rate of the commercial agar (1.55 ± 0.41%) was comparable with rates obtained in autumn, spring and summer, while pyruvate rate (0.45 ± 0.03%) was closer to that obtained in the summer (Figure 3).

The agar viscosity

The average viscosity of the agar solution (1%) at 60°C, was 11.11 ± 1.50 mPa.s. Following its evolution according to the seasons, it was shown that it remained

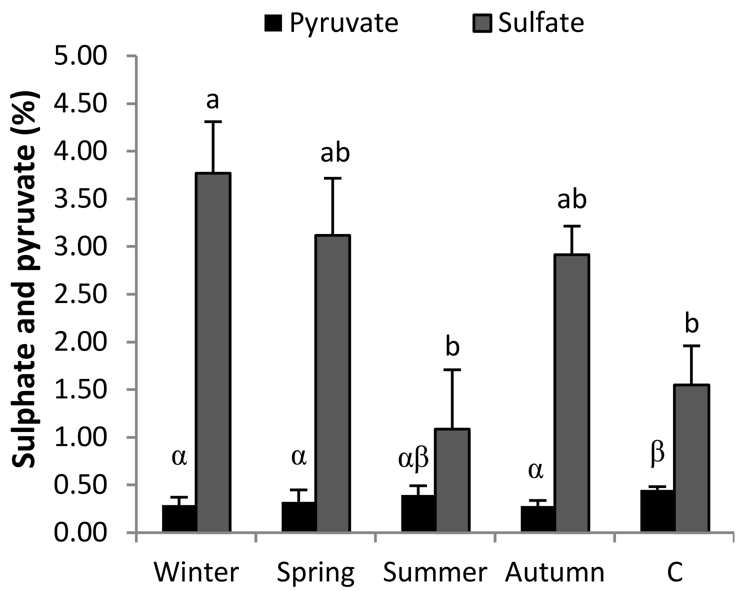

Figure 3. Seasonal variation of sulphate and pyruvate rate, n = 9, C: Commercial agar. Histograms with different subscripts are significantly different (p <0.05).

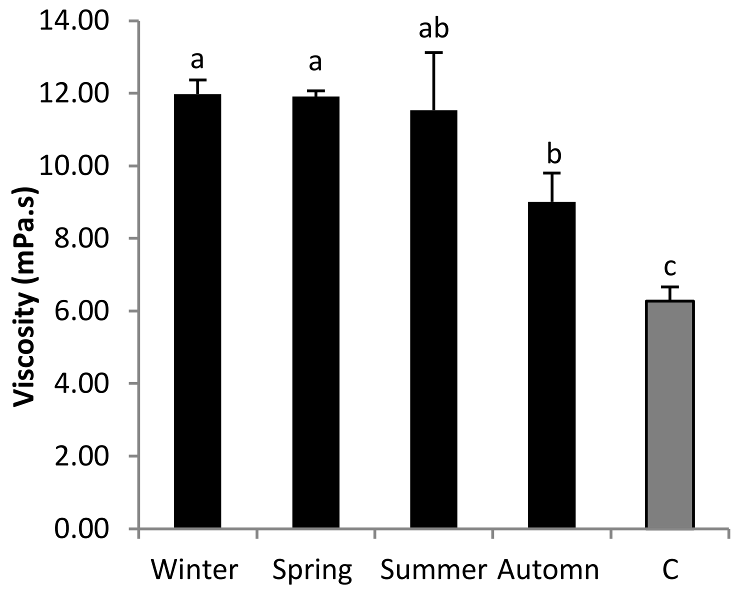

Figure 4. Seasonal variation in the viscosity of the agar. n = 9, C: Commercial agar. Histograms with different subscripts are significantly different (p <0.05).

stable from winter (11.97 ± 0.40 mPa.s) to summer and decreased significantly (P <0.05) and reached its minimum in autumn (November: 8.53 ± 1.2 mPa.s) (Figure 4). The agar extracted from *G. sesquipedale* had a viscosity significantly (P <0.05) higher than the commercial agar (6.25 ± 0.4 mPa.s).

DISCUSSION

The red algae are the subject of many studies around the world; however, the data presented in this study are the first results of the Rhodophyceae, *G. sesquipedale* of the Algerian coast.

The yield of dry matter, obtained from *G. sesquipedale,* during this study was lower than that obtained from the Atlantic species, *G. sesquipedale* of Moroccan coast (Mouradi-Givernaud et al., 1999), *Gelidium latifolium* of Rosscoff coast (Mouradi-Givernauld et al., 1992) and *Gelidium canarisiensis* of Canary Islands (Freile-Pelegrin et al., 1995) with dry matter yield fluctuating around 30% depending on the season. The highest yield of agar was registered in spring and summer (32.22 ± 1.7%). The present study results are in agreement with those of Mouradi-Givernaud et al. (1999) which showed that the maximum yield of agar from *G. sesquipedale* of Moroccan Atlantic coast, is obtained between March and August (40%). Moreover, several works on *Gelidium*, recorded the maximum yield during the spring and/or summer, such as *G. spinosum* of the Tunisian coast (Monastir) (33.37 ± 2.80%) (Ben Said et al., 2009), *G. canarisiensis* of the Canary Islands (32.6%) (Freile-Pelegrin et al., 1995) and *G. robustum* of western coast of Mexico (44.2%) (Freile-Pelegrin et al., 1999).

The maximum yield obtained during the spring and summer was related to the increase in temperature and photoperiod. Indeed, temperature and light have a synergistic effect on photosynthesis. In *G. sesquipedale,* the results of Torres et al. (1991), showed that the increase of the light's intensity was associated with cell wall polysaccharides synthesis. In addition, Freile-Pelegrin et al. (1995) linked the increase in the yield of agar to lower nutrient concentrations observed during the summer, which was also observed in this species (Zellal et al., 2015). According to Vergara et al. (1993), the nitrate regulates agar biosynthesis, when the nitrogen (N) is limited, newly fixed carbon (C) is directed towards agar biosynthesis and inversely, when there is an active assimilation of N, C is directed towards organic N compounds and away from C reserve structures. On the other hand, Mouradi-Givernaud et al. (1993) suggested that this increase in spring and summer can be explained by the fact that the new fragments apical ramifications are richer in agar than the old parts.

The decrease in the yield during winter and autumn may be the result of several factors such as the reduction of light intensity; this was observed in *Gracilaria* where the reduction in light intensity led to the decrease of agar's content (Freile-Pelegrín and Robledo, 1997). On the other hand, the stress induced by the reduction in salinity, observed in winter, may lead to a decrease in the rate of photosynthesis (Craigie, 1990; Buriyo and Kivaisi, 2003). Thus, this decrease in the rate of photosynthesis can lead to degradation of cell wall polysaccharides, according to Torres et al. (1995), cell-wall polysaccharides could act not only as a structural polysaccharide in the wall, but also as storage polymers which could be mobilized under certain conditions. Moreover, the lack of the adequate rates of CO_2, HCO^{-3} and Ca^{+2} in the rainwater, can also have a considerable effect on the photosynthesis and induce a reduction in the accumulation of carbohydrate (Buriyo and Kivaisi, 2003). Mercado et al. (2001), demonstrated that the photosynthetic rates decreased in parallel with a decrease in CO_2 concentration, and the parameters of photosynthesis are affected by the changes in dissolved inorganic carbon concentration.

The rate of total sugars varied during the seasons and reached the minimum in spring. In the agar of *G. sesquipedale* and *G. latifolium* of the Atlantic coasts, the rate of total sugars was relatively stable during the year (Mouradi-Givernaud et al., 1992, 1999). The 3,6-anhydrogalactose (3,6-AG) is the second principal component of agar. The average rate recorded during the present study was 34.24 ± 3.8% (30.65 ± 5.56% to 40.40 ± 1.34%). In general, the rate of 3,6-anhydrogalactose does did not exceed 50% in agar, this was observed in several species; *G. robustum* of the Pacific Coasts of Mexico (Hurtado et al., 2010), *G. rex* of the Chilean coast (Matshuiro and Urzua,1990) and *G. purpuascens* of the Canadian coast (Whyte and Englar, 1981). As for seasonal variations, the rate of 3,6-anhydrogalacose has not changed during the year. These results are in agreement with those obtained from *G. sesquipedale* (Mouradi-Givernauld et al., 1999) and *G. latifolium* (Mouradi-Givernauld et al., 1992).

With regard to the substituents of the agar, the rate of sulfate varied with the seasons. This rate is similar to those reported in many other species such as *G. sesquipedale* of Spain coasts (3.2%) (Young et al., 1971), *G. rex* Chilean coast (from 2.2 to 4.2%) (Matshuiro and Urzua, 1990), *G. latifolium* of the coast of Roscoff (1.6 to 2.08%) (Mouradi-Givernauld et al., 1992), *G. robustum* (1.2 - 4%) (Hurtado et al., 2010). The results of this study showed a decrease of sulfate during summer (Freile-Pelegrín and Robledo, 1997). Lahaye and Yaphe (1988) also found that the agar of *Gracilaria pseudoverrucosa* collected in winter was richer in sulfates groups. Freile-Pelegrín and Robledo (1997) attributed this variation to environmental changes.

The pyruvate; the second substituent studied, has an average rate of 0.32 ± 0.09%. Indeed, the pyruvate was not detected in *G. latifolium* of the Roscoff coast (Mouradi-Givernauld et al., 1992), neither in *G. rex* of Chilean coast (Matshuiro and Urzua, 1990), while in *G. chilense* (Matshuiro and Urzua, 1991) of these same coasts, the pyruvate was estimated between 0.42 to 0.54%. Young et al. (1971) found agar pyruvate contents to about 0.04% for *Gelidium sesquipedale*. According to Nelson et al. (1994), a highly pyruvated agar may be of use commercially, for example as the gel matrix in high electroendosmosis (EEO) electrophoresis.

With regards to the physical properties of agar gel (1% solution at 60°C), the viscosity was 11.11 ± 1.50 mPas. This value was higher than the commercial agar, but comparable to the results obtained by Millan et al. (2002) (10 mPa.s). However, a high viscosity, with an excellent agar yield, suggest that use of this colloid in the food industry is feasible, mainly in spreads and soft-texture food products (Pereira-Pacheco et al., 2007).

This study enriched the data on *G. sesquipedale* in the Algerian coast and the Western Mediterranean basin. In general, the physicochemical characteristics of the agar of *G. sesquipedale* are close to those of the commercial agar, which leads to the conclusion that this alga can be a source of production of agar at Algeria during the spring/summer period.

Conflict of interests

The authors have not declared any conflict of interest.

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