

Modeling of Dynamic Material Behavior-Based Optimization of *Gracilaria* Powder, Kappa-Carrageenan, and Tricalcium Phosphate and Sequential Acidulant Adjustment for Instant Pre-Mix Seaweed Beverage

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Abstract

The growing demand for functional beverages has led to increased use of fiber-rich ingredients such as grains and nuts. However, the use of native seaweed as a primary ingredient in beverage formulations remains underreported. Its limited application is mainly due to processing challenges and poor compatibility with industrial requirements. Therefore, developing seaweed-based premixes offers a potential solution to enhance its usability in functional beverage products. This research aimed to optimize the formulation of an instant pre-mix powdered beverage based on *Gracilaria* using the I-Optimal Mixture Design, incorporating kappa-carrageenan as a stabilizer and tricalcium phosphate as an anti-caking agent and evaluated the effects of citric acid on the physical and functional characteristics of pre-mix powder. The independent variables included *Gracilaria* powder 36% - 45%, kappa-carrageenan 0% - 1%, and tricalcium phosphate 5% - 13%, while the measured responses encompassed moisture content, flowability, cohesiveness, flow time, angle of repose, and peak viscosity. The optimization results identified the optimal formulation as *Gracilaria* powder 36.52%, kappa-carrageenan 0.48%, and tricalcium phosphate 13%, yielding the highest desirability score of 0.973 with significant prediction confidence interval. To enhance solubility during the post-brewing process, citric acid was introduced as a chelating agent to improve solubility. Citric acid significantly improved the solubility of the post-brew powder, with a concentration of 4.5% producing the best CIE- $L^*a^*b^*$ ΔE value and solubility profile yielding a sedimentation index of 32.60% (close to the ideal < 30%), water absorption index of 2.63 g/g (> 2.2 g/g), water solubility index of 33.17%, and a ΔE value of 43.65. These findings emphasize the important role of citric acid in enhancing solubility and physical properties, thereby significantly improving the stability and overall quality of the product. This preliminary modeling of dynamic material behavior in instant powdered beverages offers a promising strategy for the food industry, supporting the demand for convenient and health-oriented products.

Keywords: Citric acid, Fiber rich, Mixture design, Pre-mix, Red seaweed

Introduction

The functional beverage market is projected to continue growing. This is evidenced by the global market value reaching USD 204.8 billion in 2022 and a Compound Annual Growth Rate (CAGR) of 7.1% from 2023 to 2030 [1]. Currently, global products primarily focus on energy and sports beverages that are high in

calories, sugar, and low in fiber. This trend presents opportunities for innovation in nutraceutical beverages, driven by an increasing CAGR of 7.0% (2020 - 2028) due to the rising prevalence of chronic diseases [2]. Among degenerative diseases, diabetes mellitus remains one of the most rapidly increasing worldwide.

Increasing the intake of natural dietary fiber presents a promising strategy for diabetes prevention. In addition to reducing glucose absorption, dietary fiber contributes to lowering the risk of cardiovascular disease, obesity, and digestive disorders by modulating lipid metabolism and promoting gut health [3]. While dietary fiber is mostly sourced from terrestrial plants such as grains and legumes. Overexploitation of terrestrial plants poses a threat to sustainability and land availability [4,5]. As an alternative, seaweed provides a sustainable source of natural fiber, particularly rich in soluble fiber with health benefits [6-12]. Despite its potential, the incorporation of native seaweed into beverage formulations has received limited attention. This underutilization is largely attributed to physical challenges in processing and its inadequate alignment with standard industrial specifications [13]. Innovation is required to optimize the utilization of seaweed-derived fiber in food applications. Instant powdered beverages present a promising option, aligning with the growing consumer trend toward convenient and health-oriented products [14].

The development of seaweed-based instant powdered beverages remains unexplored, as the industry primarily focuses on producing intermediate products such as agar strips [15] and agar paper [16]. Seaweeds such as *Gelidium* sp. and *Ulva lactuca* have been widely utilized in food development. *Gelidium* sp., while capable of producing strong gels, has low solubility and limited availability due to its reliance on wild harvesting [17]. *Ulva lactuca*, despite exhibiting moderate gelling properties, remains inferior to *Gracilaria* sp. in terms of solubility and availability [18]. *Gracilaria* sp., on the other hand, is characterized by wide availability, ease of cultivation, rapid gel solubility, and a light texture [18], making it highly suitable for instant products that require high solubility and a non-viscous mouthfeel. Beyond its physical attributes, *Gracilaria* sp. is also rich in bioactive compounds such as flavonoids, soluble fiber, and phenolics, which offer additional health benefits, including antioxidant and antidiabetic effects [6,7,9]. The combination of these attributes and functional characteristics positions *Gracilaria* sp. as a primary ingredient for the development of seaweed-based premix beverage. In powdered beverage products, rehydration is a major challenge affecting the final

product's sensory quality and stability. Extensive research has been conducted on rehydration in fat-based powdered beverages, including lecithin addition to milk powder [19] and sugar incorporation into cocoa powder [20]. However, studies specifically addressing seaweed fiber-based beverages, particularly in pre-mix powdered form, remain highly limited. Fiber-based powdered beverages require stabilizers to maintain emulsion stability and ensure optimal compatibility under various conditions. Xanthan gum, while effective as a stabilizer, results in a thicker texture, making it less suitable for beverage products that prioritize rapid solubility and a light mouthfeel [21]. In contrast, alginate forms a softer gel but is highly sensitive to pH variations, limiting its applicability in certain formulations [22]. Kappa-carrageenan, however, forms a strong yet elastic gel, offering high stability in solution and better control over texture [23]. Furthermore, its ability to improve fiber suspension, along with its compatibility with calcium ions that strengthen the gel network [24], positions kappa-carrageenan as superior in maintaining both stability and textural quality in seaweed-based premix beverage. Additionally, powdered products are inherently hygroscopic, necessitating anticaking to minimize clumping and reduce moisture absorption.

The incorporation of anti-caking agents, such as silicon dioxide, has been shown to be effective in minimizing moisture absorption due to its hygroscopic properties, but provides no additional nutritional benefits and does not contribute to gel structure or system stability [25]. Magnesium stearate, while commonly used in powdered formulations, is highly hydrophobic, which can inhibit powder rehydration and lacks synergistic interaction with hydrocolloids. In contrast, tricalcium phosphate not only prevents caking but also serves as a bioavailable calcium source, Generally Recognized as Safe (GRAS) status, and actively contributes to the reinforcement of gel networks through ionic interactions with kappa-carrageenan [24,26]. These multifunctional properties make tricalcium phosphate a superior anti-caking agent for use in seaweed-based premix beverage. Optimization of formulation and analysis of material behavior are key to ensuring product performance. To the best of the author's knowledge, no prior study has optimized the combination of kappa-carrageenan and tricalcium phosphate in fiber-based seaweed powdered beverages.

Statistical modeling of material behavior is essential to determine optimal ingredient proportions for improved functionality in industrial applications. In the formulation of instant pre-mix seaweed beverage, the selection of an appropriate optimization method directly influences product development outcomes. The I-Optimal offers accurate model predictions while minimizing the number of experimental runs required. Unlike factorial design and Central Composite Design, which are more appropriate for independent variables, mixture designs are specifically intended for systems with a fixed total component proportion [27]. Compared to the D-Optimal, which emphasizes parameter estimation efficiency, the I-Optimal provides superior predictive accuracy [28]. This makes it particularly suitable for complex formulations involving ingredients such as *Gracilaria* powder, κ -carrageenan, and tricalcium phosphate. Its prior success in functional beverage development, such as turmeric powder [29] and tea-based formulations [30]. Nevertheless, for advanced post-brewing processing stages in industrial applications, the initial formulation step may be insufficient to ensure optimal performance. Therefore, targeted incorporation of acidulants is essential to improve solubility and enhance the functional properties of the final premix product.

In formulations, acidulants are widely incorporated into various products to meet specific sensory and functional requirements of the final product. Malic acid imparts a milder and more natural sourness, but tends to be more expensive and less readily available in large quantities [31]. Ascorbic acid, while beneficial for its preservative properties and as a source of vitamin C, is prone to oxidation, which can reduce its long-term effectiveness and negatively impact product stability [32]. In contrast, citric acid is advantageous due to its widespread availability, cost-effectiveness, and stability across a broad range of pH and temperature conditions [33]. Furthermore, it enhances pigment stability and limits non-enzymatic browning reactions, thereby improving the visual quality of the final product [34]. These attributes make citric acid a preferred acidulant in the food industry, particularly for products requiring rapid solubility and high stability. Hence, on the final step, we propose the incorporation of citric acid in the formulation of a seaweed-based premix beverage to

enhance its solubility, stability, and color acceptability during processing.

Previous studies have investigated instant beverage formulations using seaweed-derived ingredients [35,36], and have examined the individual roles of citric acid [33], kappa-carrageenan [37], and tricalcium phosphate [38]. However, to date, no study has explored the combined effects of these components in a *Gracilaria*-based premix formulation, thereby underscoring the novelty and significance of the present study in the development of functional beverages. Therefore, this study aims to evaluate and optimize the formulation of a *Gracilaria*-based instant beverage premix through a combination approach of kappa-carrageenan, and tricalcium phosphate. Evaluation of the effect of citric acid addition on the physical and functional characteristics of the product, particularly in terms of solubility, stability, and system compatibility under various processing conditions was also carried out. Ultimately, a deeper understanding of material behavior is crucial for enhancing processing efficiency and maximizing product stability and performance, particularly in the preparation of instant beverage premix formulations for the functional food industry.

Materials and methods

Materials

The materials used in this study were *Gracilaria* sp. seaweed powder and kappa-carrageenan, obtained from PT. Kappa Carrageenan Nusantara, located in Pasuruan, Indonesia. Other materials included food-grade tricalcium phosphate and citric acid, purchased from Asia Food Chemicals (Bangkok, Thailand). The equipment used in this study included a rapid visco analyzer (Perten RVA 4800, Perten Instruments, Stockholm, Sweden), tapped density tester (Erweka GmbH, Langen, Germany), centrifuge (BECKMAN J2-MC, Minnesota, USA), chromameter (Konica Minolta CR-310, Tokyo, Japan), moisture analyzer (Ohaus, Parsippany, NJ, USA), homogenizer (Armfield Ltd, United Kingdom, UK), vortex powder mixer (VELP Scientifica Srl, Usmate Velate, Italy), analytical balance (Ohaus, Parsippany, NJ, USA), Beaker (Pyrex, Iwaki Glass Co., Ltd., Tokyo, Japan), magnetic stirrer (DLAB Scientific Co., Ltd., Beijing, China), and other glassware for analytical purposes.

Determination of proximate composition

The proximate composition was analyzed based on standardized AOAC protocols [39]. Moisture, ash, and protein contents were determined following AOAC methods 930.04, 930.05, and 2001.11.2005, respectively, with protein quantified using the Kjeldahl method (conversion factor: Nitrogen \times 6.25) and a Buchi K-355 distillation unit. Lipid content was assessed using the gravimetric method via Soxhlet extraction, in accordance with AOAC method 923.05. Carbohydrate content was determined with a by-difference method.

Total dietary fiber

Total dietary fiber was determined according to the AOAC 985.29 - 1986 (2003) [40]. Dietary fiber content was determined by enzymatic-gravimetric method, using α -amylase and amyloglu- cosidase enzyme.

Experimental methods

This study used the I-Optimal Mixture Design method to determine the optimum instant pre-mix seaweed beverage formula. The mixture design method is used to find the optimum formulation consisting of 2 - 24 components with different ranges [29]. Optimization of the instant pre-mix seaweed beverage formula is carried out by optimizing the composition of

the main ingredients to create the optimum physics characteristics of the powder. The mixture components used as process variables are *Gracilaria* powder (36% - 45%), kappa-carrageenan (0% - 1%), and tricalcium phosphate (5% - 13%), which were determined based on literature review and preliminary formulation trials in the Laboratory to ensure material compatibility and the physical and functional stability of instant pre-mix seaweed beverage. The mixture design used is the I-Optimal Mixture Design based on the exchange algorithm (lack-of-fit points = 5, replicate points = 3, additional center points = 2) with a quadratic model.

The maximum and minimum range limits were then used as input, followed by randomization, resulting in 16 treatment combinations in the formula design stage of the Design Expert 13[®] (Stat-Ease Inc., Minneapolis, USA) program to determine the formula design of the mixture components used as process variables as shown in **Table 1**. The responses (process variables) observed were moisture content, flowability, cohesiveness, flow time, angle of repose, peak viscosity in the powder formula, which were subsequently used in the instant powder beverage formulation. These 6 parameters are interrelated and need to be properly controlled to ensure that the resulting product is of high quality, physically stable, easily dissolvable, and durable.

Table 1 Design of the formula for *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate.

Formula	Material		
	<i>Gracilaria</i> powder	K-carrageenan	Tricalcium phosphate
F1	38.21	0	11.79
F2	38.44	1	10.56
F3	41.41	1	7.59
F4	40.5	0.5	9
F5	44	1	5
F6	37	0	13
F7	39.66	0	10.34
F8	44.23	0	5.77
F9	39.99	1	9
F10	40.5	0.5	9
F11	36.1	0.9	13
F12	40.5	0.5	9

Formula	Material		
	<i>Gracilaria</i> powder	K-carrageenan	Tricalcium phosphate
F13	36.1	0.9	13
F14	42.96	0	7.04
F15	44.43	0.6	5
F16	40.5	0.5	9

Response analysis to variables

ANOVA analysis was conducted on the variables and responses. The selected model was the one that produced significant ANOVA values and demonstrated the highest level of accuracy. Two ANOVA models were used: Linear and quadratic. Responses were analyzed using the model that produced significant ANOVA values and non-significant values for the lack-of-fit parameter.

Moisture content

The moisture content (%) of the samples was measured using the method described by [41]. A small amount of sample (1 - 2 g) was weighed and placed in an aluminum cup of the moisture analyzer, ensuring it was evenly spread across all sides of the cup. The aluminum cup was then positioned in the sensing groove of the water activity meter and analyzed using the biosensor located on the lid of the moisture analyzer.

Flowability and cohesiveness

The flowability and cohesiveness (%) of the product are expressed in terms of the Carr Index (CI) and Hausner Ratio (HR), respectively [42]. CI and HR based on the tapped density (ρ_T) and bulk density (ρ_B) of the powder was calculated using Eqs. (1) and (2);

$$\text{Carr index (CI) (\%)} = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \quad (1)$$

$$\text{Hausner ratio (\%)} = \frac{\rho_T}{\rho_B} \quad (2)$$

Flow time

Flow time (g/s) testing was conducted by placing 100 g of *Gracilaria* powder, combined with kappa-carrageenan and TCP, into a funnel with a closed bottom. The bottom of the funnel was then opened while simultaneously starting a stopwatch to measure the flow

time (t). The powder is considered to have good flow properties if 100 g flows in ≤ 10 s or if the flow rate is ≥ 10 g/s [43]. The flow time formula was calculated using Eq. (3);

$$\text{Flow time (s)} = \frac{\text{Weight}}{\text{Time}} \quad (3)$$

Angle of repose

Gracilaria powder with a combination of kappa-carrageenan and tricalcium phosphate samples were poured through the walls of a funnel, which was positioned exactly 2.0 cm above a hard surface to measure the angle of repose ($^\circ$) [44]. The samples were poured until the top of the pile touched the bottom tip of the funnel. The angle of repose was then calculated using the Eqs. (4) and (5);

$$\tan \theta = \frac{h}{r} \quad (4)$$

$$\theta = \tan^{-1} [h/r] \quad (5)$$

where, θ - angle of repose, h - height in cm, and r- radius in cm.

Peak viscosity

A total of 3 g of *Gracilaria* powder was mixed with kappa-carrageenan and tricalcium phosphate, then weighed and placed into an RVA pan, followed by the addition of 25 mL of distilled water. Finally, the further step and the peak viscosity of the sample was measured using the method described by [45].

Response optimization and verification

Variable and response data were input into the Design Expert 13[®] (Stat-Ease Inc., Minneapolis, AS) program to generate an output in the form of a new optimal formula recommendation with a maximum

desirability value, approaching 1.0 [46]. This value indicates that the final product aligns with the desired criteria. The next step involved verifying the recommended formula. Instant pre-mix seaweed beverage was prepared based on the recommended formula (in triplicate), and quality parameters were analyzed, including physical criteria such as moisture content, flowability, cohesiveness, angle of repose, flow time, and peak viscosity. The results were compared to the response values predicted by the program to assess their alignment.

Post-brewing physical characteristics of pre-mix instant powder with citric acid addition

To optimize formula performance and address powder solubility challenges during the post-brewing process, an acidulant must be incorporated. Citric acid is proposed as the preferred acidulant due to its greater efficacy in enhancing solubility and imparting a milder sourness compared to alternatives such as tartaric or malic acid [47]. This study examined the effect of citric acid at concentrations of 0%, 1.5%, 4.5%, and 7.5% on the physical characteristics of instant pre-mix seaweed beverage after brewing. An experimental approach was employed using a CRD. Data analysis was performed using ANOVA, and the optimal treatment was determined through a post hoc Duncan's test. Evaluations include the solubility, absorption, sedimentation index, and CIE $L^*a^*b^*$ color parameters to assess the final product quality after brewing.

Water solubility index

A total of 1 g powder sample was weighed and mixed with 25 mL of distilled water at room temperature. The mixture was homogenized using a homogenizer for 5 min. Finally, the further step and the peak viscosity of the sample was measured using the method described by [48].

Water absorption index

A total of 1 g powdered sample was weighed and placed into a centrifuge tube containing 15 mL of distilled water, then vortexed until fully homogenized. The sample was then centrifuged at 3000 rpm for 10 min using a centrifuge with a JA-14 rotor at 21 °C. After centrifugation, the supernatant was separated from the mixture, and the remaining residue was weighed [49].

Sedimentation index

The sedimentation index was determined by diluting 10 g of the powdered sample in 100 mL of distilled water using a 100 mL measuring cylinder. The decrease in sedimentation height was then observed over 30 min. Sedimentation index (%) was calculated as the ratio of the transparent volume to the total volume [50].

CIE $L^*a^*b^*$ color

The equipment used for the analysis was chromameter. The obtained data from the color measurement consisted of L^* , a^* , b^* value [51].

Results and discussion

Chemical compositions of *Gracilaria* powder

Proximate analysis is a quantitative chemical analysis used to determine the basic nutritional composition of a food or biological sample [39]. To substantiate the claim that *Gracilaria* sp. serves as a significant source of dietary fiber, a proximate analysis was conducted on the commercial *Gracilaria* powder used in the formulation. The analysis encompassed measurements of moisture, ash, fat, protein, carbohydrate (by difference), and dietary fiber content. Additionally, a separate evaluation was performed to quantify the fiber fraction remaining after the dissolution process, aiming to assess the stability and functionality of the fiber under brewing conditions. The results of these analyses are presented in **Table 2**.

Table 2 Comparison of proximate and dietary fiber content of *Gracilaria* powder pre- and post-dissolution.

	Proximate composition					
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Dietary fiber (%)
<i>Gracilaria</i> powder	1.85 ± 0.14	3.09 ± 0.02	0.51 ± 0.00	5.57 ± 0.17	87.89 ± 0.30	59.07 ± 0.58
Post-dissolution						4.06 ± 0.09

The results of the proximate analysis of *Gracilaria* powder as shown in **Table 2** indicate an moisture content of 1.85% (d.b), ash 3.09%, crude protein 5.57%, crude fat 0.51%, and carbohydrate content (by difference) of 87.89%. The dietary fiber content, measured at 59.07% per gram of dry sample, highlights its potential as a significant source of functional fiber in the formulation of healthy, value-added beverages. However, following the post-dissolution process, the fiber content decreased markedly to approximately 4.06%. This reduction suggests that the majority of the fiber in *Gracilaria* consists of water-soluble fractions, which are prone to dissolution or degradation during the process. The post-dissolution procedure, typically involving elevated temperatures and specific solvent media, facilitates the solubilization of polysaccharide components such as pectin and hemicellulose, leading to their removal through the liquid phase [52]. The residual fiber content indicates the predominance of insoluble components, such as lignin and crude cellulose, which are more resistant to solvent treatment [53]. Therefore,

processing methods for *Gracilaria* should be optimized to preserve its functional fiber fractions. Despite the reduction, the remaining fiber content of 4.06% still satisfies the minimum threshold for the “contains fiber” claim ($\geq 3\%$) according to both by the Indonesian National Standard [54] and international regulatory standards [55].

Analysis of material response optimization

In the initial stage, a response analysis is conducted, and the most appropriate model is selected to accurately describe the relationship between independent and dependent variables. The measurement results of the optimization response for instant pre-mix seaweed beverage formula in **Table 3** indicate that the product’s moisture content (dry basis) values ranged from 8.7% to 11.44%, flowability ranged from 26% to 35%, cohesiveness ranged from 1.31 to 1.59 mg/L, flow time ranged from 12.22 to 21.94 g/s, angle of repose ranged from 31.52° to 34.11°, and peak viscosity ranged from 833 to 2005 cP.

Table 3 Response values in various optimization treatments of *Gracilaria* powder combined with kappa-carrageenan and tricalcium phosphate.

Formula	Response					
	Moisture Content (% d.b.)	Flowability (%)	Cohesiveness (g/mL)	Flow time (g/s)	Angle of repose (°)	Peak viscosity (cP)
1	9.3	28.72	1.33	12.35	32	872
2	9.7	31.2	1.37	12.76	32.43	1312
3	10.23	33.5	1.47	16.17	33.4	1350
4	10.1	31	1.41	14.38	32.97	1325
5	11.44	35	1.59	21.55	34.09	2001
6	8.9	27.43	1.31	12.22	31.52	833
7	9.5	29.91	1.36	13.13	32.53	1312

Formula	Response					
	Moisture Content (% d.b.)	Flowability (%)	Cohesiveness (g/mL)	Flow time (g/s)	Angle of repose (°)	Peak viscosity (cP)
8	11.13	34.72	1.55	20.51	33.92	1471
9	10.03	31.2	1.41	14.15	32.99	1327
10	10.34	32.8	1.4	14.37	33.01	1335
11	8.7	27	1.32	12.34	31.54	1319
12	9.8	32	1.42	14.35	32.98	1337
13	8.8	26	1.32	12.36	31.54	834
14	10.9	34.41	1.49	17.74	33.58	1349
15	11.3	34.91	1.58	21.94	34.11	2005
16	10.36	31	1.41	14.36	32.99	1325

Based on the analysis of variance, the selected model for the moisture content and peak viscosity response was linear, while the responses for flowability, cohesiveness, flow time, and angle of repose follow a quadratic model, showing a highly significant effect ($p < 0.0001$) as shown in **Table 4**. *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate

significantly influenced response, with an error probability below 0.01%. The non-significant lack-of-fit value ($p > 0.05$) indicates the model accurately represents the response without substantial inaccuracies. The selected model was then utilized to determine the optimal parameters for formulating instant pre-mix seaweed beverage.

Table 4 ANOVA results for trial responses.

Responses	Model	Significant ($p < 0.001$)	Lack of fit ($p > 0.05$)	R ²	Adj. R ²	Pred. R ²
Moisture content (%)	Linear	< 0.001	0.875	0.964	0.9587	0.9523
Flowability (%)	Quadratic	< 0.001	0.701	0.957	0.9350	0.9060
Cohesiveness (mg/L)	Quadratic	< 0.001	0.954	0.998	0.9969	0.9963
Flow time (g/s)	Quadratic	< 0.001	0.139	1.000	1.0000	0.9999
Angel of repose (°)	Quadratic	< 0.001	0.738	0.999	0.9997	0.9996
Peak viscosity (cP)	Linear	< 0.001	0.613	0.789	0.756	0.645

Note: Adj. = Adjusted; Pred. = Predicted.

Modelling and analysis response

In this section, the model is selected based on its suitability in representing a well-distributed dataset, as all points (runs) closely follow a linear trend (normal residual plot) and illustrating the correlation between variables as shown in **Figures 1** and **2**. Furthermore, response analysis is conducted based on the

interpretation of response results to evaluate the influence of each ingredient on the physical characteristics of the instant pre-mix seaweed beverage formulation. The selected model is then utilized to determine the optimal parameters for achieving the best formulation.

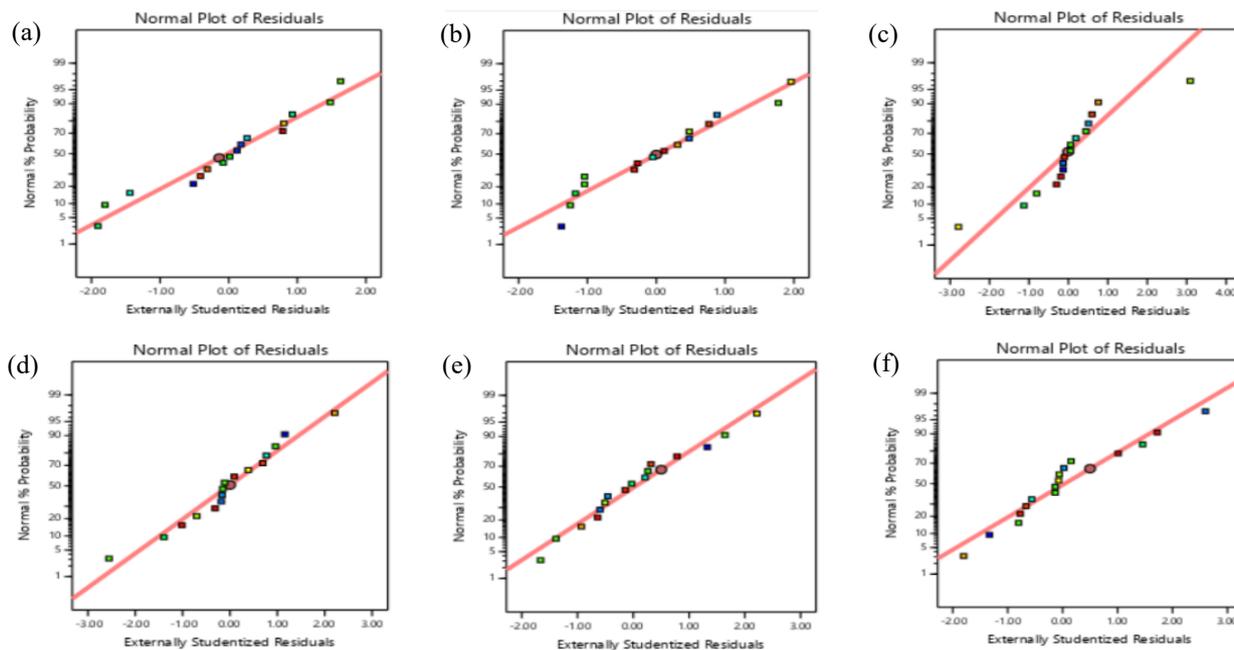


Figure 1 Normal graph plot of residual response (a) moisture content, (b) flowability, (c) cohesiveness, (d) flow time, (e) angle of repose and (f) peak viscosity.

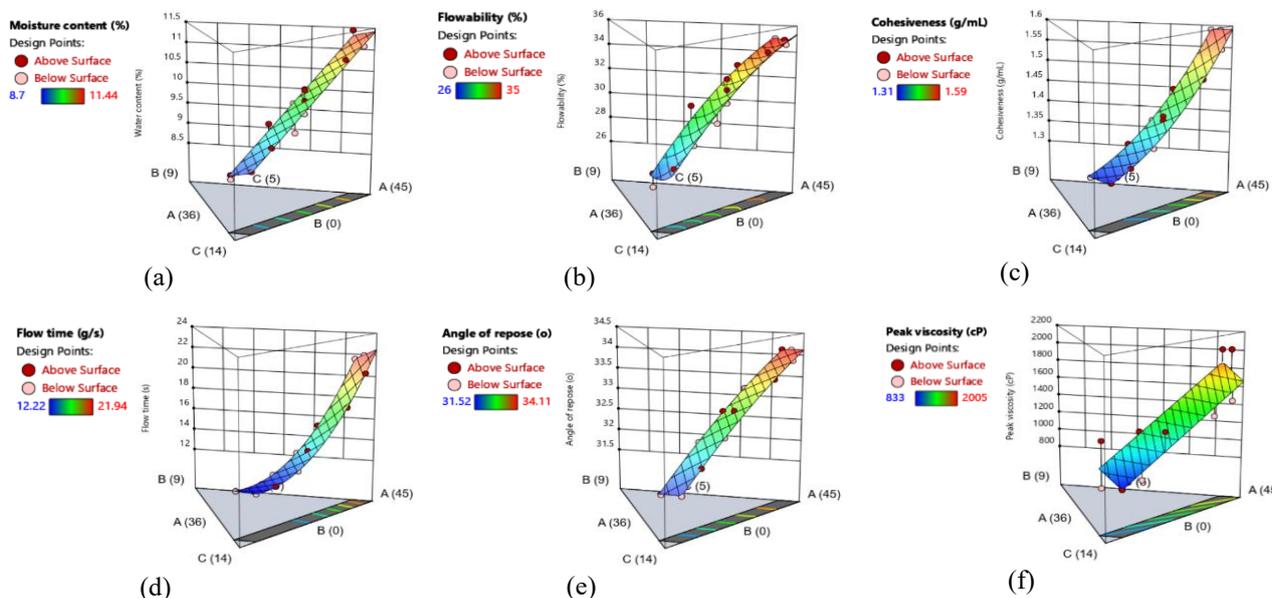


Figure 2 3D Surface Plot of *Gracilaria* powder (A), kappa-carrageenan (B), and tricalcium phosphate (C) against (a) moisture content, (b) flowability, (c) cohesiveness, (d) flow time, (e) angle of repose and (f) peak viscosity value.

Moisture content

The R^2 value of 0.9642 indicates that the factor (X) explains 96.42% of the response (Y). An R^2 value approach 1 suggests a strong correlation between variables and homogeneous data distribution [56]. The difference between adjusted R^2 (0.9587) and predicted

R^2 (0.9523), which is less than 0.2, confirms the model’s reliability [57]. **Figure 1(a)** presents a normal residual plot, demonstrates a well-distributed dataset with all points (runs) closely align with the linear line.

Moisture content influences storage stability and shelf life by promoting chemical reactions and microbial

growth at higher levels [41]. The moisture content of the instant pre-mix seaweed beverage formula ranged from 8.7% to 11.44% (d.b.), as shown in **Table 2**, below the 30% maximum limit set by the Indonesian National Standard for powdered beverages. The lowest moisture content was observed in F11, which contained of 36.1% *Gracilaria* powder, 0.9% kappa-carrageenan, and 13% tricalcium phosphate. The reduced proportion of *Gracilaria* powder combined with a higher level of tricalcium phosphate likely contributed to the lower moisture content, due to the lower hygroscopicity and limited water-binding capacity of these components. In contrast, F5 showed the highest moisture content, consisting of 44% *Gracilaria* powder, 1% kappa-carrageenan, and 5% tricalcium phosphate. The increased level of *Gracilaria* in this formulation is presumed to enhance water retention, supported by the presence of kappa-carrageenan, which may reinforce the gel matrix and facilitate water entrapment within the product structure. The effects of *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate on moisture content as shown in **Figure 2(a)**, with synergistic and antagonistic interactions interpreted by the final equation in Eq. (6).

$$Y_{\text{moisture content (\%)}} = +11.42A + 10.51B - 8.55C \quad (6)$$

Based on the response interpretation, high moisture content is influenced by the hydrophilic nature of *Gracilaria sp.* (coefficient A) and the hygroscopic balance of kappa-carrageenan (coefficient B). The sulfate ester group ($-\text{OSO}_3^-$) in kappa-carrageenan facilitates water binding through hydrogen bonding and electrostatic interactions [58]. In contrast, tricalcium phosphate (coefficient C) has minimal influence due to its hydrophobic properties, which reduce moisture adsorption by coating powder particles and preventing agglomeration [59]. The moisture content results of the instant seaweed beverage premix were consistent with those reported for other instant powders, such as those based on turmeric, red ginger, and palm sugar, which typically range between 8% - 12% [29]. This indicates that the optimized formulation demonstrates favorable storage stability, comparable to other nutraceutical powder products. Further reducing moisture content can be achieved through various strategies, such as

controlling relative humidity, modifying the packaging atmosphere, and implementing other approaches.

Flowability

The R^2 value of 0.9566 indicates that factor (X) explains 95.66 % of the variation in the response (Y). An R^2 value close to 1 indicates a strong correlation between variables and a homogeneous dataset [56]. The small difference between the adjusted R^2 (0.9350) and the predicted R^2 (0.9060), which is less than 0.2, confirms the model's reliability [57]. **Figure 1(b)** presents a normal residual plot, indicating a well-distributed dataset where all points (runs) closely follow the linear line.

Flowability influences packaging, transportation, and usability, as poor flow can cause clogging, segregation, and filling inconsistencies [60]. The flowability of the instant pre-mix seaweed beverage formula ranged from 26% to 35%, as shown in **Table 2**, indicating relatively good flowability based on the Carr Index (CI). Among the tested formulations, F13 exhibited the lowest flowability, consisting of 36.1% *Gracilaria* powder, 0.9% kappa-carrageenan, and 13% tricalcium phosphate. The limited amount of *Gracilaria* powder, which generally contributes to smoother flow, combined with the high content of tricalcium phosphate known for its granular texture and higher interparticle friction is presumed to be the primary factor reducing flow performance. In contrast, F15 demonstrated the highest flowability, with a formulation of 44.43% *Gracilaria* powder, 0.6% kappa-carrageenan, and 5% tricalcium phosphate. The dominant presence of *Gracilaria* powder, which exhibits hydrophilic properties, likely enhanced particle cohesion and uniformity, while the reduced levels of tricalcium phosphate may have minimized interparticle resistance, thereby improving the overall flow characteristics of the powder. The effects of *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate on flowability as shown in **Figure 2(a)**, with synergistic and antagonistic interactions interpreted by the final equation in Eq. (7).

$$Y_{\text{flowability (\%)}} = +141.79A + 35.34B + 25.41C + 116.37AB - 4.93AC - 121.95BC \quad (7)$$

Based on the response interpretation, the high flowability was primarily influenced by the hydrophilic

nature of *Gracilaria* powder (coefficient A), which exhibited a significant synergistic effect. The hydrophilic properties of *Gracilaria* powder tend to reduce porosity, increase density, promote water binding, and enhance gel strength [58]. In contrast, the interaction between kappa-carrageenan and tricalcium phosphate (coefficients B and C) demonstrated a significant antagonistic effect. Calcium ions released from tricalcium phosphate interact with the sulfate groups of kappa-carrageenan through interchain associations, disrupting the gel network and thereby reducing compressibility [61]. The flowability test results of the instant seaweed premix beverage showed values comparable to the flow characteristics of seaweed kombucha powder derived from *Sargassum* which were reported to range between 25% - 32% [10]. This suggests that the developed formulation exhibits flow properties similar to those of other high-fiber-based powders, indicating its suitability as a functional ingredient in instant beverage applications due to its favorable flow performance.

Cohesiveness

The R^2 value of 0.9980 indicates that factor (X) influences the response (Y) by 99.80%. An R^2 value near 1 signifies a strong relationship between variables and a uniform dataset [56]. The minimal difference between the adjusted R^2 (0.9969) and the predicted R^2 (0.9963), which is below 0.2, validates the model's reliability [57]. **Figure 1(e)** shows a normal residual plot, illustrating a well-distributed dataset in which all points (runs) closely align with the linear line.

Cohesiveness reflects particle adhesion in powders, influencing mechanical stability, clumping tendency, and flowability [62]. The cohesiveness of the instant pre-mix seaweed beverage formula ranged from 1.31 to 1.59 g/mL, as shown in **Table 2**, indicating moderate to high cohesiveness based on compressive and bulk density as reflected by the Hausner Ratio (HR). The lowest cohesiveness was observed in F6, which contained 37% *Gracilaria* powder, 0% kappa-carrageenan, and 13% tricalcium phosphate. The low proportion of *Gracilaria* powder, which plays a critical role in the structural integrity of the powder matrix, likely contributed to reduced interparticle adhesion. Additionally, the absence of kappa-carrageenan a known gelling agent may have further diminished the

compactness of the powder structure. In contrast, the highest cohesiveness was observed in F5, formulated with 44% *Gracilaria* powder, 1% kappa-carrageenan, and 5% tricalcium phosphate. The higher content of *Gracilaria* powder, combined with the presence of kappa-carrageenan, is presumed to enhance interparticle bonding through the development of a denser gel matrix, thereby improving the overall cohesiveness of the powder. The effects of *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate on cohesiveness as shown in **Figure 2(a)**, with synergistic and antagonistic interactions interpreted by the final equation in Eq. (8).

$$Y_{\text{cohesiveness (g/mL)}} = +1.59A + 1.30B - 0.3812C + 1.87AB - 0.2165AC - 0.3233BC \quad (8)$$

Based on response interpretation, the high cohesiveness is primarily influenced by the synergistic interaction between *Gracilaria* powder and kappa-carrageenan (coefficient AB). Kappa-carrageenan forms a thin surface layer on the surface of powder particles, enhance cohesion and minimize interparticle voids [58]. Additionally, water bound by *Gracilaria* powder acts as a plasticizer, promoting agglomerate formation through fluid bridges and reducing compression resistance [63]. In contrast, tricalcium phosphate (coefficient C) exhibited a significant antagonistic effect on cohesiveness. The calcium ions released from tricalcium phosphate tend to disrupt the gel matrix by altering the ionic charge distribution, thereby weakening gel interactions and reducing material compactness [64]. A comparable cohesiveness value (~1.4 g/mL) has also been reported for bael (*Aegle marmelos*) fruit powder [64], suggesting that the cohesiveness level observed in the instant pre-mix seaweed beverage is relatively stable. This stability highlights its potential for further development in the storage and processing of functional beverage products.

Flow time

The R^2 value of 1.000 indicates that factor (X) explains 100% of the variation in response (Y). An R^2 value approach 1 signifies a strong correlation between variables and homogeneous data distribution [56]. The difference between adjusted R^2 (1.0000) and predicted R^2 (0.9999) is less than 0.2, confirms the model's

reliability [56]. **Figure 1(d)** presents a normal residual plot, demonstrates a well-distributed dataset with all points (runs) closely aligning with the linear line.

Flow time reflects powder fineness during transfer, impacting industrial efficiency and consumer convenience [43]. The flow time of the instant pre-mix seaweed beverage formula ranged from 12.22 to 21.94 g/s, as shown in **Table 2**, indicating that the powders did not meet the optimal standard of ≤ 10 s per 100 g (equivalent to a flow rate ≥ 10 g/s) [43]. The shortest flow time was observed in F6, which contained 37% *Gracilaria* powder, no kappa-carrageenan, and 13% tricalcium phosphate. This composition is presumed to result in particles with lower density and weaker interparticle cohesion, thereby facilitating improved flowability. In contrast, the longest flow time was observed in F15, containing 44.43% *Gracilaria* powder, 0.6% kappa-carrageenan, and 5% tricalcium phosphate. The higher *Gracilaria* content, combined with the viscoelastic properties imparted by kappa-carrageenan, likely enhanced interparticle cohesion and resistance to flow, thus slowing down powder discharge. The influence of *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate on flow time is depicted in **Figure 2(d)**, with synergistic and antagonistic interactions described by the final regression model in Eq. (9).

$$Y_{\text{flow time (g/s)}} = +12.51A + 12.83B - 14.13C + 22.47AB - 1.95AC - 1.62BC \quad (9)$$

Based on the response interpretation, high flow time is influenced by the synergistic interaction between *Gracilaria* powder and kappa-carrageenan (coefficient AB). The presence of polysaccharides and dietary fiber in these materials can enhance moisture absorption, promote the formation of a thin surface layer that facilitates agglomeration, strengthen interparticle cohesion, and reduce void spaces, thereby contributing to a denser powder structure [60,61]. In contrast, tricalcium phosphate (coefficient C) exhibited a significant antagonistic effect. Acting as an anti-caking agent, tricalcium phosphate modifies particle size distribution and morphology, producing a smoother surface and improving powder flow characteristics, ultimately resulting in reduced flow time [64]. These findings are consistent with previous studies on high-fiber cocoa-based powdered beverages, which reported

that elevated fiber content and strong interparticle cohesion tend to increase flow time beyond 12 s [20]. Thus, deviation from the optimal flow time standard appears to be a typical feature of fiber-rich powdered products.

Angle of repose

The R^2 value of 0.9998 indicates that factor (X) explains 99.98% of the response (Y). An R^2 value near 1 signifies a strong correlation between variables and a consistent data distribution [56]. The slight difference between the adjusted R^2 (0.9997) and the predicted R^2 (0.9996), remaining below 0.2, verifies the model's reliability [57]. **Figure 1(e)** presents a normal residual plot, demonstrates a well-distributed dataset with all points (runs) closely aligned with the linear line.

Angle of repose reflects powder flowability; a smaller angle indicates better powder flow, enhancing processing, packaging, and end-use efficiency [60]. The angle of repose for the instant pre-mix seaweed beverage formula ranged from 31.52° to 34.11° , as shown in **Table 2**, indicating that the powder is classified as free-flowing based on Carr's classification, which defines an angle of repose between 30° and 38° as indicative of good flowability [60]. The lowest angle of repose was observed in F6, which contained 37% *Gracilaria* powder, no added kappa-carrageenan, and 13% tricalcium phosphate, demonstrated improved flowability. The absence of kappa-carrageenan, which typically enhances interparticle cohesion through its gelling capability, along with a relatively high concentration of tricalcium phosphate an anti-caking agent known to reduce particle adhesion and alter morphology likely contributed to lower interparticle resistance, thus producing a shallower angle of repose. In contrast, the highest angle of repose was observed in F15, which contained 44.43% *Gracilaria* powder, 0.6% kappa-carrageenan, and 5% tricalcium phosphate. The increased proportion of *Gracilaria*, rich in polysaccharides and dietary fiber, combined with the viscoelastic nature of kappa-carrageenan, is believed to enhance interparticle bonding and matrix compaction, consequently impeding powder flow and resulting in a steeper repose angle. The effects of *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate on angle of repose as shown in **Figure 2(e)**, with synergistic and

antagonistic interactions interpreted by the final equation in Eq. (10).

$$Y_{\text{angle of repose}} (^{\circ}) = +34.11A + 31.58B - 31.11C + 2.73AB + 0.8188AC + 3.66BC \quad (10)$$

Based on the interpretation of response, the high angle of repose is influenced by the polysaccharide content, such as agar, *Gracilaria* powder (coefficient A), which exhibits hygroscopic properties. These properties promote the formation of a thin surface layer on powder particles, thereby increasing interparticle adhesion. The presence of moisture induces a liquid bridge effect, wherein a thin film of water acts as an adhesive, enhancing Van der Waals interactions and thus increasing particle cohesion [65]. In contrast, tricalcium phosphate (coefficient C) demonstrated a significant antagonistic effect. Its role as a lubricant is attributed to its spherical morphology and relatively larger particle size, which reduce interparticle friction and facilitate smoother particle movement [63]. A comparable angle of repose, ranging from 32° to 36°, has been reported for seaweed-based instant kombucha powder [10], supporting the favorable handling properties observed in the optimized formulation of this study. These findings underscore that the interplay between hygroscopic biopolymers and flow-enhancing agents significantly governs the flow behavior of fiber-rich functional beverage powders.

Peak viscosity

The R^2 value of 0.7886 indicates that factor (X) explains 78.86% of the response (Y). An R^2 value close to 1 indicates a strong correlation between variables and homogeneous data distribution [56]. The difference between adjusted R^2 (0.7561) and predicted R^2 (0.6448) is less than 0.2, confirm the model's reliability [57]. **Figure 1(f)** presents a normal residual plot, demonstrates a well-distributed dataset with all points (runs) closely align with the linear line.

Peak viscosity reflects hydration and gel-forming ability, influencing solubility, texture, and final product stability [66]. The peak viscosity values of instant pre-mix seaweed beverage formula ranged from 833 to 2005 cP, as shown in **Table 2**. These value indicating a moderate viscosity range appropriate for promoting rapid solubility and a light texture in instant powdered

beverages, such as fiber beverage or fiber fruit-based [67]. The lowest peak viscosity was observed in F6, which contained 37% *Gracilaria* powder, no kappa-carrageenan, and 13% tricalcium phosphate. This low viscosity is attributed to the absence of kappa-carrageenan, a known thickening agent, and the relatively low polysaccharide content of *Gracilaria*, which limited the formation of an extensive gel network. Additionally, the high concentration of tricalcium phosphate may have further contributed to the reduced viscosity by mechanically disrupting the gel matrix and weakening interactions among polymer chains. In contrast, the highest peak viscosity was observed in F15, which contained 44.43% *Gracilaria* powder, 0.6% kappa-carrageenan, and 5% tricalcium phosphate. The increased polysaccharide content, combined with the viscoelastic properties of kappa-carrageenan serving as a molecular network enhancer in the aqueous phase significantly improved the gel-forming capacity of the formulation. The effects of *Gracilaria* powder, kappa-carrageenan, and tricalcium phosphate on angle of repose as shown in **Figure 2(f)**, with synergistic and antagonistic interactions interpreted by the final equation in Eq. (11).

$$Y_{\text{peak viscosity}} (\text{cP}) = +3509.37A + 1658.38B - 726.21C \quad (11)$$

Based on the response interpretation, the high peak viscosity was primarily influenced by the polysaccharide content of *Gracilaria* powder (coefficient A), which contributes to the formation of a highly cohesive macromolecular network that limits water mobility. The hydrophilic nature of *Gracilaria* enhances molecular hydration via osmotic mechanisms, thereby increasing viscosity [38,62]. In contrast, tricalcium phosphate (coefficient C) exhibited a significant antagonistic effect. The Ca^{2+} ions released from tricalcium phosphate interact with the functional groups of polysaccharides, reducing the water retention capacity of the gel matrix. Furthermore, excessive Ca^{2+} can induce molecular aggregation, disrupt the structural integrity of the gel, and ultimately lead to a decrease in peak viscosity [68,69].

Optimization and validation formula

Based on the responses discussed in the previous paragraph, all significant parameters were incorporated into the optimization process. This optimization was conducted to determine the optimal combination of variables in the formulation of pre-mix instant seaweed beverage to achieve the desired response. All components and the peak viscosity parameter were set

to the “range” category, while for moisture content, compressibility, cohesiveness, flow time, and angle of repose parameter were set to “minimize”, as shown in **Table 5**. These criteria were established to optimize the product’s physical characteristics. The desirability function was applied to achieve the optimal results based on variable criteria and to determine the accuracy of the optimum solution [70].

Table 5 Target formulation and response criteria for the study.

Criteria	Target	Lower limit	Upper limit
Gracilaria powder	<i>is in range</i>	36	45
Kappa-carrageenan	<i>is in range</i>	0	1
Tricalcium Phosphate (TCP)	<i>is in range</i>	5	13
Moisture content (% d.b.)	<i>minimize</i>	8.69	11.44
Flowability (%)	<i>minimize</i>	24	35
Cohesiveness (mg/L)	<i>minimize</i>	1.31	1.48
Flow time (s)	<i>minimize</i>	12.26	14.72
Angle of repose (°)	<i>minimize</i>	31.52	34.11
Peak viscosity (cP)	<i>is in range</i>	833	1500

The optimal solution for this formulation is presented in **Figure 3** and **Table 6**. The solution has a desirability score of 0.973, indicating that the predicted factors can achieve the maximum expected response, with approximately 97.3% of the variation influenced by the selected factors. The predicted formula was validated using a T-test analysis in Minitab, which

showed no significant difference between the predicted and actual values. This statistical validation confirms that the optimized formula has been successfully verified, ensuring the production of pre-mix instant seaweed beverages with consistent moisture content, flowability, cohesiveness, flow time, angle of repose, and peak viscosity

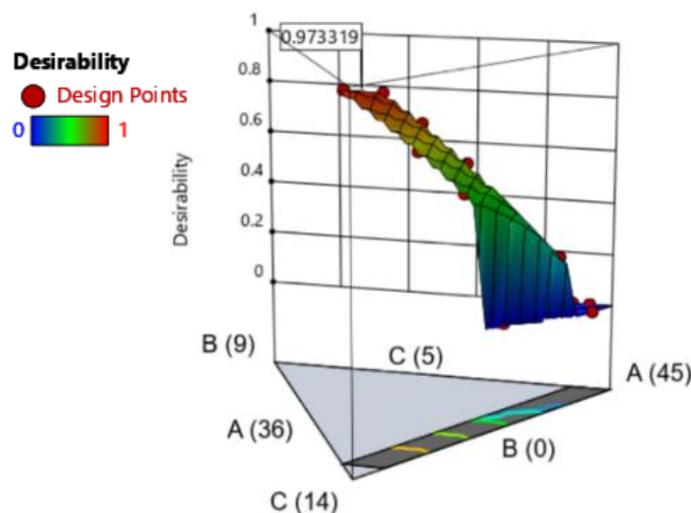


Figure 3 3D graph of desirability.

Table 6 The solution formula of instant pre-mix seaweed beverage all responses.

<i>Gracilaria</i> sp. Powder (%)	Kappa-carrageenan (%)	Tricalcium phosphate (%)	Moisture content (% d.b.)	Flowability (%)	Cohesiveness (mg/L)	Flow time (g/s)	Angle of repose (°)	Peak viscosity (cP)	Desirability
36.52	0.480	13	8.82	26.54	1.31	12.29	31.532	928.5	0.973

Evaluation of the post-brewing physical characteristics of instant pre-mix seaweed beverage with citric acid addition

Based on the optimization results and response verification as shown in **Table 6**, the optimal formulation meeting the established criteria was

obtained. The selected formulations were further utilized in subsequent experiments to assess their effectiveness and stability as shown in **Figure 4** and color visualization as shown in **Table 6**, ensuring their suitability for the development of pre-mix instant seaweed beverages.

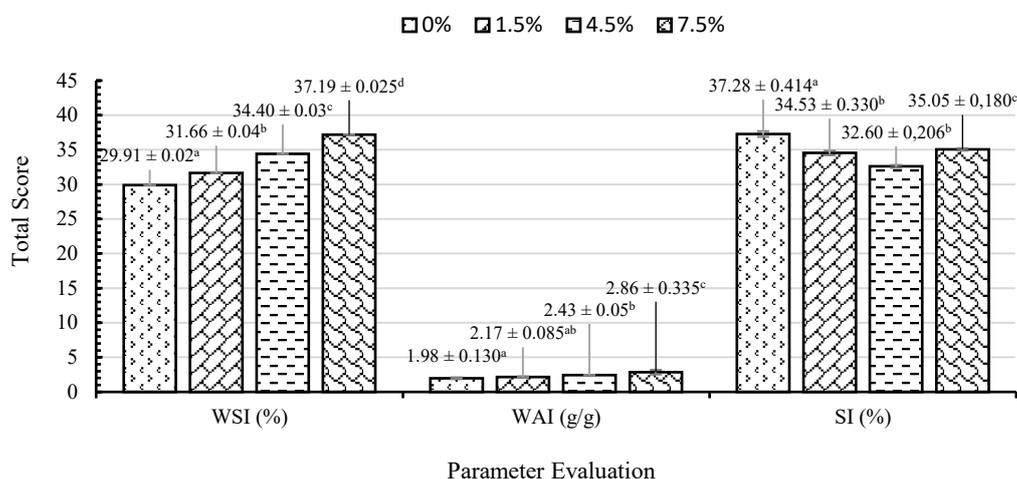


Figure 4 The result of the parameter evaluation in instant pre-mix seaweed beverage with different *citric acid* compositions. Numbers followed by superscript of different letters are significantly different ($p < 0.05$).

Water solubility index

Water solubility reflects the ability of food substances to dissolve, affecting residue formation, consumption, and digestion [48]. The water solubility index of instant pre-mix seaweed beverage ranged from 29.91% to 37.19% as shown in **Figure 4**, below the Indonesian National Standard requirement of $\geq 75\%$ for powdered beverages. Based on the analysis results, the low solubility index is likely due to the high content of fiber and natural colloids from seaweed, such as agar or carrageenan, which are difficult to dissolve completely in water.

Citric acid ($C_6H_8O_7$) disrupts the hydrogen bond (H-X) between fiber and polysaccharide molecules in *Gracilaria* powder and kappa-carrageenan powders by

lowering the pH, increasing dispersibility. As a chelating agent, it partially dissolves calcium tricalcium phosphate ($Ca_3(PO_4)_2$), releasing Ca^{2+} ions that can induce fiber and polysaccharide aggregation, thus potentially increasing solubility [47]. Similar solubility of 45% to 50% was reported in instant beverages made from *Sargassum wightii* and *Ulva Lactuca* [36], highlighting the general challenges of achieving high solubility in fiber-rich seaweed beverages. In addition, suboptimal drying or milling processes can also result in less fine particles, thus inhibiting dissolution.

Water absorption index

Water absorption assesses the hydrophilicity, gelation, and hydration capacity of macromolecules

such as fibers [49]. The water absorption index ranged from 1.95 to 2.86 g/g as shown in **Figure 4**, with the water absorption index increasing significantly with the addition of citric acid. At citric acid concentrations of 4.5% and 7.5%, the water absorption index exceeded the ideal threshold of > 2.2 g/g. A water absorption index value of > 2.2 g/g is considered good, especially in the context of flour or other food ingredients that are considered good for rehydration [50].

Based on the analysis results, citric acid ($C_6H_8O_7$) dissolves hydrophobic tricalcium phosphate, releasing Ca^{2+} ions that interact with kappa-carrageenan, affecting gel cross-linking and water absorption [71]. As a triprotic acid, citric acid lowers the pH, releasing H^+ ions that disrupt hydrogen bonds in *Gracilaria* and kappa-carrageenan powders, increasing the availability of hydrophilic groups and improving the water absorption index [72]. Similar water absorption index values of 2.5 to 2.8 g/g were reported in *Gracilaria*-enriched hybrid snacks [11]. A high water absorption index is beneficial for instant powders, improving hydration, texture and solubility during consumption.

Sedimentation index

The sedimentation index reflects the stability of the dispersion in instant beverages, with lower values indicating better suspension [73]. The sedimentation index ranged from 32.60% to 37.28% as shown in **Figure 4**, with 4.5% citric acid producing 32.60%, close

to the ideal target of < 30%. The sedimentation index of instant pre-mix seaweed beverage decreased up to 4.5% citric acid, but increased again at 7.5%. At concentrations of 0% - 4.5% citric acid, fine particles remained homogeneously dispersed, thus slowing down sedimentation. However, at a lower pH of 7.5%, large agglomerates formed due to decreased surface charge, thus accelerating sedimentation.

At optimum pH, decreasing pH decreases the surface charge of particles, reducing repulsive forces and stabilizing the dispersion. Conversely, further decreasing pH brings the system closer to the isoelectric point, weakening repulsion and increasing attraction, leading to floc formation [59]. Similar levels of 35% to 38% were reported in *Sargassum*-based kombucha beverages [10]. Although slightly above ideal, the dispersion profile supports the role of citric acid in enhancing post-brewing stability.

Color (CIE- $L^*a^*b^*$)

Color is a key factor in the visual appeal of a product, with L^* (lightness), a^* (red-green), and b^* (yellow-blue) values, which influences consumer perception of quality and freshness in instant pre-mix seaweed beverage [51]. The control (0% citric acid) appeared darker and duller, while higher citric acid concentrations increased brightness. The mean values of L^* , a^* , and b^* are presented in **Table 7**.

Table 7 Average value L^* , a^* and b^* of instant pre-mix seaweed beverage using various concentrations of citric acid.

Addition citric acid (%)	L^*	a^*	b^*	ΔE	Colour description	Colour
0	76.41 ± 1.58 ^a	-10.95 ± 0.32 ^c	26.74 ± 0.46 ^a	As reference	Chartreuse	
1.5	83.31 ± 1.05 ^b	-13.87 ± 0.67 ^{ab}	63.28 ± 1.79 ^b	37.30 ± 0.30 ^b	Lemon-lime	
4.5	93.15 ± 3.85 ^c	-16.14 ± 3.01 ^a	66.72 ± 3.26 ^b	43.65 ± 0.39 ^a	Neom-lime	
7.5	90.78 ± 2.00 ^c	-11.05 ± 0.97 ^{bc}	32.76 ± 2.16 ^a	15.58 ± 0.35 ^c	Pastel-lime	

Note: Numbers followed by superscripts of different letters are significantly different ($p < 0.05$).

The addition of citric acid significantly increased the L^* value of instant pre-mix seaweed beverage, from 76.41 to 90.78, indicating enhanced brightness. This improvement in lightness can be attributed to the chelating ability of citric acid, which effectively binds

divalent and monovalent metal ions such as K^+ , Ca^{2+} , and Na^+ , thereby inhibiting the formation of dark-colored complexes that could otherwise diminish visual clarity [33]. Furthermore, the acidification of the matrix resulting from citric acid addition lowers the system's

pH, which suppresses non-enzymatic browning reactions, including the Maillard reaction. This inhibition reduces the formation of brown pigments and promotes a shift toward a lighter and more appealing visual appearance in the final product [74].

The a values progressively decreased from -10.95 to -16.14 as the citric acid concentration increased up to 4.5%, indicating a spectral shift toward a more dominant green hue. This phenomenon may be attributed to the increased acidity of the matrix, which influences the molecular configuration and interactions of active compounds, while also altering the light reflectance properties within the red–green wavelength range [75]. However, at a citric acid concentration of 7.5 %, an increase in the a values was observed. This reversal is likely due to the formation of new molecular assemblies or spatial reorientation of color-soluble compounds under extreme pH conditions, thereby modifying the optical characteristics of the system.

The b value gradually increased from 26.74 to 66.72 as the citric acid concentration increased up to 4.5%, indicating a shift in the spectrum towards a more dominant yellow hue. An increase in the b value was observed as the citric acid concentration increased up to 4.5%, suggesting that a certain level of acidity enhances the spectral reflectance in the yellow wavelength range. This phenomenon is attributed to the modification of the chemical structure and interactions between the active compounds in the beverage matrix, which results in a more favorable optical configuration for light reflection at the optimum pH level [76]. However, at a citric acid concentration of 7.5%, the b value decreased. This change indicates by pigment degradation or ionic interactions or oxidative reactions triggered by the acidic conditions that occur due to extreme pH conditions [75]. As a result, the yellow hue becomes paler and less intense, which has the potential to reduce the visual appeal of the product.

ΔE (Delta E) is a quantitative parameter used to measure the color difference between 2 samples in the CIE- $L^*a^*b^*$ color space [77]. As a comparative assessment, the color difference (ΔE) values clearly demonstrate the visual impact of citric acid concentration on the appearance of instant pre-mix seaweed beverage. Using 0% citric acid as a reference sample, the addition of 1.5% and 4.5% citric acid resulted in ΔE values of 37.30 and 43.65, respectively,

indicating a very pronounced and significant color shift. This shift can be attributed to the increased dispersion of solutes, matrix clarity, and pH-induced changes in light reflectance [75]. These values were higher than the ΔE range of 5.8 - 22.3 reported in green tea powder beverages during storage [78], indicating superior color clarity in the initial blend. Interestingly, the ΔE value decreased to 15.58% at 7.5% citric acid, indicating a less intense color deviation compared to lower concentrations. This phenomenon can be attributed to increased molecular interactions or aggregation at higher acid levels, potentially reducing light scattering or causing partial precipitation of colored compounds [76]. Overall, the data indicate that citric acid at 4.5% produced the most visually distinct and stable color profile, which may contribute to product appeal. These findings support the role of citric acid not only in changing solubility and functional attributes but also in optimizing visual quality as an important factor in consumer acceptance of functional beverages.

Conclusions

In conclusion, the optimization of the *Gracilaria*-based instant beverage formulation using the I-Optimal Mixture Design successfully identified the optimal composition: 36.52% *Gracilaria* powder, 0.48% kappa-carrageenan, and 13% tricalcium phosphate, with a high desirability value of 0.973. This formulation demonstrated good compatibility with the key physical parameters of the premix. The addition of citric acid significantly enhanced post-brewing solubility by chelating Ca^{2+} ions, thereby inhibiting gel formation and improving dispersion. The pH reduction further reinforced ionic interactions and improved the solubility of kappa-carrageenan, contributing to reduced flocculation and increased system stability. A citric acid concentration of 4.5% yielded the most favorable functional profile, with a sedimentation index of 32.60% (approaching the ideal value of $< 30\%$), water absorption index of 2.63 g/g (> 2.2 g/g), water solubility index of 33.17%, and a ΔE value of 43.65. Although the solubility has not meet the SNI threshold of $\geq 75\%$, these findings indicate considerable potential for further formulation improvement. Additionally, citric acid contributed to visual stability by minimizing colour degradation and Maillard browning, resulting in a clear and stable soft green appearance. These results highlight

the critical role of the I-Optimal Mixture Design in efficiently identifying statistically and practically optimal ingredient combinations, thereby accelerating product development. Moreover, citric acid functioned not only as an acidulant but also as a multifunctional agent that improved solubility, system stability, and visual quality. Future studies are recommended to assess the long-term stability and functional performance of the product under various storage conditions, as well as consumer acceptance testing to support its application as a functional beverage.

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Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (QuillBot and ChatGPT by OpenAI) solely for language refinement and grammar correction during the preparation of this manuscript. These tools were not used for content creation or data interpretation. The authors take full responsibility for the content and conclusions presented in this work.

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Mochamad Alauddin Perdana Putra: Methodology, Data curation, Formal analysis, Investigation, Validation, Visualization, and Writing –original draft.

Sri Purwaningsih: Conceptualization, Supervision, Validation, Funding acquisition.

Wahyu Ramadhan: Conceptualization, Supervision, Validation, Writing –review & editing.

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