



Optimization of alkali-treated *Gracilaria verrucosa* chips production using response surface methodology: Physicochemical characterization and agar quality assessment

[Optimización de la producción de chips de *Gracilaria verrucosa* tratados con álcali mediante la metodología de superficie de respuesta: Caracterización fisicoquímica y evaluación de la calidad del agar]

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Abstract

Context: Agar, a hydrocolloid derived from red seaweed such as *Gracilaria verrucosa*, is widely used in food and pharmaceutical industries. In addition to dried seaweed, several countries now utilize alkali-treated *Gracilaria* (ATG) chips as a semi-finished product for agar production. However, the alkali pre-treatment process for producing ATG chips remains suboptimal and requires refinement for improved efficiency.

Aims: To optimize the production of ATG chips from *G. verrucosa* using response surface methodology (RSM) to obtain optimal product quality.

Methods: A central composite design was applied with two factors: alkali concentration and heating temperature, and six responses: ATG yield, moisture content, ash content, sulfate content, agar yield, and agar gel strength. Design Expert 11 software was used for experimental design, model selection, and process optimization.

Results: Optimal conditions were found at 10.30% NaOH concentration and 67.76°C heating temperature, achieving a desirability value of 0.87. Under these conditions, the ATG chips showed a yield of 45.60%, 21.18% moisture, 4.42% ash, and 1.42% sulfate content. The derived agar had a yield of 54.74% and a gel strength of 100.91 g/cm².

Conclusions: The validation results confirmed the predictive accuracy of the RSM model. The optimized alkali pre-treatment process improves the quality and efficiency of ATG chip production, offering a promising alternative for sustainable agar manufacturing.

Keywords: agar yield; alkali-treated chips; gel strength; process optimization; seaweed processing; response surface methodology.

Resumen

Contexto: El agar, un hidrocoloide derivado de algas rojas como la *Gracilaria verrucosa*, se utiliza ampliamente en las industrias alimentaria y farmacéutica. Además de algas secas, varios países utilizan actualmente virutas de *Gracilaria* tratadas con álcali (ATG) como producto semiacabado para la producción de agar. Sin embargo, el proceso de pretratamiento con álcali para la producción de chips de ATG sigue siendo subóptimo y requiere un perfeccionamiento para mejorar su eficiencia.

Objetivos: Optimizar la producción de chips de ATG a partir de *G. verrucosa* utilizando la metodología de superficie de respuesta (RSM) para obtener una calidad óptima del producto.

Métodos: Se aplicó un diseño compuesto central con dos factores: concentración de álcali y temperatura de calentamiento, y seis respuestas: Rendimiento de ATG, contenido de humedad, contenido de cenizas, contenido de sulfato, rendimiento de agar y resistencia del gel de agar. Se utilizó el software Design Expert 11 para el diseño experimental, la selección del modelo y la optimización del proceso.

Resultados: Las condiciones óptimas se encontraron con una concentración de NaOH del 10,30% y una temperatura de calentamiento de 67,76°C, alcanzando un valor de deseabilidad de 0,87. Bajo estas condiciones, las astillas de ATG mostraron un rendimiento del 45,60%, 21,18% de humedad, 4,42% de ceniza y 1,42% de contenido de sulfato. El agar derivado tuvo un rendimiento del 54,74% y una fuerza de gel de 100,91 g/cm².

Conclusiones: Los resultados de la validación confirmaron la exactitud predictiva del modelo RSM. El proceso optimizado de pretratamiento con álcali mejora la calidad y la eficiencia de la producción de chips de ATG, ofreciendo una alternativa prometedora para la fabricación sostenible de agar.

Palabras Clave: fuerza del gel; rendimiento de agar; optimización del proceso; procesamiento de algas; metodología de superficie de respuesta; virutas tratadas con álcali.

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INTRODUCTION

Seaweed is a marine resource with significant development potential in Indonesia, owing to the country's abundant and suitable coastal growing areas. Seaweed is found along nearly all Indonesian coastlines, making it a key export commodity of the fisheries sector. In fact, Indonesia ranks as the world's second-largest seaweed exporter after China, with an export volume of 232,081.2 tons reported in 2022 (BPS, 2023).

Seaweed is rich in nutrients such as vitamins, minerals, polyunsaturated fatty acids, amino acids, fiber, and bioactive compounds that offer various health benefits (Francavilla et al., 2013). These properties support its broad application across food, cosmetic, and pharmaceutical industries (El-Beltagi et al., 2022; Vieira et al., 2018). Moreover, due to its antioxidant activity, seaweed-derived products are frequently utilized in pharmaceutical formulations (Insani et al., 2022).

Agar, a polysaccharide extracted from red seaweeds such as *Gracilaria* spp. and *Gelidium* spp., is widely used in pharmaceutical manufacturing. It serves as an excipient in various dosage forms, including tablet binders, gel bases, and cream formulations (Kurniawan et al., 2016).

In addition to dried seaweed, agar can also be obtained from alkali-treated *Gracilaria* (ATG) chips, which serve as a semi-processed intermediate product. These chips are produced through a pretreatment and drying process of *Gracilaria* that involves desulfation—specifically, the conversion of galactose-6-sulfate to 3,6-anhydro-galactose. This reaction enhances the gel strength of agar and is typically induced using sodium hydroxide (NaOH) under controlled temperature and time conditions (Arvizu-Higuera et al., 2008; Freile-Pelegrín and Robledo, 1997; Orduña-Rojas et al., 2008).

The use of ATG chips as an industrial raw material for agar production presents several advantages: it shortens processing time and reduces the generation of wastewater from raw seaweed washing, thus offering environmental benefits. However, the ATG production process in Indonesia remains suboptimal and requires further improvement.

To address this, the present study applies Response Surface Methodology (RSM) to optimize two key processing parameters—alkali concentration and heating temperature—in the production of ATG chips. RSM is a statistical and mathematical tool frequently used for modeling and analyzing problems in which multiple variables influence a response of in-

terest (Nuryanti and Salimy, 2008). The objective of this study is to determine the optimal process conditions for producing high-quality ATG chips efficiently and sustainably.

MATERIAL AND METHODS

Vegetal material

The raw material used in this study was the entire thallus of dried seaweed *Gracilaria verrucosa* (Hudson) Papenfuss, 1950 (AphiaID: 163501). The material was sourced from a local seaweed farmer's warehouse located in Randusanga, Brebes, Central Java, Indonesia (coordinates: 6.85° S, 109.07° E). A total of 50 kg of dried seaweed was packed in plastic sacks and transported by vehicle to the Research Center for Marine and Fisheries Product Processing and Biotechnology (RCMFPPB), Jakarta—a distance of approximately 200 km. Prior to processing, the seaweed was stored at ambient temperature (approximately 25°C).

To produce ATG chips, the dried seaweed was immersed in NaOH solutions at three different concentrations and subjected to two different heating temperatures. After treatment, the seaweed was sun-dried to obtain ATG chips.

The following parameters were analyzed:

- ATG chip yield and moisture content according to Indonesian National Standards (BSN, 2015a),
- Ash content following BSN (2010),
- Sulfate content according to AOAC (2005),
- Agar yield extracted from the ATG chips, using the AOAC (2005) procedure,
- Gel strength of the extracted agar, determined following the method of Armisen and Galatas (1987), with slight modifications.

Experiment design and data analysis

RSM is a statistical and mathematical technique used to evaluate and optimize processes influenced by multiple variables. In this study, RSM was employed to determine the optimal conditions for the production of ATG chips. The experimental design followed a Central Composite Design (CCD) (Nuryanti and Salimy, 2008) using Design Expert software version 11 (Stat-Ease Inc., USA), with two independent variables: alkali concentration (X_1) and heating temperature (X_2).

The response variables analyzed were yield, moisture content, ash content, and gel strength of the ATG

chips, and also yield and gel strength of the extracted agar.

CCD consists of three main components, as described by Jeff and Hamada (2011):

1. Factorial points (corner points), with coded values of $x_i = \pm 1$, forming a full factorial design,
2. Center points, with $x_i = 0$, replicated five times to estimate experimental error,
3. Axial (star) points, with $x_i = \pm \alpha$, where α is calculated based on the number of factors as $\alpha = (2^k)^{1/4}$. For this study ($k = 2$), $\alpha = 1.414$.

The range for NaOH concentration was set between 2.17% and 7.83%, while the heating temperature ranged from 53.79°C to 96.21°C. These limits were defined based on preliminary trials and previous literature and were refined using Design Expert software. The experimental design generated 13 runs, including five replicates at the center point. The treatment combinations and coded levels are presented in Tables 1 and 2.

Each experimental response was analyzed to fit a suitable polynomial regression model (linear, two-factor interaction [2FI], or quadratic). The model selection was based on statistical criteria, including:

- Model p-value < 0.05 (significant),
- Lack-of-fit p-value > 0.05 (not significant),
- Predicted R^2 and adjusted R^2 difference ≤ 0.2 ,
- Adequate precision > 4.

Analysis of variance (ANOVA) was performed to evaluate the significance and suitability of each model. The relationships between the independent variables and responses were illustrated using three-dimensional (3D) surface plots and contour plots generated by the software.

Optimization was then conducted using desirability functions. Each response was assigned a goal (e.g., maximize, minimize, target, in range, equal to) and an importance ranking from 1 (least important) to 5 (most important). The software generated a list of optimal solutions, each associated with a desirability index (0–1), where values closer to 1 indicate more favorable combinations of factors.

Finally, model validation was performed by comparing the experimental results under the optimized conditions with the predicted values from the software to confirm the model's accuracy and practical applicability.

RESULTS AND DISCUSSION

Chemical composition of *G. verrucosa*

Prior to the alkali treatment, the chemical composition of *G. verrucosa* was analyzed to assess the initial quality of the raw material used in the production of ATG chips. The seaweed samples were cultivated in Randusanga, Brebes, Central Java, under environmental conditions characterized by a water salinity of 20 ppt and harvested at 35 days of age. The parameters assessed included moisture content, ash content, and sulfate content. The corresponding results are summarized in Table 3.

The moisture content of *G. verrucosa* was found to be 20.76%, slightly higher than those reported by Amalia (2013) (19.01%) and Rasyid et al. (2019) (19.4%). This discrepancy may be attributed to differences in post-harvest handling and drying techniques, as supported by Kumesan et al. (2017), who reported that variations in drying conditions significantly influence residual moisture levels. Elevated moisture content in dried seaweed may increase the risk of microbial contamination during storage, thereby potentially compromising the quality of the resulting ATG chips. Nevertheless, the observed moisture content complies with the Indonesian National Standard (SNI) for dried *Gracilaria* spp., which permits values between 12% and 40% (BSN, 2015b), indicating the raw material is suitable for further processing.

The ash content of the dried *G. verrucosa* was 1.63%, which is notably lower than previously reported values of 14.18% by Amalia (2013) and 6.78% by Rasyid et al. (2019). Ash content reflects the mineral composition of the biomass, with higher values suggesting elevated inorganic residue accumulation.

Tapotubun (2018) noted that elevated ash levels in seaweed may result from environmental mineral uptake, while Mwalugha et al. (2015) emphasized the role of cell wall polysaccharides and proteins in facilitating metal ion binding. Variations in ash content are likely attributable to differences in cultivation sites, seasonal fluctuations, and surrounding water quality, all of which influence the mineral profile of the seaweed.

Process optimization results using RSM

The alkali treatment process was optimized using RSM, evaluating six key responses across 13 treatment combinations (Table 4). These responses included the yield of ATG chips, moisture content, ash content, sulfate content, agar yield, and gel strength. The experimental treatments involved varying concentrations of NaOH and heating temperatures.

Table 1. Treatment variable relationships.

Treatment	Treatment codes				
	-2 ^{1/2}	-1	0	1	2 ^{1/2}
NaOH concentration (X1) (%)	2	3	5	7	8
Heat temperature (X2) (°C)	54	65	75	90	96

Table 2. Combination design.

Run	Factor code		Actual factor		Response variables
	X1	X2	NaOH (%)	Heating temperature (°C)	
1	0	0	5	75	Y1
2	0	2 ^{1/2}	5	96	Y2
3	2 ^{1/2}	0	8	75	Y3
4	0	0	5	75	Y4
5	0	2 ^{1/2}	5	54	Y5
6	2 ^{1/2}	0	2	75	Y6
7	0	0	5	75	Y7
8	-1	-1	3	60	Y8
9	0	0	5	75	Y9
10	0	0	5	75	Y10
11	1	-1	7	60	Y11
12	1	1	7	90	Y12
13	-1	1	3	90	Y13

Table 3. The main quality parameters of dry *G. verrucosa* seaweed.

Parameter	Value (%)
Water content	20.76 ± 0.27
Ash content	1.63 ± 0.12

Among the tested conditions, Treatment 5 (5% NaOH, 54°C) produced the highest ATG chip yield, reaching 52%, whereas Treatment 12 (7% NaOH, 90°C) resulted in the lowest yield at 36%. The maximum moisture content was recorded in Treatment 4 (5% NaOH, 75°C) at 18.12%, while the lowest was in Treatment 8 (3% NaOH, 60°C) at 15.89%.

In terms of ash content, Treatment 8 yielded the highest value at 5.11%, whereas Treatment 12 again showed the lowest ash content at 3.72%. The sulfate content was most elevated in Treatment 8 (2.69%) and minimized in Treatment 13 (3% NaOH, 90°C), which exhibited 1.46%.

Regarding agar yield, the best outcome was observed in Treatment 3 (8% NaOH, 75°C), achieving 53.4%, while Treatment 6 (2% NaOH, 75°C) had the lowest yield at 24.41%. The gel strength of the extracted agar ranged widely, with the highest value of 139.53 g/cm² obtained in Treatment 2 (5% NaOH, 96°C) and the lowest value of 14.64 g/cm² again recorded in Treatment 8.

These variations underscore the significant influence of both NaOH concentration and processing temperature on the physicochemical characteristics of ATG chips and their derived agar. Further statistical modeling and interpretation of each response variable are provided in the following sections.

Table 4. The results of the central composite design of ATG chips using response surface methodology.

Run	X1	X2	ATG chips yield (%)	Water content (%)	Ash content (%)	Sulfate content (%)	Agar yield (%)	Gel strength (g/cm ²)
1	5	75	46	17.47	3.91	1.94	36.64	36.91
2	5	96	38.8	17.56	4.07	1.58	39.64	139.53
3	8	75	48	17.31	3.98	1.81	53.4	128.31
4	5	75	45.2	18.12	4.17	1.96	39.93	21.98
5	5	54	52	16.35	4.89	2.06	32.21	37.64
6	2	75	45.2	17.58	4.88	2.24	24.41	16.25
7	5	75	46	17.13	4.50	2.00	41.46	28.54
8	3	60	50.4	15.89	5.11	2.69	31.35	14.64
9	5	75	48.4	17.31	4.24	1.90	46.48	35.88
10	5	75	48.4	17.05	4.27	2.02	46.76	40.65
11	7	60	48.4	15.88	4.41	1.63	35.63	18.50
12	7	90	36	17.23	3.72	2.13	41.76	88.05
13	3	90	41.2	17.35	4.32	1.46	39.52	116.97

X1: NaOH concentration (%), X2: Heating temperature (°C).

Yield of ATG chips

The yield of ATG chips was effectively modeled using a linear regression model, as recommended by the Design Expert version 11 software. The model demonstrated strong statistical validity, with a sequential p-value of 0.0002, a lack of fit p-value of 0.1632, an adjusted R² of 0.7731, and a predicted R² of 0.6446, indicating that the linear model was appropriate for describing the relationship between the independent variables and the yield response (StatEase, 2020).

ANOVA further confirmed the model's significance, with a model F-value of 21.44 ($p = 0.0002$), suggesting that the probability of obtaining such a high F-value due to random variation (noise) is only 0.02%. As per Anderson and Whitcomb (2016), a p-value < 0.05 signifies a statistically significant model. Additionally, the lack of fit test produced an F-value of 2.87 and a p-value of 0.1632, which was not significant, confirming that the model adequately fits the data without unexplained variability.

The derived regression equation [1] was as follows:

$$Y = 45.69 - 0.4050A - 5.03B \quad [1]$$

Where Y: ATG chips yield response value (%); A: Linear effect of alkali concentration (%); and B: Linear influence of heating temperature (°C).

The negative coefficients of both variables indicated that increasing either the NaOH concentration or

the heating temperature led to a linear decrease in yield. This trend is consistent with previous reports by Zatnika and Istini (2008), who noted that excessive alkali concentrations and higher processing temperatures reduce agar yields due to degradation of extractable components. Similarly, Chapman and Chapman (1980) and Armisen and Galatas (1987) emphasized that extraction conditions play a critical role in optimizing agar recovery from seaweed.

This relationship is illustrated in Fig. 1, which presents a 3D surface response plot. In the figure, red regions correspond to the highest yield values (up to 52%), while blue regions indicate the lowest yields (as low as 36%). The flat surface of the plot reflects the linear nature of the model.

Moisture content of ATG chips

The moisture content response of ATG chips was also best described by a linear regression model, as selected by the Design Expert version 11 software. The model was statistically significant with a sequential p-value of 0.0359, a lack of fit p-value of 0.2919, and acceptable goodness-of-fit statistics (adjusted R² = 0.3832, predicted R² = 0.1088). Although the explanatory power is moderate, the model was deemed sufficient for predicting moisture content based on the processing variables.

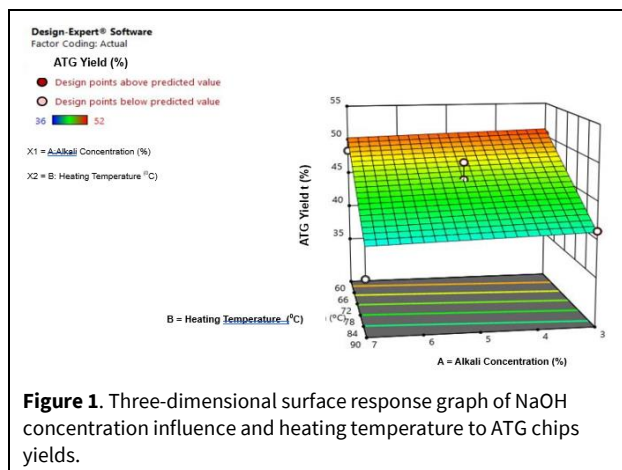


Figure 1. Three-dimensional surface response graph of NaOH concentration influence and heating temperature to ATG chips yields.

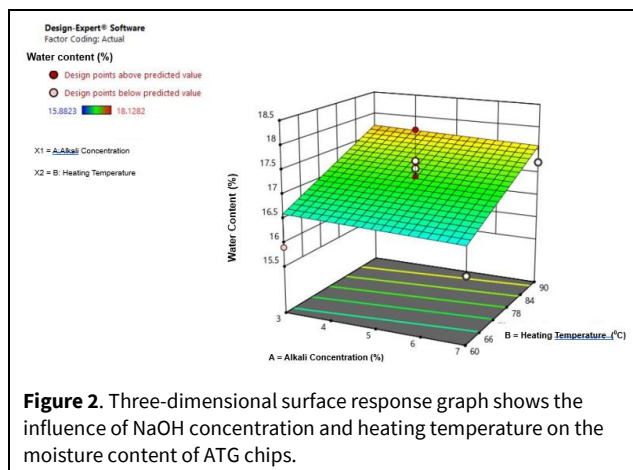


Figure 2. Three-dimensional surface response graph shows the influence of NaOH concentration and heating temperature on the moisture content of ATG chips.

ANOVA revealed a model F-value of 4.73 and a corresponding p-value < 0.0359, indicating that the linear model adequately captures the effect of the independent variables. The lack of fit p-value (0.2919) was not significant, supporting the model's validity in explaining moisture variations.

The fitted linear regression equation [2] for moisture content was as follows:

$$Y = 17.10 - 0.0644A + 0.5649B \quad [2]$$

Where Y: ATG chips moisture content value (%); A: Linear effect of alkali concentration (%); B: Linear influence of heating temperature (°C).

This equation suggests that increasing the alkali concentration slightly decreases the moisture content, while increasing the heating temperature results in higher moisture retention. These outcomes are consistent with expectations, as higher temperatures can cause faster surface drying and reabsorption of moisture from ambient humidity during the sun-drying process, particularly under non-controlled drying environments.

Moisture content values observed in this study ranged from 15.89% to 18.12%, which fall within the acceptable limits specified by the Indonesian National Standard (SNI), which allows up to 22% for seaweed-based products (BSN, 2015a). However, the values are higher than those reported in previous studies. For instance, Manurung et al. (2024) reported moisture content of *Gracilaria* sp. was $6.01 \pm 0.07\%$ (treated with 5% NaOH at 30°C). However, for *G. verrucosa*, Sinurat et al. (2024) reported $9.88 \pm 0.20\%$ (4% NaOH, 70-80°C for 2 hours), and Santika et al. (2014) values between 12.32% and 15.7% (5-7% NaOH, 85-90°C for 1.5 hours). These discrepancies can be attributed to differences in drying methods and environmental conditions, as the present study relied on manual sun-drying, which is sensitive to atmospheric humidity and exposure time.

The three-dimensional surface response plot (Fig. 2) illustrates the relationship between NaOH concentration and heating temperature. Moisture content response was visually represented, where red indicated the highest values (18.13%) and blue the lowest (15.88%). The planar surface contour confirmed the appropriateness of the linear model in representing this response.

Ash content of ATG chips

The ash content response of ATG chips was best modeled using a quadratic regression model, as determined by the Design Expert version 11 software. This model selection was supported by statistical indicators, including a sequential p-value of 0.0020, a lack of fit p-value of 0.9233, an adjusted R^2 of 0.8324, and a predicted R^2 of 0.7918, all of which confirm the model's robustness and predictive validity.

ANOVA analysis yielded a model F-value of 12.92 ($p = 0.0020$), indicating that the regression model was statistically significant. The lack of fit test produced a non-significant result ($F = 0.15$; $p = 0.9233$), confirming the model's suitability for predicting ash content based on the experimental variables.

The regression equation [3] derived from the model was as follows:

$$Y = 4.22 - 0.3203A - 0.3321B + 0.0229AB + 0.0869A^2 - 0.1168B^2 \quad [3]$$

Where Y: Response of ATG chips ash content value (%); A: Linear effect of alkali concentration (%); B: Linear effect of heating temperature (°C); AB: Effect of interaction of alkali concentration with heating temperature; A^2 : Quadratic effect of alkali concentration; and B^2 : Quadratic effect of heating temperature.

The model suggests that increasing both NaOH concentration and temperature generally leads to a reduction in ash content, although quadratic and interaction effects may slightly counterbalance this trend at higher treatment levels. These findings highlight the complexity of the ash removal process,

which is influenced by the interplay of thermal and chemical conditions (Tomar and Sikarwar, 2023).

In this study, the ash content ranged from 3.72% to 5.11%, values that are within acceptable limits for agar raw materials as defined by international standards such as those set by the Food and Agriculture Organization, Food Chemical Codex, United States Pharmacopeia, and European Economic Community, which permit up to 6.5% ash in commercial seaweed-based products (Wongwai, 1996). A three-dimensional surface plot of the ash content response (Fig. 3) illustrates the variation across treatment combinations, with the lowest values (blue) observed at moderate temperatures and alkali concentrations and highest values (red) occurring under more extreme conditions.

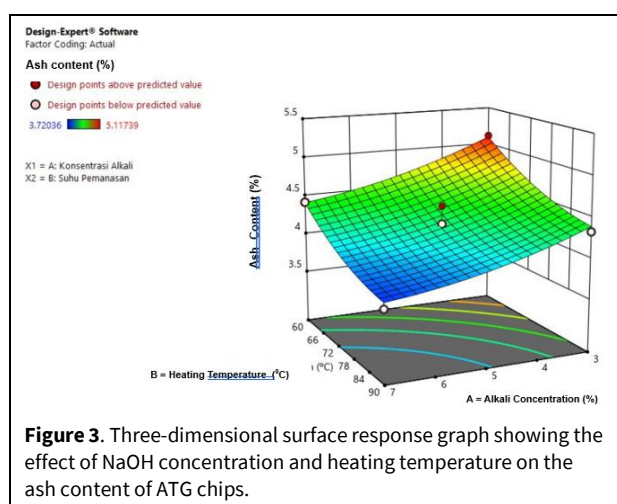


Figure 3. Three-dimensional surface response graph showing the effect of NaOH concentration and heating temperature on the ash content of ATG chips.

Comparative data from other studies show some variability. Sinurat et al. (2024) reported a lower ash content of $3.57 \pm 0.04\%$, while Santika et al. (2014) documented a range of 1.48–3.35%. Freile-Pelegri n and Robledo (1997) observed even lower values (2.39–3.06%) in *G. cornea*. On the contrary, Manurung et al. (2024) reported a significantly higher ash content of $8.92 \pm 0.24\%$, exceeding that observed in the present study. These discrepancies may be attributed to differences in species, environmental conditions, or post-harvest handling, particularly in the efficiency of washing and filtration processes, which play a critical role in minimizing inorganic residues.

In summary, the quadratic model accurately predicts the ash content of ATG chips and provides insights into the optimal processing conditions that minimize mineral content, thereby enhancing the purity and quality of the final agar product.

Sulfate levels of ATG chips

The sulfate content response of ATG chips was optimally described by a quadratic model, as deter-

mined by Design Expert version 11. This model demonstrated statistical robustness, with a sequential p-value of 0.0001, lack of fit p-value of 0.2411, adjusted R^2 of 0.9679, and predicted R^2 of 0.9068. These indicators suggest that the model has both high predictive accuracy and minimal error (Anderson and Whitcomb, 2016).

ANOVA confirmed the significance of the model, with a model F-value of 73.27 and a p-value < 0.0001 , indicating that the probability of the observed results arising from random variation was less than 0.01%. Furthermore, the lack of fit F-value was 2.11 with a p-value of 0.2411, demonstrating that the model's residual variance was not significantly different from that of pure error, and thus, the model is deemed statistically adequate.

The fitted regression model was expressed by the following equation [4]:

$$Y = 1.97 - 0.1243A - 0.1243B + 0.4299AB + 0.0441A^2 - 0.0563B^2 \quad [4]$$

Where Y: Response of ATG chips sulfate content value (%); A: Linear effect of alkali concentration (%); B: Linear effect of heating temperature ( C); AB: Effect of interaction of alkali concentration with heating temperature; A^2 : Quadratic effect of alkali concentration; and B^2 : Quadratic effect of heating temperature.

According to this model, the sulfate content of ATG chips generally decreases with increasing NaOH concentration and temperature (linear terms), as well as with the quadratic effect of temperature. However, the interaction between alkali concentration and temperature and the quadratic effect of alkali concentration can increase sulfate retention under certain conditions.

In this study, the sulfate content ranged from 1.46% to 2.69%, a notable reduction compared to that of commercial dried *Gracilaria* seaweed, which typically ranges from 12.94% to 27.27%. These findings align with those of Xiao et al. (2020), who reported that alkaline pre-treatment facilitates desulfation by converting galactose-6-sulfate to 3,6-anhydro-L-galactose, thereby improving the gelling properties of agar. However, the sulfate levels in the ATG chips remain higher than those found in refined commercial agar, which typically ranges between 0.79% and 0.87% (Yuliani et al., 2017), reflecting the intermediate processing stage of the chips.

The results are visually represented in Fig. 4, which illustrates the sulfate response surface in a three-dimensional contour plot. The highest sulfate content (2.69%), depicted in red, was observed at lower alkali concentrations and moderate temperatures, whereas the lowest sulfate content (1.46%), shown in blue, occurred under conditions of elevated alkali concentration and temperature. These observa-

tions confirm that effective desulfation is facilitated by more intensive alkaline-thermal conditions, within limits that avoid excessive degradation of the biomass.

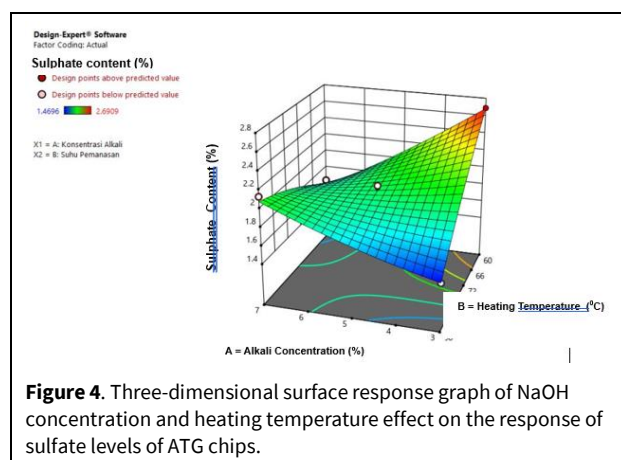


Figure 4. Three-dimensional surface response graph of NaOH concentration and heating temperature effect on the response of sulfate levels of ATG chips.

In summary, the quadratic model accurately predicts sulfate content based on alkali concentration and heating temperature, providing a reliable framework for optimizing ATG chip processing conditions to reduce sulfate levels and improve the suitability of the raw material for high-quality agar production.

Agar yield

The response for agar yield in ATG chips was best described by a linear model, as recommended by Design Expert version 11. This model was selected based on its sequential p-value of 0.0220, lack of fit p-value of 0.2465, adjusted R^2 of 0.4406, and predicted R^2 of 0.1601. Although the predictive power is moderate, the statistical indicators suggest the model is acceptable for use in yield prediction.

Analysis of variance (ANOVA) revealed a model F-value of 5.73 and a p-value of 0.0220, indicating that the probability of observing such results due to random error is only 2.2%. The lack of fit test yielded a F-value of 2.10 with a p-value of 0.2465, confirming that the model adequately fits the data without significant deviation.

The regression equation [5] generated by the model is as follows:

$$Y = 39.18 + 5.95A + 3.1B \quad [5]$$

Where Y: Agar yield response value (%); A: Linear effect of alkali concentration (%); and B: Linear effect of heating temperature (°C).

According to equation [5], agar yield increases linearly with both alkali concentration and heating temperature. This outcome aligns with prior reports that highlight the role of thermal and alkaline conditions in enhancing the solubilization of agarose from sea-

weed cell walls (Chapman and Chapman, 1980; Zatinika and Istini, 2008). The alkaline pre-treatment facilitates partial hydrolysis and desulfation, enabling improved release of polysaccharides.

In this study, the agar yield ranged from 24.41% to 53.45%, depending on the treatment conditions. These values are consistent with literature reports where extraction temperature and NaOH concentration were key factors influencing yield. Seaweed extraction is a temperature-sensitive process; extraction at insufficient temperatures results in incomplete polysaccharide release, while excessively high temperatures may degrade the agar structure, both negatively affecting yield.

The results are visualized in Fig. 5, which presents a three-dimensional surface plot showing the effect of NaOH concentration and heating temperature on agar yield. The highest yield (53.45%), shown in red, occurred under conditions of high NaOH concentration and elevated temperature, while the lowest yield (24.41%), shown in blue, was observed at lower treatment intensities. The flat contour observed in the figure reflects the linear nature of the fitted model.

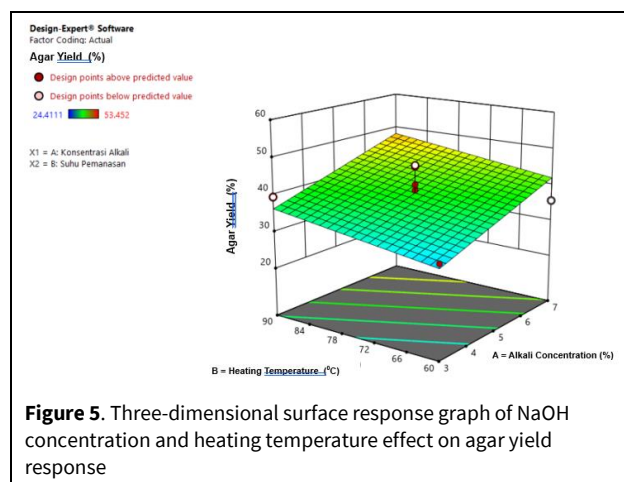


Figure 5. Three-dimensional surface response graph of NaOH concentration and heating temperature effect on agar yield response

These findings confirm that careful modulation of both alkaline concentration and heating temperature during ATG preparation can significantly improve agar recovery, providing a practical framework for industrial-scale optimization.

The agar gel strength

The response of agar gel strength in this study was best fitted using a linear model, as recommended by Design Expert version 11. This model selection was based on statistical metrics, including a sequential p-value of 0.0115, a lack of fit p-value of 0.0028, an adjusted R^2 of 0.5087, and a predicted R^2 of 0.2141. The ANOVA results demonstrated a model F-value of 7.21 and a p-value of 0.0115, suggesting that the observed

variance in gel strength could be significantly explained by the model at the 95% confidence level.

Despite its statistical significance, the lack of fit was found to be significant (F-value = 29.78, $p = 0.0028$), indicating that some variability in the response is not captured by the model. This may be attributed to experimental noise or other uncontrolled variables influencing gel strength.

The regression equation derived from the model is as follows:

$$Y = 55.68 + 16.68A + 39.50B \quad [6]$$

Where Y: Agar gel strength response value (%); A: Linear effect of alkali concentration (%); and B: Linear effect of heating temperature (°C).

According to equation [6], the gel strength of agar increases linearly with both alkali concentration and heating temperature. This trend aligns with the theoretical understanding of agarose gel formation: elevated temperatures and stronger alkaline environments promote the conversion of galactose-6-sulfate to 3,6-anhydro-L-galactose, a structural motif that facilitates gel network formation via intermolecular hydrogen bonding (Glicksman, 1983).

The measured gel strength of agar in this study ranged from 14.64 to 139.53 g/cm². While this upper range falls slightly below the Japanese export quality standard (100–150 g/cm² for Grade III agar) (Armisen and Galatas, 1987), it is still within acceptable limits for many industrial applications. Suboptimal gel strength may result from several factors, including extended storage of the dried seaweed, which can degrade polysaccharide integrity, and incomplete filtration, allowing seaweed residue to remain in the extract and disrupt proper gel formation.

Fig. 6 illustrates the 3D response surface showing the combined effect of NaOH concentration and heating temperature on agar gel strength. As depicted, the highest strength (139.53 g/cm²) was achieved at high concentrations and temperatures (red region), while the lowest (14.64 g/cm²) occurred under milder conditions (blue region). The flat contour of the surface is consistent with a linear model.

Compared to previous studies, the gel strength observed here was lower than the 479.50 ± 16.84 g/cm² reported by Sinurat et al. (2024), and 355.35 g/cm² reported by Santika et al. (2014), despite achieving higher agar yields. These discrepancies may stem from variations in filtration technique, seaweed freshness, and extraction protocols. For instance, Wang et al. (2017) reported an exceptional gel strength of 1068.15 g/cm² from *G. tenuistipitata*, highlighting the substantial effect of species selection.

Indeed, *G. tenuistipitata* is recognized for producing agar with superior gel strength compared to other *Gracilaria* species.

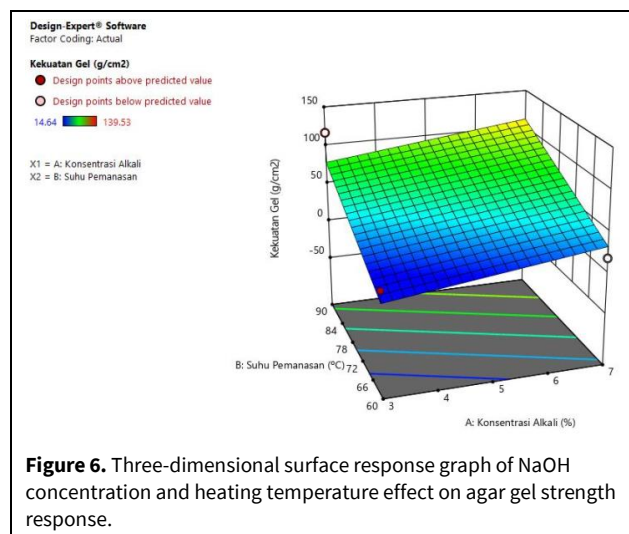


Figure 6. Three-dimensional surface response graph of NaOH concentration and heating temperature effect on agar gel strength response.

Optimization results of all responses simultaneously using RSM

A comprehensive multi-response optimization was conducted using RSM to determine the most favorable processing conditions for producing high-quality ATG chips. The response variables included ATG chip yield, moisture content, ash content, and sulfate content, as well as agar yield and agar gel strength—all considered essential quality parameters in agar production.

Optimization was performed using Design Expert version 11, which enabled simultaneous evaluation of the combined effects of NaOH concentration and heating temperature. The software generated multiple solutions, each with an associated desirability score, a metric ranging from 0 (least desirable) to 1 (most desirable), used to evaluate how well a combination of input variables meets the desired response criteria.

The analysis produced five optimal formulations (Table 5), each with varying degrees of desirability. Among these, the best-performing formulation was identified at a NaOH concentration of 10.303% and a heating temperature of 67.762°C, resulting in a high desirability value of 0.87. This indicates a robust and balanced combination of input parameters capable of achieving favorable outcomes across all measured response variables.

A detailed breakdown of the optimization criteria and corresponding response values for each proposed solution is provided in Table 6, facilitating reproducibility and further validation in industrial-scale applications.

Table 5. Simultaneous optimization of all responses.

Parameter	Achievement	Range	
		Lowest	Highest
Alkali concentration (%)	In range	0	11
Extraction temperature (°C)	In range	60	90
ATG chips yield (%)	Maximum	36	52
ATG chips water content (%)	In range	15.8823	18.1282
ATG chips ash content (%)	In range	3.72036	5.11739
ATG chips sulphate content (%)	In range	1.46962	2.6909
Agar yield (%)	Maximum	24.4111	53.452
Agar gel strength (g/cm ²)	In range	14.64	139.53

Table 6. Optimization solution of Design Expert 11 analysis.

Alkali concentration (%)	Extraction temperature (°C)	ATG chips yield (%)	Water content (%)	Ash content (%)	Sulfate content (%)	Agar yield (%)	Gel strength (g/cm ²)	Desirability
10.30	67.76	47.04	16.65	4.14	1.47	53.45	80.84	0.870
10.29	67.85	47.01	16.66	4.13	1.47	53.45	81.03	0.869
10.57	67.93	46.93	16.65	4.15	1.47	54.28	83.54	0.867
10.76	68.03	46.86	16.65	4.17	1.47	54.88	85.41	0.865
10.79	68.04	46.85	16.65	4.17	1.47	54.96	85.66	0.864

Table 7. Comparison of actual response values, prediction, and CI values.

Parameter	Actual	Prediction	95% CI	
			Low	High
ATG chips yield (%)	45.60	47.0474	42.2246	51.8702
ATG chips moisture content (%)	21.18	16.6577	15.5033	17.8122
ATG chips ash content (%)	4.42	4.14195	3.07	5.21391
ATG chips sulphate content (%)	1.42	1.4696	1.11281	1.82638
Agar yield (%)	54.74	53.452	41.0547	65.8494
Agar gel strength (g/cm ²)	97.37	80.842	10.243	151.441

Validation of optimal conditions

Validation is a critical step in confirming that the optimal conditions predicted by the RSM model are applicable under actual experimental conditions. This step assesses the reliability of the model and its predictive accuracy by comparing the experimental results to the expected values within a defined confidence interval (CI). In this study, a 95% CI was employed, as recommended by Anderson and Whitcomb (2016).

The ATG chips produced under the optimized conditions—10.303% NaOH concentration and 67.762°C extraction temperature—exhibited physical characteristics consistent with those predicted by the model, as visually confirmed in Fig. 7. The experi-

mental results demonstrated a chip yield of 45.60%, moisture content of 21.18%, ash content of 4.42%, and sulfate content of 1.42%. When these chips were further processed into agar, the resulting agar yield was 54.74%, and the gel strength reached 100.91 g/cm².

A comparison of these experimental outcomes with the predicted values—alongside their respective confidence intervals—is presented in Table 7. The yield and sulfate content of the ATG chips were slightly lower than the predicted values; however, they remained within the 95% CI, confirming the model's validity. In contrast, ash content, agar yield, and gel strength slightly exceeded the predicted values but also fell within the acceptable range. The only response that deviated beyond the prediction was moisture content, which was marginally higher. This

deviation is likely attributable to environmental variability affecting the drying process, such as ambient humidity and sunlight intensity during sun-drying.



Figure 7. Appearance of ATG chips.

Overall, the validation results support the robustness of the RSM optimization model, affirming its suitability for guiding industrial-scale production of ATG chips from *Gracilaria verrucosa*. Future studies are warranted to investigate additional independent variables—such as extraction time or pH—to further refine the production process and enhance product consistency and quality.

CONCLUSION

This study successfully optimized the production of alkali-treated *Gracilaria verrucosa* (ATG) chips using Response Surface Methodology (RSM), considering two critical process variables: NaOH concentration and extraction temperature. The optimal conditions—10.303% NaOH and 67.762°C—resulted in a high desirability score of 0.87, indicating strong model adequacy for multi-response optimization.

Validation under these optimal conditions produced ATG chips with a yield of 45.60%, moisture content of 21.18%, ash content of 4.42%, and sulfate content of 1.42%, all within acceptable ranges. When processed into agar, the product demonstrated a yield of 54.74% and a gel strength of 100.91 g/cm², approaching the minimum Japanese quality standards.

The results confirmed the effectiveness of RSM as a predictive and optimization tool in improving ATG chip production and agar quality. Moreover, the model's predictive capability was verified through experimental validation within a 95% confidence interval, demonstrating its practical applicability in industrial settings.

Future work should explore additional process variables such as extraction duration, solid-liquid ratio, and drying methods, which may further im-

prove yield and gel strength. Additionally, evaluating the cost-efficiency and environmental impact of the optimized process would provide valuable insights for large-scale applications in the agar industry.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Farand SFD	Sumaryanto H	Uju U	Subaryono S	Sinurat E	Mulyasari M	Purnomo AH	Noviendri D
Concepts or ideas	x	x	x	x	x	x	x	x
Design	x	x	x	x				
Definition of intellectual content	x	x	x	x				
Literature search	x				x	x		x
Experimental studies	x			x			x	
Data acquisition	x			x				
Data analysis	x			x				
Statistical analysis	x			x				
Manuscript preparation	x			x		x		
Manuscript editing	x	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x

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