

Improving the Texture of Low-Sodium Fish Cakes Using Agar Extract from *Gracilaria fisheri* by Hot Water and Alkaline Methods

Peerapong Wongthahan^{1*}, Darren Christian Natanael², Lyket Chuon¹ and Pakanun Charoensri³

¹Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand

²School of Life Science, Indonesia International Institute for Life Sciences, Jakarta, Indonesia

³Culinary Science and Technology Program, Faculty of Technology, Khon Kaen University, Khon Kaen, Thailand

Received : 22 December 2025, Received in revised form : 22 February 2026, Accepted : 23 February 2026

Available online : 4 March 2026

Abstract

Background and Objectives: Conventional fish cakes typically contain approximately 2–3% salt to promote myofibrillar protein solubilization and gel formation; however, salt reduction often compromises texture, water holding capacity, and overall product quality. Thailand's coastal regions are rich in edible red seaweeds, particularly *Gracilaria fisheri* (*G. fisheri*) in southern Thailand, presenting strong potential for health oriented food applications. The functional properties of agar are highly dependent on extraction methods, which in turn affect its physicochemical characteristics and performance in food systems, particularly under sodium-reduced conditions. Despite extensive documentation of individual extraction techniques, comparative evidence linking extraction induced physicochemical differences of agar to textural performance in low sodium food systems remains limited. In addition, *G. fisheri* derived agar offers sustainability benefits by supporting coastal livelihoods and environmental health. However, its application as a texture modifying agent in low sodium seafood products where salt reduction compromises protein gelation has not been systematically investigated. In particular, the comparative performance of agar obtained via the hot water extraction method (HWEM) and the pre-alkaline extraction method (PAEM) in restructured fish cakes under low sodium conditions, as well as their relationships with key textural attributes hardness, springiness, cohesiveness, and chewiness remain unclear. Therefore, this study evaluates *G. fisheri* agar extracted by HWEM and PAEM as functional texturizers in low sodium fish cakes, focusing on physicochemical properties, textural performance, and potential for sustainable sodium reduced product development.

Methodology: Fresh *G. fisheri* seaweed was collected from southern Thailand, washed, and dried before agar extraction. Two methods the hot water extraction method (HWEM) and the pre-alkaline extraction method (PAEM) were applied. For HWEM, dried seaweed was extracted in deionized water, while PAEM involved NaOH pretreatment followed by hot water extraction. Extracted agar solutions were filtered, cooled, and stored for analysis. Agar yield, color (L^* , a^* , b^*), and gel strength were determined using standard analytical methods. Low sodium fish cakes were prepared from tilapia fillets using five formulations (1% salt), varying by agar type (HWEM or PAEM)

and concentration (5% and 10%), and compared with a control (no agar) and a commercial product (2% salt). Fish cakes were steamed, cooled, and analyzed for pH, color, cooking loss, and texture profile parameters, including hardness, springiness, and gumminess.

Main Results: This study evaluated the effects of two agar extraction methods, HWEM and PAEM, on the physicochemical properties of agar derived from *G. fisheri* and its functionality as a texturizing agent in low sodium fish cakes. Agar yield, gel characteristics, and color were first examined. PAEM (29.12%) produced a significantly higher agar yield than HWEM (19.94%), confirming the role of alkaline pretreatment in enhancing agar recovery. However, agar obtained by HWEM exhibited superior gel strength at 0.46 ± 0.02 gforce/mm², indicating better gel network integrity, which are desirable attribute for food applications requiring visual clarity. In contrast, PAEM agar showed lower gel strength at 0.32 ± 0.02 gforce/mm², likely due to polysaccharide degradation and structural modification during alkaline treatment. Food application part, Low sodium fish cakes (1% salt) were formulated with agar extracted by HWEM or PAEM at 5% and 10% concentrations and compared with a control and a commercial product (2% salt). Agar addition significantly influenced textural and physicochemical properties ($p < 0.05$). Fish cakes containing HWEM agar exhibited increased hardness and gumminess with increasing agar concentration (from 5% to 10% agar), while springiness remained relatively unchanged. In contrast, PAEM agar resulted in slightly reduced hardness (207.49 ± 21.58 N at 5% agar and 202.66 ± 29.22 N at 10% agar) and gumminess at higher concentrations, possibly due to over aggregation and weakened gel cohesiveness. These results demonstrate that agar can compensate for reduced salt-induced protein gelation and maintain desirable texture in low sodium formulations. Cooking loss decreased with increasing agar concentration for both extraction methods, reflecting improved water holding capacity. Agar supplemented samples showed cooking loss values comparable to the commercial product, was not significant differences ($p > 0.05$), despite containing half the salt level, highlighting agar's effectiveness in yield retention. Fish cake pH values were slightly higher in PAEM treatments due to residual alkali, but remained within acceptable limits. No significant differences in lightness (L^*) were observed among fish cake samples containing 5% and 10% agar from either extraction method ($p > 0.05$); however, the commercial product showed the highest L^* value. From a commercial and sustainability perspective, HWEM offers a clean label, environmentally friendly approach suitable for health-oriented and premium food products, while PAEM provides higher yields and firmer gels for industrial applications. Together, these findings demonstrate the potential of *G. fisheri* agar to support sodium reduction, enhance product quality, and contribute to sustainable food and bioeconomy development in Thailand.

Conclusions: Both HWEM and PAEM produced functional agars from *G. fisheri* suitable for low sodium meat products, though with distinct advantages. PAEM yielded a higher extraction efficiency and enhanced water

retention, supporting its applicability in industrial scale processing despite the requirement for alkaline neutralization. In contrast, HWEM offered an environmentally friendly alternative with simpler processing and lighter color, supporting health oriented and sustainable product development. Adopting a dual path strategy enables Thailand to reduce dependence on imported agar, enhance local value chains, and support coastal community livelihoods. The utilization of locally sourced seaweed aligns with BCG economy principles, food security objectives, and sustainable marine resource management.

Keywords: agar extracted, hot water extraction method, pre-alkaline extraction method, low sodium fish cake, texture

*Corresponding author. E-mail: peerwo@kku.ac.th

Introduction

Excessive dietary sodium intake has become a major global health concern due to its strong association with hypertension, cardiovascular disease, and stroke. As a result, the World Health Organization (WHO) and various national health authorities have called for significant reductions in sodium consumption, particularly from processed foods, which contribute disproportionately to daily sodium intake. Among these, restructured seafood products such as fish cakes are especially challenging to reformulate because sodium chloride (NaCl) plays a critical multifunctional role—not only enhancing flavor, but also improving protein solubility, emulsification, and gelation, all of which directly influence textural integrity and consumer acceptance (Hwang *et al.*, 2013; Ramirez *et al.*, 2011). Sodium reduction in meat and fish-based products typically leads to undesirable changes in texture, including increased hardness, reduced cohesiveness, and compromised water-holding capacity. These effects are primarily attributed to the decreased solubilization of myofibrillar proteins, which are essential for forming stable gel matrices and emulsified structures (Fiorentini *et al.*, 2020; Sipahutar *et al.*, 2020). In the context of fish cakes, where a delicate balance of firmness, elasticity, and moisture retention defines product quality, reducing sodium often results in brittle or rubbery textures that diminish consumer appeal (Wongthahan & Thawornchinsombut, 2015). Therefore, there is growing interest in identifying novel, functional ingredients that can compensate for these textural deficiencies while supporting the development of low-sodium, health-oriented food products.

Hydrocolloids, particularly polysaccharides, have shown promise as effective texturizing agents in sodium-reduced formulations. When incorporated into meat and seafood matrices, hydrocolloids can enhance gel strength, improve water-holding capacity, and promote favorable textural attributes such as chewiness and resilience (Ramirez *et al.*, 2011; Santana *et al.*, 2013). Examples of widely studied hydrocolloids include carrageenan, xanthan

gum, konjac glucomannan, and microbial transglutaminase (TGase). TGase, for instance, catalyzes covalent cross-links between protein molecules, reinforcing the gel network and restoring firmness in reduced-salt systems (Pirsa & Hafezi, 2023). However, the high cost, regulatory limitations, and supply constraints of some commercial hydrocolloids have spurred interest in more sustainable, marine-based alternatives.

Agar, a sulfated galactan extracted from red seaweeds such as *Gelidium* and *Gracilaria*, is widely used in food, pharmaceutical, and microbiological industries due to its strong gelling ability and thermal reversibility. These properties stem from the agarose fraction, which forms double helical structures that aggregate into three-dimensional gel networks (Sinthusamran *et al.*, 2016). Although agar from *Gelidium* typically exhibits superior gel strength, *Gracilaria*-derived agar is more cost-effective and easier to cultivate, making it attractive for commercial-scale extraction (Martínez-Sanz *et al.*, 2019a; Wang *et al.*, 2017). In Southeast Asia, *Gracilaria fisheri* (*G. fisheri*) has gained attention as a promising raw material due to its local abundance and suitability for aquaculture (Masniyom & Benjama, 2012; Yarnpakdee *et al.*, 2015). Despite this potential, Thailand remains heavily dependent on agar imports, exceeding 1,800 tons annually, mainly from the United States, India, Brazil, and France, reflecting high domestic demand but limited local production capacity (Agar Agar Imports in Thailand, 2023). In 2023, Thailand's food processing ingredient imports reached USD 3.3 billion, with agar import values increasing sharply from USD 10.3 million in 2021 to USD 18.1 million in 2022 (NSDA, 2023). These trends highlight a strategic opportunity to develop domestic agar extraction technologies using locally available *G. fisheri* to enhance supply security, reduce import dependence, and support Thailand's sustainable bioeconomy.

The functional properties of agar are strongly influenced by the extraction method. Alkaline extraction, which involves sodium hydroxide treatment, increases the 3,6-anhydro-L-galactose content, thereby enhancing gel strength and thermal stability (Wang *et al.*, 2017). Meanwhile, hot water extraction often results in agar with higher solubility and lower molecular weight, influencing its behavior in food systems (Martínez-Sanz *et al.*, 2019a). Although both methods are well-documented, comparative studies that evaluate their effects on food structure, particularly in sodium-reduced systems, remain limited.

Beyond technical merits, the use of *Gracilaria fisheri*-derived agar aligns with broader goals of sustainability and socio-economic development. In coastal regions of Thailand and neighboring countries, *Gracilaria* cultivation supports integrated aquaculture, enhances nutrient cycling, and provides supplementary income to small-scale seaweed farmers (Masniyom & Benjama, 2012), as seen in Figure 1. Additionally, seaweed farming contributes to environmental health by absorbing excess nitrogen and serving as a nursery habitat for marine life (Yarnpakdee *et al.*, 2015). Thus, promoting the use of *Gracilaria fisheri*-based agar as a food-grade functional ingredient offers both ecological and economic advantages.



Figure 1 *Gracilaria fisheri* (Source: The original image was taken by the author, 2025)

Despite these promising attributes, the application of *Gracilaria fisheri* agar as a functional texturizer in low-sodium seafood products remains insufficiently explored. Sodium reduction in restructured fish products often compromises myofibrillar protein solubilization and gel formation, thereby increasing the functional importance of hydrocolloids in maintaining texture and water-holding capacity. However, the performance of agar in such systems is highly dependent on its extraction method, which governs polymer structure and gel-forming behavior. To date, no systematic comparison has been conducted between agar extracted via the hot water extraction method (HWEM) and the pre-alkaline extraction method (PAEM) in low-sodium restructured fish cakes. Moreover, the relationships between the physicochemical characteristics of these agars and their effects on key textural attributes, including hardness, springiness, cohesiveness, and chewiness, remain insufficiently understood. Addressing these gaps is essential for the rational design of sodium-reduced seafood products that retain desirable mechanical and sensory properties.

Therefore, this study aims to evaluate the application of agar extracted from *Gracilaria fisheri* using two hot water-based extraction techniques (HWEM and PAEM) as functional texturizers in low-sodium fish cakes, as seen in Figure 2. The objectives are threefold: (1) to compare the physicochemical properties of the extracted agars, (2) to assess their effects on the textural attributes of restructured fish cakes, and (3) to explore the potential of *Gracilaria fisheri*-based agar as a sustainable solution for sodium-reduced food product development. It is

hypothesized that agar extracted by HWEM and PAEM will exhibit different physicochemical characteristics, leading to distinct effects on texture and water retention in low-sodium fish cakes.

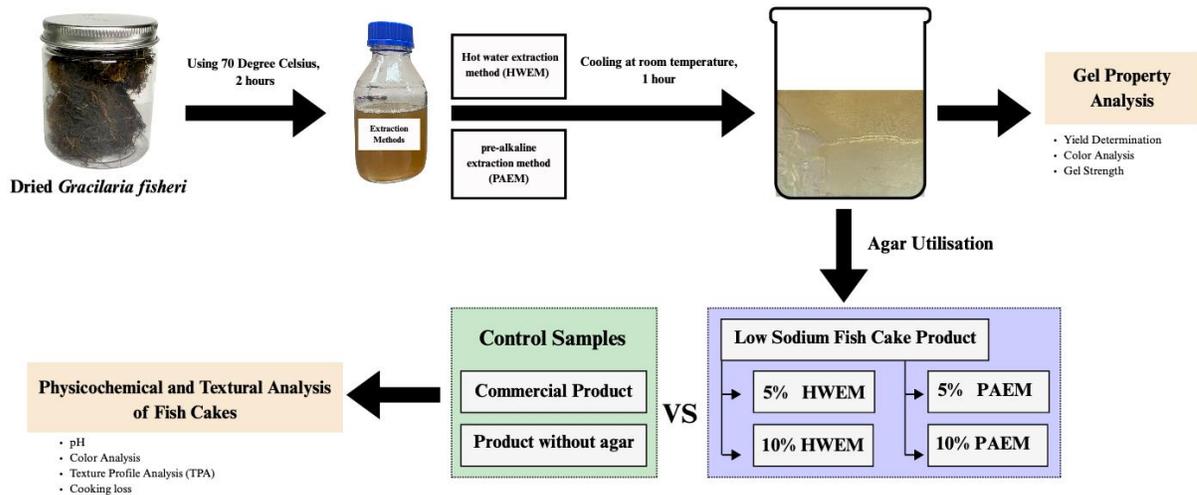


Figure 2 Research Framework (Source: The original image and design were taken by the author, 2025)

Methodology

Fresh *Gracilaria fisheri* seaweed was sourced from the coastal areas of southern Thailand. The fresh sample was washed with water to remove impurities and then dried in a hot air oven at a temperature of 60°C for 8h. Chemicals used for extraction included sodium hydroxide (NaOH) and deionized water. Ingredients for fish cake production, including tilapia fillets, flour, salt, sugar, seasoning, and flavoring agents, were purchased from a local supermarket in Khon Kaen Province, Thailand.

1. Extraction of Agar and Gel Characterization

1.1 Agar Extraction Procedures

Two extraction methods (Hot water extraction method (HWEM) and pre-alkaline extraction method (PAEM)) were employed for agar preparation, with minor modifications from Freilepelegrin (2005); Martínez-Sanz *et al.*, 2019a; ; Praiboon *et al.* (2006). Dried *Gracilaria fisheri* was cleaned and submerged in deionized water at a ratio of 1:20 (seaweed to solution, w/v) in a 1L of duran bottle at 70°C for 2 h.

In the alkaline treatment group, dried *Gracilaria fisheri* was cleaned and soaked in a 4% (w/v) sodium hydroxide (NaOH) solution at a ratio of 1:20 (seaweed to solution, w/v) into a 1L of duran bottle at 70 °C for 2 h.

After pretreatment, the seaweed was thoroughly washed with deionized water until the pH of the rinse water reached a neutral range (pH 7.0–8.0).

The pretreated seaweed was mixed with water in a ratio of 1:10 (w/v) and extracted using an electronic hotplate at 80 °C for 2 h. The agar-rich solution was subsequently filtered through an 80-mesh filter, cooled to room temperature (approximately 1 hour), and stored at 4°C for further analysis. For the application, the extracted agar was oven-dried at 60 °C for 24 h and weighed. Agar quantity was determined in terms of agar yield, expressed as a percentage of dry weight (%).

1.2 Gel Property Analysis

The extracted agar gels were analyzed for their physicochemical characteristics:

1.2.1 Agar Yield

Agar yield was determined by comparing the dry weight of the initial *Gracilaria fisheri* sample to the weight of the extracted agar. The yield (%) was calculated using the following formula:

$$\text{Agar Yield (\%)} = \frac{(\text{Weight of extracted agar (g)} - \text{Weight of dried seaweed (g)}) \times 100}{\text{Weight of dried seaweed (g)}}$$

1.2.2 Agar Color Analysis

The color characteristics of agar samples were measured using an UltraScan XE HunterLab colorimeter, as described by Sutloet & Sompongse (2016). Before measurement, the instrument was calibrated with a standard white and black calibration plate to ensure accuracy. Agar samples were placed on the sample port, and color readings were obtained through the connected software interface.

Color evaluation was based on the CIE Lab color space, reporting three parameters: L* (lightness), a* (red-green axis), and b* (yellow-blue axis). Each sample was analyzed in triplicate, and the mean values were recorded for further comparison.

1.2.3 Gel Strength

Gel strength was measured on agar gel samples equilibrated to room temperature using a Texture Analyzer (Model TA.XTplus, Stable Micro Systems, Surrey, UK) equipped with a cylindrical probe (P/10) following the method of Sutloet & Sompongse (2016). The gel samples were placed on the platform, and a penetration test was performed under standardized conditions to determine the force required to deform the gel. Results were recorded as gel strength values in grams (gforce/mm²), and each measurement was conducted in triplicate.

2. Fish Cake Preparation and Physicochemical Analysis

2.1 Formulation and Processing of Fish Cakes

Low-sodium fish cakes were prepared based on five formulations (1% Salt), varying in both extraction method and agar concentration, and compared with formular without agar (0% Agar) and a commercial product (COM), (2% regular salt). All formulations were based on a standard base mix of 1000 g of tilapia fillets. Fish fillets were blended with salt and ice using a food processor. Once homogenized, flour, sugar, seasoning, garlic powder, onion powder, and flavor enhancer were added incrementally with additional ice. Finally, agar extract obtained from either PAEM or HWEM was incorporated at levels of 5% or 10% (w/w), with the formulation ratios modified from Wongthahan & Thawornchinsombut, (2015), and sesame oil was incorporated. The mixture was transferred to a steam tray lined with parchment paper and steamed at 100 °C for 30 minutes. The samples were cooled down and kept for further analysis.

Table 1 Five fish cake formulas

Ingredients	Formular 1	Formular 2	Formular 3	Formular 4	Formular 5
	0% agar (CONTROL)	5% HWEM	10% HWEM	5% PAEM	10% PAEM
Tilapia fish fillets (g)	1000	1000	1000	1000	1000
Salt (%)	1	1	1	1	1
HWEM (%)	-	5	10	-	-
PAEM (%)	-	-	-	5	10
Seasoning blend (%)	16.5	16.5	16.5	16.5	16.5
Ice cubes (g)	400	400	400	400	400

Note: All formulations were compared with a commercial formulation containing 2% salt and comparable ingredients

3. Physicochemical and Textural Analysis of Fish Cakes

3.1 pH Measurement

The pH of the fish cake samples was measured following Wongthahan & Thawornchinsombut (2015). Eight grams of each sample were blended with 72 mL of deionized water. pH was measured using a calibrated pH meter (FiveEasy FE20-1, Mettler-Toledo AG, Schwerzenbach, Switzerland). Each sample was measured in triplicate, expressed as the mean±standard deviation.

3.2 Color Analysis

Steamed fish cakes were sliced and measured for L*, a*, and b* values using the HunterLab colorimeter. ΔE was also calculated to assess color deviation between treatments and commercial sample (Jiang *et al.*, 2022). Each sample was analyzed in triplicate.

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

Where:

L^* represents the lightness

a^* represents the red–green color axis

b^* represents the yellow–blue color axis

Subscripts 1 and 2 refer to the reference sample (e.g., commercial product) and the test sample (treatment), respectively

3.3 Texture Profile Analysis (TPA)

Texture analysis was performed on cooked fish cake samples equilibrated to room temperature using a Texture Analyzer (Model TA.XTplus, Stable Micro Systems, Surrey, UK) following the method from (Jiang *et al.*, 2022; Wongthahan & Thawornchinsombut, 2015). The samples were cut into uniform cubes measuring 2×2×2 cm and subjected to a two-cycle compression test. A cylindrical probe (P/50, 50 mm diameter) was used to compress the samples to 30% of their original height, with a 5-second interval between cycles. The pre-test, test, and post-test speeds were set at 2 mm/s, 1 mm/s, and 1 mm/s, respectively, and a trigger force of 5 g was applied. The texture parameters recorded included hardness (N), springiness, and gumminess (N). Each sample was analyzed in triplicate.

3.4 Cooking Loss

Cooking loss was determined by weighing 20 g portions of each sample, which were placed in beakers covered with aluminum foil and heated in a water bath at 90°C for 20 minutes. After heating, the samples were cooled to room temperature and allowed to rest for 10 minutes. Cooking loss (%) was calculated as the percentage difference between the initial raw weight (W_1) and the final cooked weight (W_2), representing weight reduction after heat processing (Wongthahan & Thawornchinsombut, 2015). Each sample was analyzed in triplicate.

$$\% \text{ Cooking loss} = 100 \times \frac{(W_1 - W_2)}{W_1}$$

4. Statistical Analysis

All data on physical, chemical properties of agar analysis and quality of fish cake were analyzed with SPSS (version 29.0.2.0, IBM SPSS Statistics). T-test technique and One-way ANOVA with Tukey's HSD post-hoc test were

applied to normally distributed data, as the experimental design involved multiple independent treatment groups differing in agar extraction method and concentration. Significance was considered at $p < 0.05$.

Results

Extraction of Agar and Gel Characterization

The study investigated the efficiency and effects of two extraction methods, the Hot Water Extraction Method (HWEM) and the Pre-alkaline Extraction Method (PAEM), on the yield and gel characteristics of agar extracted from *Gracilaria fisheri*. The yield and color analysis from two agar extraction methods were described in Table 2. The extraction yield of HWEM (19.96%) was significantly lower than that of PAEM (29.12%) ($p < 0.05$). Agar extracted with HWEM in the present study had noticeably greater gel strength than PAEM. In addition, Color measurements showed significantly higher L^* values in PAEM-derived gels, indicating greater lightness and transparency ($p < 0.05$), whereas HWEM-derived gels exhibited lower L^* values and an opaquer appearance.

Table 2 The yield and gel characterization of agar extracted from HWEM and PAEM

Extraction methods	Yield (%)	Color analysis			Gel strength (gforce/mm ²)
		L^*	a^{*ns}	b^{*ns}	
HWEM	19.94±2.98 ^b	39.71±0.16 ^b	-0.68±0.08	1.77±0.50	0.46±0.02 ^a
PAEM	29.12±1.05 ^a	40.33±0.21 ^a	-0.64±0.04	1.66±0.24	0.32±0.03 ^b

Note: Different letters in the same column indicate significant differences by T-test ($p < 0.05$)

All measurements were performed in triplicate, and results are expressed as mean ± SD

ns = non-significant, HWEM = Hot Water Extraction Method, PAEM = Pre-alkaline Extraction Method

Physicochemical Analysis of Low Sodium Fish Cakes

Low-sodium fish cakes were prepared using agar extracted by both the HWEM and PAEM methods, at concentrations of 5% and 10% of fish cake. Two control samples were made without agar and purchased from a commercial product. The addition of agar significantly affected the physicochemical characteristics of the fish cakes. The texture profile analysis, cooking loss, and pH of the fish cakes are presented in Table 3.

Three primary parameters were determined to present the mechanical properties of the foods, including hardness, springiness, and gumminess. The control sample (0% agar and 2% salt) exhibited the highest hardness (395.32±2.54 N) and gumminess values (2,992.66 ± 43.07 N), even higher than COM (added commercial agar) and containing 2% salt (hardness 240.67±0.71 N and gumminess 2,075.82 ± 82.40 N). Increasing the agar

concentration from 5% to 10% resulted in a significantly increased hardness (from 251.76±51.82 to 352.63±20.77 N) and gumminess (from 1,882.34 ± 345.61 to 2,628.30 ± 280.66 N) in HWEM. However, the agar concentrations did not have a significant effect on the springiness, even when compared to commercial treatment ($p < 0.05$). Interestingly, in PAEM, the hardness was slightly decreased (from 207.49±21.58 to 202.66±29.22 N), and the gumminess decreased (from 1,580.19 ± 230.79 to 1,635.61 ± 136.36 N) ($p < 0.05$). Cooking loss result is generally considered an indicator of water-holding capacity (WHC) in food systems. A lower cooking loss reflects an improved WHC, as less water is released during the thermal processing (Huff-Lonergan & Lonergan, 2005). Cooking loss was expressed as (%), which varied significantly across the treatments. The control sample (with 0% agar and 2% salt) had the highest cooking loss, followed by 5% HWEM, while COM, 10% HWEM, 5%, and 10% PAEM were not significantly different ($p < 0.05$). Overall, cooking loss decreased with increasing agar concentration in both extraction methods. Interestingly, cooking loss values of the agar-supplemented groups were comparable to the commercial sample (6.37±0.02%), suggesting that agar addition can effectively maintain the yield. The pH of fish cakes with *Gracilaria fisheri* agar extracts ranges from 8.24±0.01 to 8.33±0.02, which is higher than that of the commercial sample (7.13±0.44). The highest pH was found in the 5% and 10% PAEM groups. In general, the pH of fish cakes containing agar from HWEM remained close to the control.

Table 3 Physicochemical evaluation from different treatments of fish cake induced with agar extracted by the Hot Water Extraction Method (HWEM) and Pre-alkaline Extraction Method (PAEM)

Treatments	Texture Profile Analysis			Cooking loss (%)	pH
	Hardness (N)	Springiness ^{ns}	Gumminess (N)		
COM	240.67±0.71 ^c	0.98±0.02	2,075.82±82.40 ^{bc}	6.37±0.02 ^c	7.13±0.44 ^c
0% Agar	395.32±2.54 ^a	0.86±0.08	2,992.66±43.07 ^a	12.78±0.06 ^a	8.24±0.01 ^{ab}
5% HWEM	251.76±51.82 ^{bc}	0.92±0.07	1,882.34±345.61 ^{bc}	7.55±0.03 ^b	8.23±0.04 ^b
10% HWEM	352.63±20.77 ^{ab}	0.88±0.03	2,628.30±280.66 ^{ab}	6.04±0.01 ^c	8.28±0.04 ^{ab}
5% PAEM	207.49±21.58 ^c	0.94±0.00	1,635.61±136.36 ^c	7.00±0.02 ^c	8.31±0.03 ^{ab}
10% PAEM	202.66±29.22 ^c	0.83±0.01	1,580.19±230.79 ^c	6.66±0.04 ^c	8.33±0.02 ^a

Note: Different letters in the same column indicate significant differences by ANOVA ($p < 0.05$)

ns = non-significant, HWEM = Hot Water Extraction Method, PAEM = Pre-alkaline Extraction Method

The color of fish cake is an important quality attribute that influences consumer perception and acceptance. Table 4 presents the color analysis (L^* , a^* , b^* , and ΔE) of fish cakes prepared with the different amounts of agar extracts (0%, 5%, and 10%) from HWEM and PAEM, as well as a commercial sample. In the current

study, despite adding equal concentrations of agar from both extraction methods (HWEM and PAEM), no statistically significant differences were observed in lightness (L^*) between them. Similarly, increasing agar concentration from 5% to 10% did not produce a statistically significant change in lightness, though a slight decreasing trend with higher agar load was noted.

Table 4 Color analysis of fish cakes from different treatments

Treatments	L^*	a^*	b^*	ΔE
COM	78.16±0.80 ^a	0.32±0.28 ^d	21.36±0.41 ^a	-
0% Agar	66.42±0.20 ^b	1.28±0.08 ^c	19.58±0.38 ^b	11.92 ^d
5% HWEM	65.52±0.52 ^{bc}	1.80±0.11 ^{ab}	18.16±0.25 ^c	13.12 ^c
10% HWEM	65.07±0.86 ^{bcd}	1.68±0.22 ^{bc}	18.87±0.65 ^{bc}	13.39 ^c
5% PAEM	64.23±0.27 ^{cd}	1.20±0.22 ^c	17.58±0.45 ^c	14.46 ^b
10% PAEM	63.92±0.50 ^d	2.25±0.08 ^a	18.62±0.69 ^{bc}	19.21 ^a

Note: Different letters in the same column indicate significant differences by ANOVA ($p < 0.05$)

HWEM = Hot Water Extraction Method, PAEM = Pre-alkaline Extraction Method

Discussion

Effect of Extraction Method on Agar Yield and Gel Properties

The extraction yield results indicate the findings from previous studies, suggesting that alkaline pretreatment can enhance agar recovery from red algae (Freile-Pelegrín & Madera-Santana, 2017; Ziedan *et al.*, 2018). According to Xiao *et al.* (2021), alkali-treated agar can exhibit lower gel strength than acid or bleaching methods due to polysaccharide degradation and reduced dissolution, even though alkali pretreatment is typically seen to increase gel strength by eliminating sulfate groups and creating 3,6-anhydrogalactose. This confirms our findings, which showed that HWEM maintained superior gel strength when compared to PAEM. These differences highlight the impact of extraction methods on gel clarity, which is a critical quality attribute for applications where visual transparency and color consistency are desired. These findings agree with the previous research of Xiao *et al.* (2021), which reported that alkaline pre-treatment of the *Gracilaria tenuistipitata* resulted in lighter agar compared to water pre-treatment because pigments such as chlorophyll, carotenoids, and phycoerythrobilin are likely leached out during the extraction.

Textural Modification and Water-Holding Capacity of Low-Sodium Fish Cakes

This suggests that agar concentration positively affects the texture profile, so it could be used to reduce salt without compromising hardness and gumminess (Imeson, 2010). However, at lower incorporation levels, agar may initially exert a dilution effect, in which the added hydrocolloid and associated water partially disrupt protein–protein interactions and weaken network continuity, resulting in reduced hardness and gumminess (Cebrián-Lloret *et al.*, 2024). As the HWEM level increased to 10%, the agar concentration became sufficient to form a more continuous hydrocolloid gel phase, thereby reinforcing the composite protein–polysaccharide network and restoring hardness and gumminess. This aligns with findings by Smirnov *et al.* (2025), who reported that increasing agar concentration from 2% to 6% led to higher hardness and gumminess in the agar gels, while the springiness remained relatively unchanged with increasing agar concentration. As the hardness increased or decreased, the gumminess changed accordingly (Wongthahan & Thawornchinsombut, 2015). These reduced textural values, particularly when compared with HWEM, may be attributed to over-aggregation of agar molecules and decreased cohesiveness at higher concentrations, which disrupts gel network integrity and water retention despite alkaline pretreatment, a behavior consistent with the effect of polymer structure reported for different *Gracilaria species* (Freile-Pelegrín & Murano, 2005). In the case of PAEM-derived agar, alkaline pretreatment may further promote over-aggregation by reducing sulfate substitution and increasing chain rigidity, thereby favoring the formation of dense and localized junction zones rather than a homogeneous gel network (Zhang *et al.*, 2023). Such structural heterogeneity can weaken protein–polysaccharide interactions and impair water entrapment, leading to reduced hardness and gumminess despite higher polymer content. In addition to agar-related effects, the commercial product showed lower hardness than the 1% salt control, despite containing a higher salt level (2%), which may be attributed to differences in formulation and processing that influence protein gelation independently of salt concentration. Overall, these outcomes affirm that agar enhances the structural integrity of low-sodium formulations, which otherwise suffer from reduced salt-induced protein gelation (Zhu *et al.*, 2021).

Cooking loss is generally considered an indicator of water-holding capacity (WHC) in food systems (Huff-Lonergan & Lonergan, 2005). This demonstrates agar’s water-holding capability and aligns with previous research on hydrocolloids in surimi-based products (Yakhin *et al.*, 2013; Santana *et al.*, 2013; Kraithong & Rawdkuen, 2020). The increased pH values (>8.0) observed across all formulations, including the agar-free control, likely reflect the buffering contribution of formulation ingredients (e.g., seasoning/flavor enhancer components). The PAEM may have further increased pH slightly due to residual alkalinity from pretreatment. Although optimal myofibrillar protein gelation in fish cakes is typically reported at pH 6.0–6.5, higher pH conditions increase electrostatic repulsion and reduce protein aggregation, resulting in a more unfolded and water-binding protein state (Du *et al.*, 2021; Ni *et al.*, 2014; Shen *et al.*, 2019). In surimi-based systems, elevated pH has been shown to enhance gel expansion and

water immobilization, partially compensating for reduced salt levels (Huff-Lonergan & Lonergan, 2005; Park, 2013; Zhou *et al.*, 2021). In the present study, the acceptable texture and reduced cooking loss observed in agar-containing samples indicate that protein–polysaccharide interactions effectively stabilized the gel network despite the elevated pH. The increase in ΔE values with agar addition is likely due to the presence of naturally dark pigments in *Gracilaria* seaweed (as seen in Figure 1), which were partially retained during extraction, particularly under alkaline conditions. When incorporated into fish cakes, these pigments contributed to a darker appearance compared with the control and commercial samples. According to Martínez-Sanz *et al.* (2019a), pigment and phenolic content influence agar color. In this study, there are no significant differences in fish cake lightness, which may therefore be related to the relatively low level of agar incorporation.

Commercial and Market Development Implications of HWEM and PAEM Agar Extraction Methods in the Context of Sustainable Bioeconomy

The comparison between hot water extraction (HWEM) and pre-alkaline extraction (PAEM) methods for agar production from *G. fisheri* highlights important commercial and strategic implications for Thailand's food and bioeconomy sectors beyond physicochemical performance. As global food markets increasingly emphasize sustainability, clean-label ingredients, and health-oriented products, extraction technologies play a critical role in determining market positioning and long-term competitiveness (Pandya *et al.*, 2022).

From a clean-label and green technology perspective, HWEM represents a highly promising approach. The use of only water and heat without chemical reagents aligns well with global trends toward natural, additive-free, and environmentally responsible food ingredients (Awad *et al.*, 2021; Plaza & Turner, 2015). Agar produced via HWEM can therefore be positioned for premium applications such as low-sodium foods, functional beverages, and clear gel-based products, where ingredient transparency and visual quality strongly influence consumer acceptance. In addition, HWEM offers advantages in operational simplicity, reduced waste generation, and lower environmental impact, making it accessible to small and medium-sized enterprises (SMEs) and community-based processors (Knierim *et al.*, 2024; Li *et al.*, 2025). These attributes align closely with Thailand's Bio-Circular-Green (BCG) economy framework and national strategies promoting seaweed as a "future food" under the UN Ocean Decade (2021–2030).

Conversely, PAEM remains an important and robust extraction method for industrial food applications. Alkaline extraction typically provides higher agar yields and stronger gel properties, which are essential for products requiring high mechanical strength, elasticity, and thermal stability, such as meat analogues, confectionery, and restructured seafood products (Ahmad *et al.*, 2011). Despite its performance advantages, the use of alkaline chemicals (e.g., NaOH) generates processing waste and necessitates post-treatment neutralization. These factors

can increase production costs and environmental impact, which may hinder alignment with sustainability frameworks. However, PAEM remains indispensable for manufacturers demanding high functionality, elasticity, and texture durability in large-scale systems

Conclusions

Both the HWEM and PAEM methods yield functional agars capable of improving the quality of low-sodium fish cake products by enhancing textural properties and water retention, thereby compensating for reduced salt levels. PAEM-derived agar provided a higher extraction yield and was more suitable for applications requiring strong structural integrity, although its use involves alkaline treatment and subsequent neutralization. In contrast, HWEM offered a clean-label, environmentally friendly alternative with simpler processing and lighter color, making it particularly attractive for health-oriented and sustainable food products. Method selection should depend on end-use requirements, regulatory considerations, and market positioning to maximize product functionality and application potential. Overall, the findings demonstrate the potential of *Gracilaria*-derived agar as an effective functional ingredient for the development of low-sodium fish cake products.

Limitations and Future Work

This study was limited to physicochemical and textural evaluations, and consumer acceptability was not assessed. Therefore, future research should include sensory evaluation to identify optimal formulations for commercial development. In addition, more in-depth investigations into protein–polysaccharide interactions are recommended to further elucidate the mechanisms underlying quality improvement in low-sodium fish cakes. Such studies would support the effective utilization of locally produced agar and contribute to sustainable food innovation and bioeconomic development.

Implications and Sustainability

The utilization of *Gracilaria fisheri*, a locally available seaweed, not only contributes to functional food innovation but also supports coastal communities economically. Encouraging seaweed cultivation and extraction could promote sustainable marine resource utilization, aligning with SDG goals on responsible consumption and aquatic ecosystem preservation.

Further research should explore microbial safety, storage stability, and consumer acceptability through sensory trials. Moreover, the scalability of extraction and integration into food systems remains essential for commercial application.

Acknowledgements

The authors would like to thank the Department of Food Technology, Faculty of Technology, Khon Kaen University, Thailand, for laboratory facilities and for providing *Gracilaria fisheri* samples sourced from the coastal areas of southern Thailand, as well as the School of Life Science, Indonesia International Institute for Life Sciences, Jakarta, Indonesia, and Assoc. Prof. Amporn Sae-Eaw, Ph.D. Department of Food Technology, Faculty of Technology, Khon Kaen University, Thailand, for their support.

References

- Agar agar Imports in Thailand—Import data with price, buyer, supplier, HSN code. (2023). Retrieved from <https://www.volza.com/p/agar-agar/import/import-in-thailand/>
- Ahmad, R., Surif, M. B., Ramli, N., & Yahya, N. (2011). A Preliminary Study on the Agar Content and Agar Gel Strength of *Gracilaria manilaensis* Using Different Agar Extraction Processes. Retrieved from <https://www.semanticscholar.org/paper/A-Preliminary-Study-on-the-Agar-Content-and-Agar-of-Ahmad-Surif/49a502892292d465605e474785aad0665adcb7d5>
- Awad, A. M., Kumar, P., Ismail-Fitry, M. R., Jusoh, S., Ab Aziz, M. F., & Sazili, A. Q. (2021). Green Extraction of Bioactive Compounds from Plant Biomass and Their Application in Meat as Natural Antioxidant. *Antioxidants*, 10(9), 1465.
- Cebrián-Lloret, V., Martínez-Abad, A., López-Rubio, A., & Martínez-Sanz, M. (2024). Exploring alternative red seaweed species for the production of agar-based hydrogels for food applications. *Food Hydrocolloids*, 146, 109177. doi.org/10.1016/j.foodhyd.2023.109177
- Du, X., Zhao, M., Pan, N., Wang, S., Xia, X., & Zhang, D. (2021). Tracking aggregation behaviour and gel properties induced by structural alterations in myofibrillar protein in mirror carp (*Cyprinus carpio*) under the synergistic effects of pH and heating. *Food Chemistry*, 362, 130222. doi.org/10.1016/j.foodchem.2021.130222
- Fiorentini, M., Kinchla, A. J., & Nolden, A. A. (2020). Role of Sensory Evaluation in Consumer Acceptance of Plant-Based Meat Analogs and Meat Extenders: A Scoping Review. *Foods*, 9(9), 1334.

- Freilepelegrin, Y. (2005). Agars from three species of *Gracilaria* (*Rhodophyta*) from Yucatn Peninsula. *Bioresource Technology*, 96(3), 295–302.
- Freile-Pelegrin, Y., & Madera-Santana, T. J. (2017). Biodegradable Polymer Blends and Composites from Seaweed(Cebrián-Lloret et al., 2024)s. In Handbook of Composites from Renewable Materials (pp. 419–438). John Wiley & Sons, Ltd.
- Freile-Pelegrin, Y., & Murano, E. (2005). Agars from three species of *Gracilaria* (*Rhodophyta*) from Yucatán Peninsula. *Bioresource Technology*, 96(3), 295–302.
- Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 51st International Congress of Meat Science and Technology (ICoMST)*, 71(1), 194–204. doi.org/10.1016/j.meatsci.2005.04.022
- Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role postmortem biochemical and structural changes. *Meat Science*, 71(1), 194–204.
- Hwang, H.-J., Choi, S.-Y., & Lee, S.-C. (2013). Preparation and quality analysis of sodium-reduced fried fish cakes. *Preventive Nutrition and Food Science*, 18(3), 222–225. doi.org/10.3746/pnf.2013.18.3.222
- Imeson, A. (2010). Food Stabilisers, Thickeners and Gelling Agents. Wiley.Com. Retrieved from <https://www.wiley.com/en-hk/Food+Stabilisers%2C+Thickeners+and+Gelling+Agents-p-9781405132671>
- Jiang, C., Chen, Y., Li, S., Shang, S., Fu, B., Wang, L., Dong, X., & Jiang, P. (2022). Ready-to-Eat Fish Cake Processing Methods and the Impacts on Quality and Flavor. *Foods*, 11(21), 3321.
- Kim, T.-K., Yong, H. I., Jang, H. W., Kim, Y.-B., Sung, J.-M., Kim, H.-W., & Choi, Y.-S. (2020). Effects of hydrocolloids on the quality characteristics of cold-cut duck meat jelly. *Journal of Animal Science and Technology*, 62(4), 587–594.
- Knierim, L., Uhl, A., Schmidt, A., Flemming, M., Höß, T., Treutwein, J., & Strube, J. (2024). Pressurized Hot Water Extraction as Green Technology for Natural Products as Key Technology with Regard to Hydrodistillation and Solid–Liquid Extraction. *ACS Omega*, 9(29), 31998–32010.
- Kraithong, S., & Rawdkuen, S. (2020). Effects of food hydrocolloids on quality attributes of extruded red Jasmine rice noodle. *PeerJ*, 8, e10235.

- Li, J., Ye, G., Wang, J., Gong, T., Wang, J., Zeng, D., Cifuentes, A., Ibañez, E., Zhao, H., & Lu, W. (2025). Recent advances in pressurized hot water extraction/modification of polysaccharides: Structure, physicochemical properties, bioactivities, and applications. *Comprehensive Reviews in Food Science and Food Safety*, 24(1), e70104.
- Martínez-Sanz, M., Gómez-Mascaraque, L. G., Ballester, A. R., Martínez-Abad, A., Brodkorb, A., & López-Rubio, A. (2019a). Production of unpurified agar-based extracts from red seaweed *Gelidium sesquipedale* by means of simplified extraction protocols. *Algal Research*, 38, 101420.
- Masniyom, P., & Benjama, O. (2012). Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. *Songklanakarin Journal of Science and Technology (SJST)*, 34(2), 223–230.
- Ni, N., Wang, Z., He, F., Wang, L., Pan, H., Li, X., Wang, Q., & Zhang, D. (2014). Gel properties and molecular forces of lamb myofibrillar protein during heat induction at different pH values. *Process Biochemistry*, 49(4), 631–636. doi.org/10.1016/j.procbio.2014.01.017
- Pandya, Y. H., Bakshi, M., & Sharma, A. (2022). Agar-agar extraction, structural properties and applications: A review. *The Pharma Innovation Journal*, 11(6S), 1151–1157.
- Park, J. W. (Ed.). (2013). Historical Review of Surimi Technology and Market Developments. In *Surimi and Surimi Seafood* (3rd ed.). CRC Press.
- Pirsa, S., & Hafezi, K. (2023). Hydrocolloids: Structure, preparation method, and application in food industry. *Food Chemistry*, 399, 133967.
- Plaza, M., & Turner, C. (2015). Pressurized hot water extraction of bioactives. *TrAC Trends in Analytical Chemistry*, 71, 39–54.
- Praiboon, J., Chirapart, A., Akakabe, Y., Bhumibhamon, O., & Kajiwara, T. (2006). Physical and Chemical Characterization of Agar Polysaccharides Extracted from the Thai and Japanese Species of *Gracilaria*. *Science Asia*, 32(s1), 011.

- Ramírez, J. A., Uresti, R. M., Velazquez, G., & Vázquez, M. (2011). Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: A review. *Food Hydrocolloids*, 25(8), 1842–1852.
- Santana, P., Huda, N., & Yang, T. A. (2013). The Addition of Hydrocolloids (Carboxymethylcellulose, Alginate and Konjac) to Improve the Physicochemical Properties and Sensory Characteristics of Fish Sausage Formulated with Surimi Powder. *Turkish Journal of Fisheries and Aquatic Sciences*, 13(4), 561–569.
- Shen, H., Zhao, M., & Sun, W. (2019). Effect of pH on the interaction of porcine myofibrillar proteins with pyrazine compounds. *Food Chemistry*, 287, 93–99. doi.org/10.1016/j.foodchem.2019.02.060
- Sinthusamran, S., Benjakul, S., & Hemar, Y. (2016). Rheological and sensory properties of fish gelatin gels as influenced by agar from *Gracilaria tenuistipitata*. *International Journal of Food Science & Technology*, 51(6), 1530–1536.
- Sipahutar, Y. H., Taufiq, T., Kristiani, M. G. E., Prabowo, D. H. G., Ramadheka, R. R., Suryanto, M. R., & Pratama, R. B. (2020). The effect of *Gracilaria* powder on the characteristics of nemipterid fish sausage. *IOP Conference Series: Earth and Environmental Science*, 404(1), 012033.
- Smirnov, V., Khramova, D., Chistiakova, E., Zueva, N., Vityazev, F., Velskaya, I., & Popov, S. (2025). Texture Perception and Chewing of Agar Gel by People with Different Sensitivity to Hardness. *Gels*, 11(1), 5.
- Sutloet P, & Sompongse W. (2016). Effect of *Gracilaria fisheri* extract on characteristics of fish emulsion sausage. Retrived from https://kukr.lib.ku.ac.th/kukr_es/index.php/covid19/search_detail/result/333381%0A%0A%0A%0A%0A
- Wang, L., Shen, Z., Mu, H., Lin, Y., Zhang, J., & Jiang, X. (2017). Impact of alkali pretreatment on yield, physico-chemical and gelling properties of high-quality agar from *Gracilaria tenuistipitata*. *Food Hydrocolloids*, 70, 356–362.
- Wongthahan, P., & Thawornchinsombut, S. (2015). Quality Improvement of Reduced-Salt, Phosphate-Free Fish Patties from Processed By-Products of Nile Tilapia Using Textural Additives and Bioactive Rice Bran Compounds. *Journal of Texture Studies*, 46(4), 240–253. doi.org/10.1111/jtxs.12122
- Xiao, Q., Wang, X., Zhang, J., Zhang, Y., Chen, J., Chen, F., & Xiao, A. (2021). Pretreatment Techniques and Green Extraction Technologies for Agar from *Gracilaria lemaneiformis*. *Marine Drugs*, 19(11), 617.

- Yakhin, L. A., Wijaya, K. M., & Santoso, J. (2013). Peningkatan Kualitas Gel Sosis Ikan Lele dengan Penambahan Tepung *Gracillaria gigas*. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 16(2).
- Yarnpakdee, S., Benjakul, S., & Kingwascharapong, P. (2015). Physico-chemical and gel properties of agar from *Gracilaria tenuistipitata* from the lake of Songkhla, Thailand. *Food Hydrocolloids*, 51, 217–226.
- Yudiati, E., Nugroho, A. A., Sedjati, S., Arifin, Z., & Ridlo, A. (2021). The Agar Production, Pigment and Nutrient Content in *Gracilaria sp.* Grown in Two Habitats with Varying Salinity and Nutrient Levels. *Jordan Journal of Biological Sciences*, 14(4), 755–761.
- Zhang, T., Yuan, Y., Wu, X., Yu, P., Ji, J., Chai, J., Kumar Saini, R., Liu, J., & Shang, X. (2023). The level of sulfate substitution of polysaccharide regulates thermal-induced egg white protein gel properties: The characterization of gel structure and intermolecular forces. *Food Research International*, 173, 113349. doi.org/10.1016/j.foodres.2023.113349
- Zhou, Y., Liu, J. J. H., Kang, Y., Cui, H., & Yang, H. (2021). Effects of acid and alkaline treatments on physicochemical and rheological properties of tilapia surimi prepared by pH shift method during cold storage. *Food Research International*, 145, 110424. doi.org/10.1016/j.foodres.2021.110424
- Zhu, Y., Lu, Y., Ye, T., Jiang, S., Lin, L., & Lu, J. (2021). The Effect of Salt on the Gelling Properties and Protein Phosphorylation of Surimi-Crabmeat Mixed Gels. *Gels*, 8(1), 10.
- Ziedan, E. S. H., Zahaby, H. M. E., Maswada, H. F., & Zoeir, E. H. A. E. R. (2018). Agar-agar a promising edible coating agent for management of postharvest diseases and improving banana fruit quality. *Journal of Plant Protection Research*, 58(3), 234–240.