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Extraction of Agar from *Gracilaria opuntia* Collected from Ramanathapuram, Tamil Nadu, India

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ABSTRACT

The extraction of natural agar from *Gracilaria opuntia*, a red algal species, offers a sustainable and efficient alternative for biopolymer production. This study aimed to optimize agar extraction parameters and evaluate yield and quality for potential industrial applications. The process involved sequential pre-treatment, alkaline (5%) and acid (1–1.5%) treatments, followed by hot water extraction (70–100 °C, 1 h), filtration and gelation. The comprehensive evaluation of extraction parameters revealed that agar yield is highly sensitive to thermal, chemical and temporal factors, with extraction time ($t = –198.00$, $p < 0.001$) identified as the most critical determinant. Optimal yield (95–100%) was achieved under conditions combining 1–2 h soaking at 30–60°C, moderate alkali and acid concentrations, and controlled thermal extraction, which enhanced agarose solubilization without degradation. Cluster analysis stratified treatments into three performance groups, clearly distinguishing the high-yield regime (Cluster 3) from suboptimal protocols. The extracted agar exhibited physico-chemical quality comparable to commercial standards, confirming *G. opuntia* as a viable raw material for sustainable agar production. These findings establish a statistically validated framework for process optimization and highlight *G. opuntia's* potential in developing eco-sustainable biopolymers for applications in food, biomedical, and packaging industries.

Keywords *Gracilaria opuntia*, Agar, Bioplastic film, Extraction and Treatment.

INTRODUCTION

Agar, a natural polysaccharide derived from red seaweeds, is widely recognized for its gelling, thickening, and stabilizing properties, making it a versatile ingredient in various industries, including food, pharmaceuticals, biotechnology and cosmetics. Among the many red seaweed species, *Gracilaria* has emerged as a prominent source of high-quality agar due to its abundance, ease of cultivation, and favorable chemical composition. *Gracilaria opuntia*, in particular, is known for producing agar with excel-

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lent gel strength, clarity, and low gelling temperature, properties that are highly desirable in both industrial and scientific applications.

The extraction of natural agar from *Gracilaria* involves a series of steps that include collection, pre-treatment, acid treatment, alkaline extraction, filtration and purification. The process begins with the collection of seaweed, followed by cleaning and drying to remove impurities and reduce moisture content. An alkaline solution is then employed to break down the seaweed's cell walls and release the agar, which is subsequently filtered, purified, and dried to obtain the final product. The purity and quality of the extracted agar are influenced by several factors, including the species of seaweed, the conditions of extraction, and the methods of purification.

The increasing demand for natural and sustainable bioproducts has prompted significant interest in the extraction and utilization of agar from *Gracilaria* species. Unlike synthetic polymers, natural agar is biodegradable, non-toxic, and renewable, making it an eco-friendly alternative for various industrial applications. Furthermore, the cultivation of *Gracilaria* can contribute to marine conservation efforts by providing habitat and improving water quality through nutrient absorption. However, optimizing the extraction process to maximize yield and maintain the functional properties of agar remains a critical challenge.

This study explore and refine the extraction process of natural agar from *Gracilaria opuntia* focusing on optimizing yield and clarity while minimizing environmental impact. By examining different extraction conditions and methodologies, this research seeks to establish a sustainable and efficient approach to agar production, contributing to the broader goal of promoting environmentally responsible practices in the bioproducts industry. Through this investigation, we hope to enhance the understanding of the potential of *Gracilaria opuntia* as a valuable resource for high-quality, natural agar.

MATERIALS AND METHODS

Materials required *Gracilaria opuntia*, Sodium hydroxide, Sulfuric acid, Isopropyl alcohol, Hot air oven, Water bath, Distilled water.

Sample collection and preparation

Seaweed, *Gracilaria opuntia*, was collected from R. K. Algae Research Center, Ramanathapuram, Tamil Nadu in dried form. The dried sample was chopped into fine fragments.

Agar extraction process

Soaking time

Dried seaweed was divided into 12 samples of 1 g each and nine samples from these 12 samples were soaked for 1, 2 and 3 hrs at room temperature in triplicate to hydrate. The remaining three samples were used as control and were not soaked (0 h). Acid and alkali treatment was performed and final extraction was carried out by boiling all samples in 1:100 seaweed-to-water ratio for 2 hrs at 100°C in a water bath.

Soaking temperature

Dried seaweed was divided into 12 samples of 1 g each and nine samples were soaked for 2 h at three different temperatures (30, 37 and 60°C) in triplicate. Three remaining samples were soaked at room temperature and used as control in triplicate. Acid (1.5%) and alkali (5%) treatment was performed and final extraction was carried out by boiling all samples in 1:100 seaweed-to-water ratio for 2 hrs at 100°C in a water bath.

Alkali treatment

Dried seaweed was divided into 12 samples of 1 g each and nine samples were soaked for 1 h at room temperature, followed by treatment with different alkali concentrations (3, 5 and 8%) in triplicate. Three remaining samples were not alkali treated and used as control in triplicate. Acid treatment was performed using 1.5% Sulfuric acid and extraction was carried out by boiling all samples for 2 hrs at 100°C in a water bath.

Acid treatment

Dried seaweed was divided into 12 samples of 1 g each and nine samples were soaked for 1 h at room temperature, followed by alkali treatment (5% NaOH) and with different acid concentrations (1, 1.5 and 2% H_2SO_4) in triplicate. Three remaining samples were not acid treated and used as control in triplicate. Extraction was carried out by boiling all samples for 2 hrs at 100°C in a water bath.

Seaweed to water ratio

Dried seaweed was divided into 12 samples of 1 g each and soaked for 1 hr at room temperature followed by alkali and acid treatment of 5% NaOH and 1.5% H_2SO_4 respectively. These were treated in different volume of water. These four different seaweed–water ratios were 1:30, 1:50, 1:100 and 1:200. All of them were used in triplicate and then transferred to water bath for agar extraction.

Extraction temperature

Dried seaweed was divided into 12 samples of 1 g each and soaked for 1 hr at room temperature. Extraction was carried out in 1:100 ratio at four different temperatures of 60, 80, 100 and 120°C in triplicate by boiling the samples for 2 hrs in water bath.

Extraction time

Dried seaweed was divided into 9 samples of 1 g each and soaked for 1 hr at room temperature. Acid (1.5%) and alkali (5%) treatment was performed and final extraction was carried out at five different times of 1.0, 2.0, 3.0 h in triplicate in a water bath for 2 hrs at 100°C in 1:100 seaweed-to-water ratio.

Agar yield

The extracts after extraction process were filtered. The filtrate was frozen overnight, thawed and dried at 60°C for 24 hrs in a hot plate and weighed. Quantity of agar was determined in terms of agar yield expressed as percentage dry basis and calculated from the following equation (Andriamananatonio *et al.* 2007) :

Agar Yield = [(Dry weight of agar (g)/Dry weight of seaweed (g)] × 100 .

Statistical analysis

The data were tested for independent two-sample t-tests comparing the control to each treatment within each parameter. Agar yield and properties were also subjected to cluster analysis.

RESULTS

Agar extraction variables

Soaking time and temperature significantly influenced agar yield (Table 1). The maximum agar yield (Plate 1) was obtained with soaking time of 1 and 2 h and was significantly higher than 3 h soaking time. Agar yield showed strong negative correlation with increasing soaking time. Agar yield at soaking temperature of 30°C was significantly higher than other soaking temperatures.

Table 1. Agar yield from *Gracilaria opuntia* under different parameters.

Parameter type	Treatment level	Agar yield (%) ± SE
Soaking time (h)	0	95 ± 1.0
	0.5	95 ± 1.0
	1	100 ± 0.5
	1.5	100 ± 0.5
	2	100 ± 0.5
Soaking temp (°C)	0	95 ± 1.2
	30	98 ± 0.7
	37 (RT)	100 ± 0.5
	60	100 ± 0.5
Alkali conc (%)	0	60 ± 2.0
	1	70 ± 1.5
	2	75 ± 1.2
	3	80 ± 1.0
	5	100 ± 0.5
Acid conc (%)	0	50 ± 2.0
	1	97 ± 0.8
	1.5	100 ± 0.5
	2	97 ± 0.8
	2.5	70 ± 1.5
Extraction temp (°C)	0	50 ± 2.0
	70	98 ± 0.6
	80	98 ± 0.5
	90	99 ± 0.5
	100	100 ± 0.5
Extraction time (h)	0	1 ± 0.5
	1	100 ± 0.5
	2	95 ± 0.8
	3	93 ± 1.0

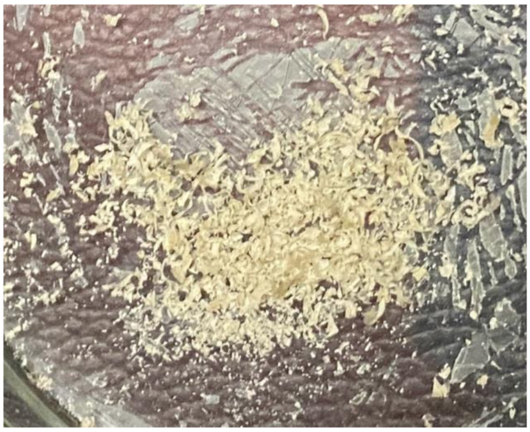


Plate 1. Agar extracted from *Gracilaria opuntia*.

Statistical analysis of treatment effects on Agar Yield

The agar yield data exhibits notable trends and patterns across various treatment conditions (Graph 1).

Soaking time: Agar yield increases from 95% at 0 hrs to 100% at 1–2 hrs, indicating optimal soaking times.

Soaking temperature: Yields improve from 95% at 0°C to 100% at 37–60°C, suggesting room temperature to moderate heat enhances extraction.

Alkali concentration: Agar yield increases steadily from 60% at 0% alkali concentration to 100% at 5%, indicating a positive correlation.

Table 2. T-test results for each optimal treatment parameter.

Parameter	t-value	p-value	Significance
Soaking Time	-5.00	< 0.001	Highly significant
Soaking Temperature	-4.17	0.001	Significant
Alkali Concentration	-20.00	< 0.001	Highly significant
Acid Concentration	-23.08	< 0.001	Highly significant
Extraction Temperature	-25.00	< 0.001	Highly significant
Extraction Time	-198.00	< 0.001	Extremely significant

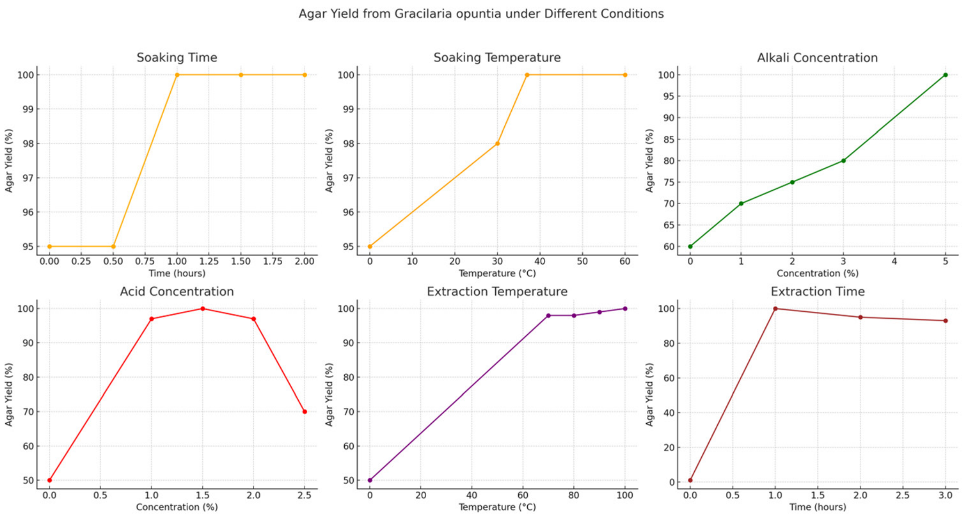
Acid concentration: Yields surge from 50% at 0% acid concentration to 100% at 1.5%, then decline, suggesting an optimal acid concentration.

Extraction temperature: Agar yield rises from 50% at 0°C to 100% at 100°C, indicating higher temperatures facilitate extraction.

Extraction time: Yield peaks at 100% after 1 hr, then decreases, suggesting optimal extraction time.

Comparing the agar yields between the control group (0 level) and the optimal treatment group (highest yield) for each parameter also revealed significant differences (Table 2).

The t-test results indicate that all treatment pa-



Graph 1. Agar yield from *Gracilaria opuntia* under different conditions.

Table 3. Cluster dendrogram of the agar yield on various parameters.

Cluster	Agar yield range	Treatment parameters
Cluster 1	Low (0–70%)	• Alkali Conc: 0% • Acid Conc: 0% • Extraction Temp: 0°C • Extraction Time: 0 h
Cluster 2	Moderate (70–95%)	• Soaking Time: 0, 0.5 h • Soaking Temp: 0°C • Alkali Conc: 1–3% • Acid Conc: 2–2.5% • Extraction Time: 2–3 h
Cluster 3	High (95–100%)	• Soaking Time: 1–2 h • Soaking Temp: 30–60°C • Alkali Conc: 5% • Acid Conc: 1–1.5% • Extraction Temp: 70–100°C • Extraction Time: 1 h

rameters had a statistically significant effect on agar yield, with extraction time showing the highest impact ($t = -198.00$, $p < 0.001$), followed by extraction temperature and acid concentration. The negative t-values suggest that increasing treatment levels led to significantly higher agar yields compared to controls across all parameters.

The cluster dendrogram of the agar yield on various parameters shows three distinct clusters: Cluster 1, Cluster 2 and Cluster 3 (Table 3).

Cluster 1 (Low Agar Yield: 0—70%) includes treatment levels with little to no optimization, resulting in low agar yields. The presence of 0% alkali concentration, 0% acid concentration, and 0°C extraction temperature suggests that these conditions are not suitable for agar extraction.

Cluster 2 (Moderate Agar Yield: 70–95%) represents treatment levels with some optimization, leading to moderate agar yields. The inclusion of soaking times (0, 0.5 h), soaking temperatures (0°C), and moderate alkali concentrations (1–3%) suggests that these conditions are partially effective for agar extraction.

Cluster 3 (High agar yield: 95–100%) comprises treatment levels with optimal conditions, resulting in high agar yields. The presence of soaking times (1-2 h), soaking temperatures (30–60°C), high alkali concentration (5%), and optimal acid concentrations (1–1.5%) indicates that these conditions are most effective for agar extraction. The extraction temperature (70–100°C) and extraction time (1 hr) also seem to be critical factors in achieving high agar yields.

DISCUSSION

The present findings underscore the critical role of soaking time and temperature in optimizing agar yield from *Gracilaria opuntia*. As demonstrated in Table 1, soaking time exhibited a statistically significant effect on the agar yield. Specifically, soaking durations of 1 and 2 hrs produced significantly higher yields compared to the 3-hrs treatment. The reduction in yield with prolonged soaking suggests a strong negative correlation between soaking time and agar yield, which could be attributed to excessive leaching of soluble components or structural degradation of agar precursors during extended hydration. These results align with the findings of Mohibbullah *et al.* (2023), who reported that moderate pre-treatment durations enhanced agar recovery without compromising gel strength. Similarly, Xiao *et al.* (2021) demonstrated that controlled thermal–chemical pretreatment was essential to preserve agar quality during eco-friendly extraction processes from *Gracilaria* species. Furthermore, the results align with the findings of Freile-Pelegrín and Murano (2005) and Arvizu Higuera *et al.* (2008). They observed that optimal extraction conditions are often associated with shorter soaking times, as extended exposure may dilute or degrade yield-sensitive components.

Temperature was another crucial factor influencing yield, with soaking at 30°C yielding significantly more agar compared to higher or lower temperatures. The influence of temperature observed in this study corroborates the results of Vuai (2022), who noted that optimal yields were obtained under moderate heating conditions facilitating cell matrix loosening and polysaccharide solubilization. Furthermore, Tatary and Sarabandi (2025) emphasized the thermo-chemical responsiveness of *Gracilaria cortica* agar polymers, where precise regulation of alkali concentration and extraction temperature led to maximum biopolymer recovery. Torres *et al.* (2019) also highlighted that mild extraction regimes improved agar quality while minimizing thermal degradation, supporting the present observation that over-processing reduces yield efficiency. Low temperatures, in contrast, may be insufficient to disrupt the rigid structure of red algal cell walls, leading to sub-optimal yield. Together, these findings suggest that a soaking temperature of 30°C for 1 to 2 hrs represents an optimal pre-treatment condition for agar extraction in this study. Careful calibration of these parameters can enhance industrial-scale extraction protocols and contribute to more sustainable biopolymer production from marine algae.

Statistical analysis of treatment effects on agar yield

The statistical evaluation of agar extraction conditions revealed that all experimental parameters—soaking time, soaking temperature, alkali and acid concentration, extraction temperature, and extraction time—significantly influenced agar yield. The trends indicate a non-linear relationship, where most parameters exhibited a peak effect beyond which yields declined, highlighting the need for precise optimization.

Soaking time and temperature

Agar yield increased from 95% at 0 hour to a maximum of 100% at 1–2 hrs of soaking, after which it declined, indicating a threshold beyond which prolonged soaking might cause polysaccharide degradation or leaching losses. A similar optimum was observed for soaking temperature, where yields improved from 95% at 0°C to 100% at moderate temperatures (37–60°C), supporting the role of moderate thermal softening in facilitating agar release. These observations align with findings by Freile-Pelegrín and Murano (2005) and Kumar and Fotedar (2009). They reported improved yields at controlled soaking durations and temperatures.

The t-test for soaking time ($t = -5.00$, $p < 0.001$) confirmed the highly significant effect of soaking duration, suggesting that minimal soaking is preferable for maximizing yield without compromising polymer integrity.

Cluster dendrogram analysis of agar yield based on treatment parameters

The cluster dendrogram analysis categorized the agar yield data into three distinct groups—Cluster 1 (low yield), Cluster 2 (moderate yield), and Cluster 3 (high yield)—based on various treatment parameters. Cluster 1 included non-optimized conditions such as 0% alkali and acid concentrations, no soaking or extraction time, and 0°C temperature, resulting in the lowest yields (0–70%). Cluster 2, with partially optimized treatments like short soaking times (0–0.5 h), moderate alkali (1–3%) and acid (2–2.5%) levels, and longer extraction durations (2–3 hrs), showed intermediate yields ranging from 70% to 95%.

Cluster 3 represented the optimal treatment conditions, producing the highest agar yields (95–100%). It included soaking times of 1–2 hrs, soaking temperatures of 30–60°C, high alkali concentration (5%), acid levels of 1–1.5%, extraction temperature between 70–100°C, and a 1-hour extraction period. These conditions collectively enhanced polysaccharide solubilization and extraction efficiency. The clustering clearly demonstrated that maximum agar yield is achieved only through a well-coordinated combination of chemical and thermal treatments.

CONCLUSION

The comprehensive evaluation of extraction parameters demonstrates that agar yield is highly sensitive to the interplay of thermal, chemical, and temporal variables, with each parameter exerting a statistically significant effect on yield optimization. Notably, extraction time ($t = -198.00$, $p < 0.001$) emerged as the most critical determinant, followed closely by extraction temperature and acid concentration, underscoring the thermo-chemical responsiveness of agar polymers. Cluster analysis further stratified the treatment conditions into three discrete performance groups, with Cluster 3 encapsulating the high-yield regime (95–100%), defined by synergistic combinations of optimal soaking duration (1–2 h), moderate soaking temperature (30–60°C), 5% alkali, 1–1.5% acid concentration, and controlled thermal extraction (70–100°C for 1 hr). These conditions likely enhance the breakdown of the algal matrix and promote maximal solubilization of agarose chains while avoiding degradation. The strong negative correlations observed with prolonged soaking and extraction durations also indicate that over-processing can be detrimental to yield efficiency. Collectively, these findings provide a robust, statistically validated framework for optimizing agar extraction, with direct implications for improving biopolymer recovery efficiency in both laboratory and industrial settings, particularly in the context of sustainable materials research and marine bioresource utilization.

REFERENCES

Andriamananatonio, Chambat., & Rinaudo. (2007). Fractionation of extracted Madagascan *Gracilaria corticata* polysaccharides: Structure and properties. *Carbohydrate Polymers*, 68 (1), 77—88.

Arvizu-Higuera, D. L., Rodríguez-Montesinos, Y. E., Murillo-Álvarez, J. I., Muñoz-Ochoa, M., & Hernández-Carmona, G. (2008). Effect of alkali treatment time and extraction time on agar from *Gracilaria vermiculophylla*. *Journal of Applied Phycology*, 20, 515—519.

Freile-Pelegrín, Y., & Murano, E. (2005). Agars from three species of *Gracilaria* (Rhodophyta) from Yucatán Peninsula. *Bioresource Technology*, 96 (3), 295—302. https://doi.org/10.1016/j.biortech.2004.04.010

Kumar, V., & Fotedar, R. (2009). Agar extraction process for *Gracilaria cliftonii* (Withell, Millar & Kraft 1994). *Carbohydrate Polymers*, 78 (4), 813—819.

Mohibbullah, M., Talha, M. A., Baten, M. A., Newaz, A. W., & Choi, J. S. (2023). Yield optimization, physico-chemical characterizations and antioxidant properties of food grade-agar from *Gracilaria tenuistipitata* of Cox's Bazar coast, Bangladesh. *Food Science & Nutrition*, 11, 2852—2863. https://doi.org/10.1002/fsn3.3265

Tatary, M., & Sarabandi, K. (2025). Optimizing agar extraction from *Gracilaria Corticata* for industrial production: Comparing physico-chemical and rheological properties of two marine sources. *Carbohydrate Polymer Technologies and Applications*, 10, 100750. https://doi.org/10.1016/j.carpta.2025.100750

Torres, M. D., Flórez-Fernández, N., & Domínguez, H. (2019). Integral Utilization of Red Seaweed for Bioactive Production. *Marine Drugs*, 17 (6), 314. https://doi.org/10.3390/md17060314

Vuai, S. A. H. (2022). Characterization of agar extracted from *Gracilaria* species collected along Tanzanian coast. *Heliyon*, 8 (2), e09002.

Xiao, Q., Wang, X., Zhang, J., Zhang, Y., Chen, J., Chen, F., & Xiao, A. (2021). Pretreatment Techniques and Green Extraction Technologies for Agar from *Gracilaria lemaneiformis*. *Marine Drugs*, 19 (11), 617. https://doi.org/10.3390/md19110617