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Biochemical Profiling of *Gracilaria edulis* Agar Waste for Aquaculture Applications

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ABSTRACT

Agar extraction from *Gracilaria edulis* generates approximately 65–75% solid waste. This study evaluated Seaweed Agar Waste (SAW) obtained through hot water extraction (T1) and alkaline pre-treatment (T2) methods for potential applications in aquaculture. Parameters analyzed included agar yield, SAW recovery, proximate composition, amino acid profile, and fatty acid composition. Agar yield was higher in T2 (77.46 ± 14.99 g; 28.17 ± 4.71%) compared to T1 (66.62 ± 14.04 g; 24.54 ± 6.41%). SAW recovery was 40.47 ± 4.76% for T1 and 36.42 ± 4.61% for T2. Proximate composition analysis revealed no significant differences in moisture, ash, fat, or fiber content between treatments. Carbohydrates constituted the predominant component, with higher concentrations in T2 (72.4 ± 2.16%) than in T1 (67.2 ± 1.00%). Conversely, crude protein content was higher in T1 (11.51 ± 0.64%) than in T2 (9.20 ± 1.01%). Total amino acid content was 20.72 ± 4.76 g kg^{-1} DW in T1 and 25.66 ± 6.76 g kg^{-1} DW in T2. Essential amino acids (EAAs) were more abundant in T1 (11.18 ± 1.61 g kg^{-1}) than in T2 (8.08 ± 3.19 g kg^{-1}), with respective EAA/Total AA ratios of 0.54 ± 0.05 (T1) and 0.31 ± 0.08 (T2). Fatty acid profiling showed saturated fatty acids (SFAs) as dominant in both treatments—40.76% in T1 and 38.93% in T2—followed by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The principal PUFA in T1 was arachidonic acid (C20:4ω6), while eicosapentaenoic acid (EPA, C20:5ω3) predominated in T2. The ω-6/ω-3 ratio was markedly higher in T1 (8.90) compared to T2 (1.45). These findings highlight the nutritional potential of SAW—particularly its protein, amino acid, and fatty acid content—and support its prospective use as an aquaculture feed additive and biofertilizer.

INTRODUCTION

In Southeast Asian countries, *Gracilaria* is primarily used for agar production **(Chan & Matanjun, 2017)**. Agar, an important industrial polysaccharide synthesized by marine algae, is widely utilized as a gelling agent in food and pharmaceutical applications

(Madera-Santana *et al.*, 2015; Munifah *et al.*, 2015). The conventional industrial process for agar extraction involves several steps: seaweed pretreatment, extraction, filtration, concentration, and dehydration. Briefly, agar is extracted and dissolved in boiling water, often under pressure **(Hernandez-Carmona *et al.*, 2013)**. The agar solution is then filtered to remove residual seaweed, and the hot filtrate is cooled to form a gel containing approximately 1% agar.

In current industrial practices, large quantities of residue are generated during the filtration step **(Madera-Santana *et al.*, 2015)**. This residue, commonly referred to as Seaweed Agar Waste (SAW), accounts for roughly 70% of the raw material used in agar production. SAW is a surplus, low-value solid by-product of the agar industry but may still retain significant amounts of polysaccharides **(Meinita *et al.*, 2017)**.

The agar extraction process therefore produces a substantial amount of waste, which has potential for conversion into valuable products. Applying the principle of a "Zero Waste Industry" by transforming agar seaweed residues into higher-value products could address public concerns regarding the environmental impact of organic waste disposal from agar processing. Proper management of SAW may reduce the organic load discharged into the sea and surrounding environments during extraction. However, limited information is available on the chemical constituents of SAW.

Most research on *Gracilaria* has focused on agar yield, gel strength, nutritional composition, chlorophyll content, and polysaccharide characterization **(Fan *et al.*, 2012; Chen *et al.*, 2017)**. In contrast, relatively few studies have investigated the utilization of agar extraction wastes, and virtually none have provided detailed characterization. This study is therefore significant, as it aims to provide valuable insights into the potential use of residues or waste generated from agar extraction as feed supplements or fertilizers in aquaculture, as well as a source of bioactive compounds for functional foods and pharmaceuticals.

MATERIALS AND METHODS

Sample collection and drying

Cuttings from 90-day-old *Gracilaria edulis* cultivated in the coastal waters of Rabon, Rosario, La Union, Philippines, using the modified culture method developed by the Don Mariano Marcos Memorial State University – College of Fisheries (DMMMSU–CF), were collected. The harvested *G. edulis* thalli were rinsed with seawater to remove debris and epiphytes, then cleaned further in freshwater before drying. Drying was carried out using a laboratory oven. A total of 20 kg of harvested *G. edulis* was oven-dried at 60°C for 72 hours.

Experimental set-up

The experiment consisted of two treatments based on commonly used agar extraction methods: hot water extraction (Treatment 1; T1) and alkaline pre-treatment extraction (Treatment 2; T2). Each treatment was performed with four replicates. The seaweed agar waste (SAW) generated from each treatment was collected and used for characterization and profiling analyses, including phytochemistry, nutraceuticals, and proximate composition.

Agar extraction using hot water

Hot water agar extraction was performed following a modified method described by **Kumar and Fotedar (2009)**. In this method, 210 g of dried seaweed was boiled at 100 °C for 1 hour. The agar solution was then filtered through a muslin cloth while still hot. The filtrate was allowed to cool at room temperature until solidification. The solidified agar was cut into strips, frozen at −20 °C for 24 hours, and subsequently thawed in tap water. The thawed agar was oven-dried at 60 °C for 48 hours, then ground using a metal coffee grinder (Sonifer SF-3537). The residual SAW was collected, oven-dried at 60 °C for 48–72 hours, and stored for further analyses and characterization.

Agar extraction using alkaline pre-treatment

Alkali pre-treatment extraction followed the method of **Luhan (1992)** with modifications. Oven-dried *G. edulis* samples (260–292 g) were soaked in a 5% sodium hydroxide (NaOH) solution at a ratio of 30 g seaweed per liter for 30 minutes at room temperature. The treated samples were washed in running tap water for 30 minutes to remove excess NaOH, then neutralized in 3% glacial acetic acid solution for 30 minutes (pH 6–7). A final wash in running tap water for 15 minutes was performed to remove residual acid. Agar was extracted by boiling the neutralized seaweed in water (~100 °C) for 1 hour. The agar solution was filtered through muslin cloth while hot, then cooled to room temperature until solidified. The agar gel was cut into strips, frozen at −20 °C for 24 hours, thawed in tap water, oven-dried at 60 °C for 48 hours, and ground using a metal coffee grinder (Sonifer SF-3537). The SAW obtained from this process was oven-dried at 60 °C for 120 hours and stored for further analyses.

Determination of agar yield

Agar yield was calculated using the equation described by **Meinita *et al.* (2017)** (Equation 1).

$$\text{Agar Yield (\%)} = \left(\frac{\text{dry weight of agar}}{\text{initial dry weight of seaweed}}\right) \times 100 \qquad (1)$$

Determination of percentage seaweed agar waste (SAW)

The percentage of SAW obtained from the two treatments was calculated based on the weight of the pulp produced after agar extraction relative to the initial dry weight of the seaweed prior to extraction, as shown in Equation 2, adapted from **Costa *et al.* (2016)**.

$$\text{SAW Yield (\%)} = \left(\frac{\text{dry weight of SAW}}{\text{initial dry weight of seaweed}}\right) \times 100 \quad (2)$$

Proximate and nutrient composition of SAW

Twenty (20) grams of dried SAW from each treatment and replicate were subjected to proximate analyses to determine carbohydrate, protein, lipid, fiber, moisture, ash, and total nitrogen content. Crude protein analysis was carried out using an in-house Macro Kjeldahl method with a Velp Scientifica UDK 129 Digester and Distiller. Determination of total nitrogen followed the Velp Scientifica in-house optimized method, referenced from the Official Methods of Analysis (AOAC), method 981.10.

For each assay, 0.5000 g of dried, homogenized sample was digested for one hour at 420°C using a $CuSO_4$:K_2SO_4 catalyst (1:20 g ratio). The digested samples were distilled for five minutes under alkaline conditions (40% NaOH), with 4% boric acid as the receiving medium, and titrated against standardized 0.1 N HCl. Crude protein content was calculated from total nitrogen values using Equation (3).

$$\text{Crude Protein (\%)} = \%\ \text{N} \times 6.25 \quad (3)$$

Amino acid analysis

Dried, homogenized samples were subjected to acid hydrolysis under elevated temperature and reduced pressure (*in vacuo*) to hydrolyze proteins and peptides into amino acid components with minimal decomposition. Hydrolysis was performed using ®Thermo Scientific Vacuum Hydrolysis Tubes (29571, 6 mL, 10 mm × 150 mm) and a ®Thermo Scientific Reacti-Therm I Heating Module (#TS-18822).

Quantitative determination of amino acids was carried out using an HPLC system (Shimadzu HPLC LC-10A/C-R7A Amino Acid Analysis System). The following amino acids were quantified: Aspartic acid (Asp), Threonine (Thr), Serine (Ser), Glutamic acid (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe),

Histidine (His), Lysine (Lys), Tryptophan (Trp), Ammonia, and Arginine (Arg).

The mobile phase consisted of a binary gradient elution of mobile phase A (sodium citrate buffer, pH 2.23) at a flow rate of 0.6 mL min^{-1} and temperature of 60 °C, mixed with reaction reagents RRA and RRB, pumped individually at 0.2 mL min^{-1}. Post-column derivatization was conducted using ortho-phthalaldehyde (OPA) to separate and identify

amino acids with an 18-amino acid column (SHIM-pack AMINO-Na, P/N 228-18837-91), packed with a styrene-divinylbenzene copolymer sulfonic acid cation-exchange resin. Quantification was based on comparison of the peak areas of the sample amino acids with those of a known amino acid standard mixture containing an internal standard. Amino acid contents were expressed as g kg^{-1} DW and mg g^{-1} sample protein. All measurements were replicated four times.

Fatty acid analysis

The fatty acid (FA) profile of *G. edulis* SAW was determined by gas chromatography–mass spectrometry (GC-MS) using a Claurus 600T® GC (PerkinElmer®) to quantify methylated fatty acids (FAMEs). Sample preparation followed AOAC Official Method 996.06 for fats (total, saturated, and unsaturated), including lipid extraction and derivatization. Sigma FAME Mix C37 standard was used for quantification. Lipid extraction was performed using a modified in-house version of the Bligh and Dyer method, with a 1:2 chloroform:methanol (MeOH) mixture. Extracted lipids were methylated with 14% boron trifluoride (BF_3) in MeOH and toluene. The derivatized FAMEs were dissolved in 1 mL hexane containing C11:0 (undecanoic methyl ester) as the internal standard (ISTD), then injected into an Elite 5MS capillary column (60 m × 0.25 mm I.D. × 0.25 µm, PerkinElmer™) suitable for semi-volatile and volatile organic analytes.

The injection volume was 1 µL (splitless mode). The oven temperature was programmed from 80 °C to 280 °C, with heating ramps of 3 °C min^{-1} and 10 °C min^{-1}. Injector and detector temperatures were set at 230 °C and 200 °C, respectively. Helium was used as the carrier gas at a flow rate of 0.75 mL min^{-1}.

Statistical analysis

All SAW samples from each treatment were analyzed in four replicates. Agar yield, SAW percentage, amino acid content, fatty acid profile, and proximate composition were statistically evaluated using independent *t*-tests to determine significant differences between treatments. Differences were considered significant at $p < 0.05$. Data analyses were conducted using the free open-source R statistical software. Results are presented as treatment means ± standard deviation (SD).

RESULTS AND DISCUSSION

Percentage agar yield and seaweed agar waste (SAW) generated

The production of agar and SAW using two extraction methods—hot water (Treatment 1; T1) and alkaline pre-treatment (Treatment 2; T2)—is summarized in Table (1). Fresh seaweed (2,500 g per treatment) was oven-dried until a constant weight was reached. The mean oven-dried weight for T1 was 276.25 ± 29.26 g, with a mean recovery

of 11.05 ± 1.17%, while T2 yielded 274.37 ± 13.60 g with a mean recovery of 10.97 ± 0.54%.

Agar yield was higher in T2 (77.45 ± 14.99 g; 28.16 ± 4.71%) compared to T1 (66.62 ± 14.03 g; 24.54 ± 6.40%). The increased yield in T2 may be attributed to chemical modifications induced by the alkaline pre-treatment, which likely enhanced gel strength and produced a more concentrated agar.

Following agar extraction, SAW was recovered for biochemical analyses. The mean SAW weight was 112.77 ± 25.21 g in T1 and 100.35 ± 17.21 g in T2. Percentage recovery was higher in T1 (40.47 ± 4.76%) compared to T2 (36.42 ± 4.61%). Thus, while T2 yielded more agar, T1 produced more recoverable SAW.

Despite numerical differences in agar yield and SAW recovery between treatments, statistical analysis revealed no significant differences. The higher agar yield observed in T2 was consistent with the elevated carbohydrate content identified in its proximate composition. Similarly, a study on *Gelidium sequispedale* reported that alkaline pre-treatment prior to agar extraction did not significantly affect the properties of films produced, thereby suggesting that hot water residues may represent a more sustainable and economically viable option **(Martinez-Sanz *et al.*, 2020)**.

Table 1. Agar yield and SAW generated using two different agar extraction methods

Parameters	Hot water (T1)	Alkaline (T2)
Fresh wt. (g)	2500.00±00.00^{A}	2500.00±00.00^{A}
OVD wt. (g)	276.25±29.26^{A}	274.38±13.60^{A}
OVD recovery (%)	11.05±01.17^{A}	10.98±00.54^{A}
Agar wt. (g)	66.62±14.04^{A}	77.46±14.99^{A}
Agar recovery (%)	24.54±06.41^{A}	28.17±04.71^{A}
SAW wt. (g)	112.77±25.21^{A}	100.35±17.21^{A}
SAW recovery (%)	40.47±04.76^{A}	36.42±04.61^{A}

OVD = Ovendried; SAW = Seaweed Agar Waste

Means with the same letters are not significantly different at alpha = 0.05

Hii *et al.* (2016) reported agar yields of 10.68% (alkali extraction) and 9.83% (photobleaching) from *G. salicornia*, which were lower than the yields obtained in the present study and in other *Gracilaria* species **(Freile-Pelegrín & Murano, 2005; Marinho-Soriano, 2001)**. In contrast, **Li *et al.* (2008)** reported higher yields: 29.7% (native), 25.8% (alkali-modified), and 25.4% (bleached agar). Nevertheless, the yield observed in this study falls within the range reported by **Hurtado-Ponce and Umezaki (1988)** for Philippine *Gracilaria* species. It is well established that agar yield is strongly influenced by extraction method, species, season, and environmental conditions **(Arvizu-Higuera *et al.*, 2007)**.

The percentage of SAW generated from hot water extraction (40.47%) was higher than that obtained from alkaline pre-treatment (36.42%). However, statistical analysis revealed no significant differences between the treatments ($P < 0.05$). The results suggest an inverse relationship between agar yield and SAW recovery: higher agar yield corresponds with lower SAW recovery, and vice versa. The SAW recoveries reported here were higher than those from *Gracilaria* sp. in the study of **Basmal *et al.* (2020)**, where recovery was 32.8%. Similarly, **Nurhayati *et al.* (2020)** reported SAW recovery using 0.3% KOH at 80 °C for 4h. In comparison, **Pei *et al.* (2012)** reported an agar yield of 22.4% and a residue recovery of approximately 28.4% of total biomass—both lower than the yields and residues obtained in the present study under both treatments.

Proximate composition of SAW

Proximate composition results of SAW obtained from hot water (T1) and alkaline pre-treatment (T2) extractions are presented in Table (2). Moisture, ash, crude fat, total nitrogen, crude protein, and crude fiber contents were all higher in T1 compared to T2, although differences were not statistically significant. In contrast, carbohydrate content was significantly higher in T2 (72.4 ± 2.16 g 100 g^{-1}) compared to T1 (67.2 ± 1.00 g 100 g^{-1}). Total nitrogen in T1 (1.84 ± 0.10%) was greater than in T2 (1.47 ± 0.16%), resulting in significantly higher crude protein content in T1 (11.51 ± 0.64%) compared to T2 (9.20 ± 1.01%). Both parameters differed significantly at the 5% level.

Variation in moisture content between treatments may be attributed to differences in extraction method and oven-drying conditions. **Rosemary *et al.* (2019)** reported comparable values for dried red seaweed, ranging from 8.40–10.40%. By contrast, **Benjama and Masniyom (2012)** obtained lower moisture contents (3.6–5.5%) in two species of dried red seaweed. Ash content in the present study was consistent with that reported by **Rosemary *et al.* (2019)** for *G. corticata* (8.10%) and *G. edulis* (7.36%), and slightly higher than the value of 6.3% reported by **Pei *et al.* (2013)** for agar extraction waste from *G. lemaneiformis*.

Crude protein content in SAW from both extraction methods was relatively low compared to other proximate composition studies on *Gracilaria* species **(Gressler *et al.*, 2010; Benjama & Masniyom, 2012; Rosemary *et al.*, 2019)**. The crude fat content obtained here was also lower than that reported by **Rosemary *et al.* (2019)** for *G. corticata* (7.07%) and *G. edulis* (4.76%). **Norziah and Ching (2000)** similarly reported fat contents of ~3.3% in *G. changii*, which is still relatively higher than values obtained in the present study.

Table 2. Proximate chemical composition (mean±SD) of SAW at two extraction methods (*n*=4).

Component (g/100g)	Hot Water (T1)	Alkaline (T2)
Moisture	11.16 ± 0.19^{A}	10.45 ± 0.97^{A}
Crude ash	9.57 ± 0.88^{A}	7.44 ± 1.77^{A}

Total nitrogen	1.84±0.10^{A}	1.47±0.16^{B}
Crude protein	11.51±0.64^{A}	9.20±1.01^{B}
Crude fat	0.49±0.24^{A}	0.38±0.36^{A}
Carbohydrates	67.20±1.00^{A}	72.40±2.16^{B}
Crude fiber	4.97±0.04^{A}	4.80±0.12^{A}

Means with the same letters are not significantly different at alpha = 0.05

The higher carbohydrate content in SAW generated from alkaline extraction may be attributed to the elimination of sulfate groups in raw dried seaweed. Carbohydrate levels of *G. edulis* in this study were relatively higher than those reported for other *Gracilaria* species: 67.3% in *G. edulis* **(Rosemary *et al.*, 2019)** and 68.67% in tropical red seaweed *G. acerosa* **(Rasyid *et al.*, 2019)**. Crude fiber content in SAW from hot water extraction was 4.97 ± 0.04 g 100 g^{-1}, while that from alkaline pre-treatment was 4.80 ± 0.12 g 100 g^{-1}. These values were lower than reported ranges, likely because the analyzed material was waste obtained after agar extraction. Previous studies on seaweeds describe total dietary fiber contents ranging from 33% to 50% DW **(Lahaye, 1991; Rupérez & Saura-Calixto, 2001)**.

Amino acid composition of SAW

The amino acid compositions of SAW derived from hot water (T1) and alkaline pre-treatment (T2) are presented in Fig. (1). Mean total amino acid content was 20.72 ± 4.76 g kg^{-1} DW in T1 and 25.66 ± 6.76 g kg^{-1} DW in T2. Amino acid content expressed relative to protein was 179.04 ± 42.55 mg g^{-1} in T1 and 278.97 ± 73.41 mg g^{-1} in T2. Both total amino acid concentration and protein-associated amino acid levels differed significantly between treatments ($P < 0.05$).

Ten essential amino acids (EAAs) and eight non-essential amino acids (NEAAs) were detected in both treatments. In T1, EAAs were 11.18 ± 1.61 g kg^{-1} DW and NEAAs were 9.54 ± 3.17 g kg^{-1} DW, whereas in T2 EAAs were 8.08 ± 3.19 g kg^{-1} DW and NEAAs were 17.58 ± 4.79 g kg^{-1} DW. The EAA-to-total amino acid ratio was significantly higher in T1 (0.54 ± 0.05) than in T2 (0.31 ± 0.08).

Seaweeds are regarded as good protein sources as they provide all essential amino acids, acidic amino acids, and conditionally essential amino acids **(Tanna & Mishra, 2018)**. Depending on their composition, amino acids and bioactive peptides derived from seaweeds have applications in cosmeceuticals, nutraceuticals, and aquaculture feeds **(Tanna *et al.*, 2019)**. In the present study, amino acid profiles differed between treatments: alkaline pre-treatment resulted in higher levels of Cys > Arg > Pro > Glu > Asp > Gly > Phe > Ala > Trp > Leu > His > Ser > Val > Lys > Ile > Thr > Tyr > Met, while hot water extraction produced Arg > Asp > Glu > Gly > Phe > Ala > Pro > Ser > His > Leu > Lys > Trp > Val > Ile > Thr > Tyr > Met > Cys.

Notably, alkaline pre-treatment enhanced cysteine concentration, producing an opposite effect compared to hot water extraction. Although cysteine is a NEAA, it

contributes to glutathione (GSH) synthesis and antioxidant defense **(Abdul Kari, 2025)**. Supporting this, **Fatima (2025)** reported that N-acetyl-L-cysteine (NAC) and lauric acid (LA) enhanced oxidative stress resistance in Pacific white shrimp. Methionine, another sulfur amino acid, also contributes to GSH synthesis through transsulfuration pathways involving glutamate, cysteine, and glycine, thereby maintaining redox balance and stress tolerance **(Wang *et al.*, 2023; Wischhusen *et al.*, 2024)**.

From a nutritional perspective, hot water extraction favored higher EAA retention (e.g., arginine, lysine, methionine), which are vital for protein deposition and growth in fish and crustaceans **(Xing *et al.*, 2024)**. Aquatic animals require balanced dietary amino acids rather than proteins per se, as amino acids serve as substrates for protein synthesis and are critical for growth, intermediary metabolism, immune modulation, and stress regulation **(Wilson, 1986; D'Mello, 2003; Wu, 2020; Mai *et al.*, 2022)**. Functional amino acids such as tryptophan and methionine also support immune function, stress resilience, and antioxidant defense **(Salamanca *et al.*, 2025)**.

Arginine, in particular, is conditionally essential and serves as a precursor for nitric oxide and polyamines, contributing to cellular defense. Arg-enriched diets have been shown to stimulate macrophage activity in fish **(Buentello & Gatlin, 1999; Tafalla & Novoa, 2000)** and modulate innate immunity in Senegal sole (*Solea senegalensis*) **(Höglund *et al.*, 2007)**. Similarly, tryptophan deficiency has been linked to reduced voluntary feed intake in European seabass, with more pronounced effects compared to deficiencies of other EAAs **(de la Higuera, 2001; Tibaldi & Kaushik, 2005)**.

These results demonstrate that the extraction method significantly influences the amino acid profile of SAW. Hot water extraction promotes EAA enrichment, with potential benefits for growth performance and feed efficiency in aquaculture species, whereas alkaline pre-treatment enriches NEAAs such as cysteine, which may enhance antioxidant defense and stress tolerance. Together, these findings suggest that SAW-derived ingredients, depending on the extraction method, could serve as functional protein sources in aquaculture feeds.

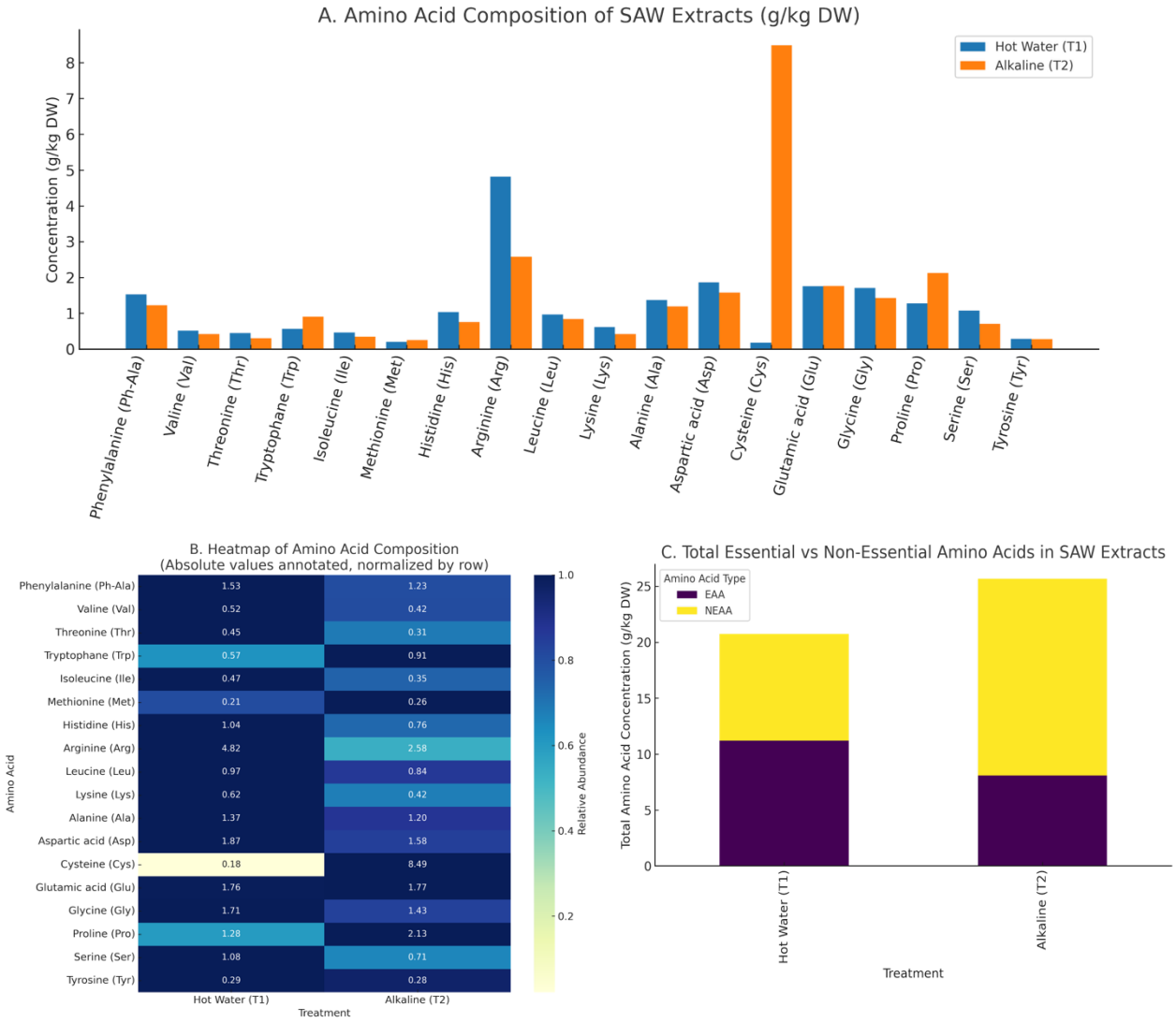

Fig. 1. Amino acid composition of hot water (T1) and alkaline (T2) pre-treated extracts of *Gracilaria edulis* SAW: (A) chart showing individual amino acid concentrations (g/kg DW) under both treatments; (B) Heatmap showing relative abundance patterns across treatments, with absolute concentrations annotated. (C) chart comparing total essential amino acids (EAA) and non-essential amino acids (NEAA)

Fatty acid profile

The fatty acid profiles of SAW obtained from hot water (T1) and alkaline pre-treatment (T2) extractions are presented in Table 3. Fatty acids were categorized into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). In total, 13 SFAs, 6 MUFAs, and 8 PUFAs were identified.

Overall, SFAs dominated the fatty acid composition, with higher levels in T1 (40.76 ± 0.10%) than in T2 (38.93 ± 0.11%). The principal SFA in T1 was palmitic acid (31.57 ± 0.03%), while stearic acid (21.36 ± 0.03%) was most abundant in T2. Among MUFAs, erucic acid (C22:1ω9) was dominant in T1 (2.66 ± 0.03%), whereas myristoleic acid (C14:1Δ9 cis) was higher in T2 (4.35 ± 0.03%). For PUFAs, arachidonic acid (C20:4ω6 cis) was highest in T1 (1.17 ± 0.03%), while eicosapentaenoic acid (EPA, C20:5ω3) was the major PUFA in T2 (3.36 ± 0.03%). The ∑PUFAs/∑SFAs ratio was greater in T2 (0.265 ± 0.23) than in T1 (0.052 ± 0.20).

Gracilaria species typically produce moderate PUFA/SFA ratios (0.42–2.12), with the exception of *G. changii*, which has been reported at 6.96 ± 0.98. The PUFA/SFA ratio in T2 falls within this reported range, whereas T1 showed the lowest proportion. Notably, alkaline pre-treatment enhanced the ω-3 PUFA fraction, particularly EPA (3.36 ± 0.03%) and DHA (0.14 ± 0.03%). In contrast, T1 contained only trace amounts of EPA (0.02 ± 0.03%) and no detectable DHA. The ω-3 PUFA levels in T2 were also higher than those reported by **Debbarma *et al.* (2016)**, who found 0.06–0.50% in *G. edulis*, *Sargassum* sp., and *Ulva lactuca*.

Fatty acids from marine algae are important precursors of bioactive metabolites, with some shown to have antibacterial activity **(Barbosa *et al.*, 2007; Oh *et al.*, 2008)**. In this study, the ω-6/ω-3 ratio further emphasized differences between extraction methods: T2 (1.45) showed a more favorable balance than T1 (8.90), indicating better representation of ω-3 relative to ω-6. This ratio is closer to the recommended dietary levels for fish, supporting growth, stress resistance, and improved nutritional quality of aquaculture species. For example, an ω-3/ω-6 ratio of ~1.43:1 has been reported to promote growth and immunity in Atlantic salmon (*Salmo salar*) **(Holen *et al.*, 2018)**.

Lipids, particularly ω-3 and ω-6 fatty acids, are essential for immune regulation. ω-3 fatty acids such as EPA and DHA exert anti-inflammatory effects that mitigate oxidative stress and chronic inflammation **(Dunbar *et al.*, 2014; Glencross *et al.*, 2024)**. In contrast, ω-6 fatty acids, including arachidonic acid, are precursors of pro-inflammatory eicosanoids that play critical roles in host defense **(Nayak *et al.*, 2020)**. Marine algae and fish oils supplying ω-3 fatty acids have been shown to modulate immune cell function, reduce inflammation, and enhance disease resistance in aquaculture species **(Yadav *et al.*, 2020)**. Thus, incorporating SAW-derived ω-3-rich extracts into aquaculture diets may promote balanced immune responses, stress tolerance, and overall health in farmed fish and shrimp.

Table 3. Fatty acid composition (means±SD) of Seaweed Agar Waste (SAW) from *G. edulis* (% total fatty acid methyl esters, FAMEs)

Fatty acids	Common name	Hot water (T1)	Alkaline (T2)
SFAs			
C8:0	Caprylic acid	0.17±0.03	0.23±0.03
C9:0	Pelargonic acid	0.56±0.03	0.43±0.03

C10:0	Capric acid	2.53±0.03	2.12±0.03
C11:0	Undecylic acid/ISTD	-	-
C12:0	Lauric acid	1.17±0.03	1.34±0.03
C13:0	Tridecylic acid	0.26±0.03	0.27±0.03
C14:0	Myristic acid	1.00±0.03	2.87±0.03
C15:0	Pentadecylic acid	1.51±0.03	1.30±0.03
C16:0	Palmitic acid	31.57±0.03	0.33±0.03
C17:0	Margaric acid	1.22±0.02	0.71±0.03
C18:0	Stearic acid	0.24±0.03	21.36±0.03
C19:0	Nonadecylic acid	0.04±0.03	2.37±0.03
C20:0	Arachidic acid	0.18±0.03	2.10±0.03
C22:0	Behenic acid	0.31±0.03	3.50±0.03
MUFAs			
C14:1Δ9 (cis)	Myristoleic acid	0.35±0.03	4.35±0.03
C16:1Δ9 (cis)	Palmitoleic acid	0.29±0.03	0.61±0.03
C18:1ω9 (cis)	Oleic acid	2.19±0.03	0.02±0.03
C18:1ω9 (trans)	Elaidic acid	0.48±0.03	0.26±0.03
C20:1Δ11	Gondoic acid	0.16±0.03	2.15±0.03
C22:1ω9	Erucic acid	2.66±0.03	1.11±0.03
PUFAs			
C18:2ω6 (cis)	Linoleic acid	0.50±0.03	1.82±0.03
C20:4ω6 (cis)	Arachidonic acid	1.17±0.03	0.22±0.03
C20:5ω3 (cis)	EPA	0.19±0.03	0.50±0.03
C20:3ω6 (cis)	Dihomo-γ-linolenic acid	0.17±0.03	3.17±0.03
C20:4ω6 (cis)	Arachidonic acid	0.03±0.03	0.58±0.03
C20:2Δ11,13 (cis)	Dihomolinoleic acid	0.04±0.03	0.52±0.03
AC20:5ω3	EPA	0.02±0.03	3.36±0.03
C22:6ω3	DHA	ND	0.14±0.03
∑SFAs		40.76±0.10	38.93±0.11
∑MUFAs		6.13±0.07	8.50±0.07
∑PUFAs		2.12±0.08	10.31±0.09
∑PUFAs/∑SFAs		0.052±0.20	0.265±0.23
ω-6/ω-3		8.90	1.45

ND = Not detected; "-" Internal standard (ISTD)

No significant difference between treatments in all parameters at alpha = 0.05

CONCLUSION

SAW extracts obtained from both hot water (T1) and alkaline pre-treatment (T2) showed no significant differences in the percentage of waste generated or in agar yield, indicating that either extraction method can be employed for agar and SAW production.

Phytochemical screening confirmed the presence of phenols, saponins, and coumarins in SAW from both treatments. Biochemical analyses revealed that SAW possesses high nutritional value, supporting its potential as a feed supplement, binder, and fertilizer in aquaculture. SAW from T1 demonstrated superior nutritional quality in terms of essential amino acids (EAAs) and proximate composition, whereas T2 extracts provided a more favorable fatty acid profile, particularly in total polyunsaturated fatty acids (∑PUFAs). Importantly, the hot water extraction method (T1) is more environmentally friendly, as it avoids the use of organic solvents. The high amino acid content of SAW highlights its potential as a nutraceutical, serving both as a source of EAAs that must be obtained through diet and as a functional ingredient for enhancing protein synthesis. These findings suggest that SAW, depending on the extraction method, could be developed into value-added products for aquaculture nutrition and sustainable industry applications.

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