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Interaction between the seaweed *Sargassum filipendula* and rice flour in the production of ready-to-eat foods by thermoplastic extrusion

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ABSTRACT

This study aimed to evaluate the effects of partially substituting rice flour (R) with 1.5 % dehydrated *Sargassum filipendula* (S) flours, using three processing methods: oven-dried flour (SD), freeze-dried flour (SL), and commercial flour (SC). The formulations prepared included a control (R) and three with substitutions (R + SD, R + SL, R + SC), which were processed in a twin-screw extruder with the temperatures of the four zones set at 70 °C, 90 °C, 120 °C, and 115 °C, a screw speed of 200 rpm, and 15 % moisture. The properties were comparatively using the *Scott-Knott* Test ($p \leq 0.05$). The substitution of R affected the torque and specific mechanical efficiency (SME) during the extrusion process. The hardness of the extrudates was higher in the R + SD, R + SL, and R + SC formulations, while porosity did not show significant differences. Microstructure analysis revealed more homogeneous surfaces for the rice-only extrudates (R), while the R + SD, R + SL, and R + SC samples showed visible areas of roughness. X-ray diffraction demonstrated the presence of type V starch and showed that the extrusion process influenced the structural organization of the materials analyzed. All pre-gelatinized flours formed cold paste, with the R + SL formulation showing the highest capacity for DPPH radical scavenging, indicating antioxidant activity potential. In the color analysis, through imaging, it was observed that extrudates and pre-gelatinized flours containing only rice were white, while the formulations with seaweed exhibited brownish tones with dark spots distributed across the sample. The results indicate that differences in composition, origin, and drying methods of seaweed influence the physicochemical properties of the rice extrudates.

1. Introduction

Rice is one of the most widely cultivated and consumed cereals in the world, serving as a staple food in various cultures, particularly in Asian countries and Brazil. Its chemical composition is predominantly starch, which influences its culinary properties. When processed into flour, rice can be utilized in various technological applications due to its gluten-free nature and neutral flavor [1]. However, given its high consumption levels, rice is also a protein source, and when combined with legumes, it provides a complete intake of essential amino acids.

Rice flour, primarily composed of starch (accounting for approximately 70 % of its total composition), can be considered a natural

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Fig. 1. The map shows the geographical location of the Brazilian seaweed sampling site in *São Sebastião* city (45° 23′W; 23°43′S), *São Paulo* State, Brazil.

hydrocolloid with thickening and stabilizing functions. Cooking rice flour in water above 50 °C disrupts the starch granule structure, leading to gelatinization and increased viscosity [43]. This property is beneficial for products such as sauces, soups, and porridges. Furthermore, according to Qian and Zhang [2], rice flour can act as a structuring agent in gluten-free bread, cakes, biscuits, pastes, and noodles [3], as well as in expanded extruded products and ready-to-eat foods.

From a sensory perspective, rice's neutral flavor and soft texture facilitate its combination with seaweed, which typically presents an umami taste. This pairing is valued for both its balanced flavor profile and its nutritional benefits, a practice that has been established for millennia in Asia and is now gaining global popularity. In Asian cuisine, rice serves as a texturizing agent in traditional dishes such as sushi, temaki, onigiri, and soups, which are considered fast-consumption foods due to their high moisture content and susceptibility to microbiological deterioration. Expanding the availability of ready-to-eat products incorporating seaweed, new formulations have been developed [4,44] and are already available on the market, including rice and seaweed crackers, snacks, and sushi preparation kits.

Moreover, growing concerns about health and the pursuit of healthier dietary habits have driven research into new nutrient sources with potential health benefits [5]. In this context, seaweed is well recognized as a healthy and sustainable food source due to their nutritional composition [6], which includes minerals, soluble fibers, and bioactive compounds such as sulfated polysaccharides.

Macroalgae are classified into three main groups - green, red, and brown - based on the predominant photosynthetic pigments in their cells. Among the brown macroalgae, species from the Sargassaceae family, such as *Sargassum filipendula*, stand out for being rich sources of polysaccharides, such as fucoidans and alginates, in addition to phenolic compounds, especially phlorotannins [7]. This group of algae is also characterized by a high concentration of micronutrients, including essential minerals (iodine, selenium, iron, and zinc), vitamins, polyphenols, and pigments such as chlorophylls and fucoxanthins [8,9]. Regarding macronutrients, brown algae provide proteins with complete amino acid profiles, carbohydrates (mostly in the form of dietary fiber), and lipids, particularly long-chain polyunsaturated fatty acids [9].

Native to the Brazilian coast, *S. filipendula* is a brown alga that is still little studied as a food ingredient, despite its wide natural availability and nutritional potential [10]. Its versatile composition allows for application in various formulations, such as breads, cakes, and soups [11]. In addition, its use contributes to sustainable strategies, in accordance with the principles of the circular economy, since by-products from this biomass can be used in the production of biofuels, cosmetics, and biofertilizers [12].

Brazil, a Western country, has significant potential for seaweed cultivation, primarily due to its extensive coastline of 8698 km [13], favorable maritime conditions, and a coastal population of 37 million people [14]. Additionally, the brown seaweed *S. filipendula* (Phaeophyceae) is abundant along the Brazilian coast. However, seaweed farming activities remain extremely limited and are concentrated in only a few areas.

Seaweed can be marketed in its natural form (with a short shelf life) or undergo a drying process to prevent microbial deterioration, reduce transportation costs [15], and extend shelf life. Drying methods include sun drying, oven drying with or without airflow, vacuum drying, and freeze-drying. However, water removal during the drying process leads to structural deformations that degrade the plant matrix and compromise the functionality of cell walls and membranes, resulting in oxidation reactions [16].

Seaweed-based products have gained attractiveness in the food industry, as highlighted by a recent review from our research group [17]. The demand for ready-to-eat and health-oriented foods has increased, leading to the study of versatile processing methods that facilitate the inclusion of novel ingredients. Among the current food processing technologies, thermoplastic extrusion stands out for providing new shapes and structures to food products [18,19].

Recent studies have investigated the use of seaweed to enhance the nutritional and functional profile of extruded products [20,21]. However, the interaction between rice flour and seaweed has not yet been explored, as other food matrices, such as corn, have been preferentially utilized.

Despite the abundance of *S. filipendula* along the Atlantic Ocean coastline below the Equator, its production for consumption and commercialization can be further developed in the Global South. This study aims to evaluate the physicochemical, techno-functional, and

Table 1
Identification and formulation of extrudates with rice flour and rice flour added of *Sargassum filipendula* and commercially *Sargassum* sp.

Identification	Composition of the mixture			
	RF[1] (%)	SD[2] (%)	SL[3] (%)	SC[4] (%)
R[5]	100.0	0	0	0
R + SD[6]	98.5	1.5	0	0
R + SL[7]	98.5	0	1.5	0
R + SC[8]	98.5	0	0	1.5

[1] RF = Rice flour.
[2] SD = *S. filipendula* oven drying.
[3] SL = *S. filipendula* lyophilized.
[4] SC = *Sargassum* sp. commercial.
[5] R = Rice flour extrudate.
[6] R + SD = extrudate of oven dryied *S. filipendula*.
[7] R + SL = extrudate of lyophilized *S. filipendula*.
[8] R + SC = extrudate of commercial *Sargassum* sp.

nutritional interactions between rice flour and *S. filipendula* seaweed in the formulation of ready-to-eat products, utilizing thermoplastic extrusion technology.

2. Materials and methods

2.1. Raw materials

In this study, the raw materials used are described below.

a) Rice flour (R)

Rice flour (SL Alimentos®, Mauá da Serra, Paraná, Brazil). The proximate composition of the flour was determined according to the official methods of AOAC - OFFICIAL METHODS OF ANALYSIS [22] for moisture (method no. 925–109) and ash (method no. 923–03). Total protein was determined by the Kjeldahl method (method no. 984.13) and calculated using a nitrogen conversion factor of 5.95. The total lipid content was determined according to Blight and Dyer [45].

b) Brazilian seaweed

The seaweed *S. filipendula* was collected at Cigarras Beach (45° 23′W; 23°43′S), located at the northern end of the São Sebastião Channel, São Paulo, Brazil (Fig. 1). After collecting, the seaweed was transported in thermal boxes with ice to the laboratory. Before drying, it was washed several times with fresh water to remove any exogenous organisms, dirt, and stones.

After cleaning, the *S. filipendula* fronds were divided into two batches: one was dried in an oven with air circulation and renewal (Tecnal, model TE-394/2, Brazil) using a stainless-steel rack at 40 °C until reaching a moisture content below 10 %, while the other batch was lyophilized in a bench-top lyophilizer (Liotop, model L108, Brazil) for over 12 h, until reaching a moisture content below 6 %.

The dried seaweed from both drying processes was ground into powder (<0.250 mm) using a commercial blender (Hamilton Beach, model HBH450, United States), packaged in polyethylene bags (primary packaging) and multilayer bags with an aluminum layer (secondary packaging), and stored at room temperature until use. Two types of seaweed flour were produced: oven-dried (SD) and lyophilized (SL).

c) Commercial seaweed (SC)

Sargassum sp. was imported from a supplier in China and ground into flour.

All raw materials were standardized to 60 mesh. The composition of SD, SL, and SC was determined in a previous study [6].

2.2. Preparation of rice and seaweed flour extrudates

Four mixtures composed of R and seaweed flour (Table 1) were weighed in 1.5 kg batches, conditioned with distilled water using a planetary mixer (Hypo, model HB 12, Brazil) to reach 15 % moisture, and stored at room temperature for 24 h to ensure uniform hydration.

A ZSK 30 co-rotating twin-screw extruder (Werner Pfleiderer Corporation, Ramsey, USA) with a 29 L/D (length/diameter ratio) was used. The process parameters were based on preliminary tests and kept constant for all four formulations: the feed rate of the mixture was maintained at 200 rpm throughout the extrusion cooking process, the screw speed was set at 160 rpm, the water feed rate was 0.7 L/h, and the temperatures of extruder zones 1, 2, 3, and 4 were fixed at 70, 90, 120, and 115 °C, respectively. The screw configuration used is shown in Fig. 2. A circular die with two holes of 4 mm in diameter was used.

During the extrusion process, torque was recorded for each sample, and the specific mechanical energy (SME) was calculated according to Pansawat et al. [23], using Eq. 1.

$$SME\ (W.h/Kg) = \frac{SS(rpm)\ x\ P(W)\ x\ T(\%)}{SSmax\ (rpm)\ x\ Q\left(\frac{Kg}{h}\right)\ x\ 100} \quad (1)$$

where: SS is the screw speed (rpm); SS max is the maximum screw speed (500 rpm); P is the nominal power of the extruder (9000 W); T is the average torque recorded during the sampling time (%); and Q is the mass flow rate (Kg/h).

The obtained extrudates were dried in an air-circulating oven (Tecnal, model TE-394/2, Brazil) at 50 °C until the moisture content was below 5 %. Subsequently, the products were stored at 20 °C in sealed metallized bags, protected from light and moisture until further analysis.

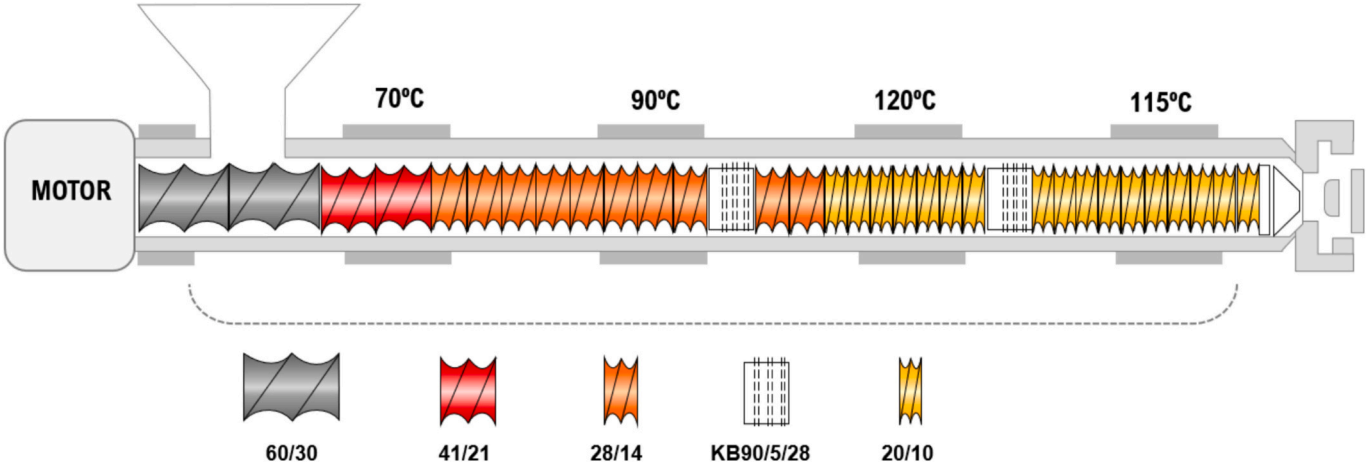

Fig. 2. Schematic representation of the screw configuration and temperatures used in the twin-screw extruder for obtention of extruded products by rice flour added of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) extrudates.

2.3. Physicochemical characterization of rice and seaweed flour extrudates

2.3.1. Moisture and protein content

The moisture and protein content of the extrudates was determined as described in section 2.1, item (a).

2.3.2. Expansion index

The expansion index is expressed as the ratio between the diameter of the extruded product and the diameter of the extruder die [24]. The diameter of the extrudate was measured using a digital caliper and calculated according to Eq. 2. The average of 10 random measurements was considered as the final extrudate diameter.

$$EI = \frac{D\ (mm)}{Do\ (mm)} \tag{2}$$

where: EI is the expansion index, D is the diameter of the extrudate (mm) and Do is the diameter of the die (mm).

2.3.3. Bulk density

The bulk density (BD) was determined by measuring the dimensions of the extrudates using a digital caliper and calculated based on the mass of the extrudate (Eq. 3), represented in g/cm^3 [25].

$$BD = \frac{4\ x\ m}{\pi\ x\ D^2 x\ L} \tag{3}$$

where: BD is the bulk density, in g/cm^3, m is the mass (g), D is the diameter (cm), and L is the length (cm) of the extrudates.

2.3.4. Hardness

The extrudates were standardized to a length of 5 cm, and the hardness (N) of the extrudates was evaluated using a TA-XT2i Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, United Kingdom) equipped with a Warner-Bratzler shear blade with a 'V' notch, which penetrated to a distance of 20 mm to obtain sample rupture, with a test speed of 2 mm/s. The results were obtained as an average of fifteen measurements.

2.3.5. Cross-sectional image analysis of extrudates

The evaluation of cross-sectional images was performed using a scanner equipped with HP PreciseScan version Pro 3.1 software (HP Scanjet 4400C, Hewlett-Packard, USA), using blue background paper. The diameter (mm), perimeter (mm), area (mm^2), number of air cells, porosity (%), extrudate circularity (0–1), and air cell circularity (0–1) were determined using Image-J (National Institute of Health, Bethesda, MD, USA), where the circularity value indicates that a scale of 0 is non-circular and a scale of 1 is a perfect circle. The results were obtained as an average of ten extrudates.

2.3.6. Scanning Electron Microscopy

The internal microstructure of all samples was analyzed using Scanning Electron Microscopy (SEM) (TM4000plus, Hitachi, Japan). For the analysis, the extrudates were cut vertically and horizontally, fixed onto an aluminum stub, and stabilized with double-sided carbon tape. Images were taken in the central area of the extruded piece at magnifications ranging from 30× to 150×, with several captures made to select the most representative ones.

2.4. Techno-functional characterization of pre-gelatinized rice and seaweed flours

To obtain the pre-gelatinized flour from R and the flour mixed with seaweed, the 4 types of extrudates were ground in a commercial blender (Hamilton Beach, model HBH450, USA), standardized to 60 mesh (0.250 mm), and stored in polyethylene bags under atmospheric conditions for analysis.

2.4.1. Hydration properties

The water absorption index (WAI) and water solubility index (WSI) were determined using the Anderson [26] methodology, with modifications. For the analysis, 2.5 g of the sample were weighed into 50 mL Falcon® tubes, 30 mL of distilled water were added, and shaken for 30 min. Afterwards, the tubes were centrifuged at 3000 rpm for 15 min. The supernatant resulting from the centrifugation was transferred to an aluminum plate and dried in an air-circulating oven at 105 °C until constant weight. The calculations were performed according to Eqs. 4 and 5.

$$WAI = \frac{weight\ of\ the\ centrifugation\ residue\ (g)}{dry\ sample\ weight\ (g)} \tag{4}$$

$$WSI\ (\%) = \frac{weight\ of\ evaporation\ residue\ (g)}{dry\ sample\ weight\ (g)} * 100 \tag{5}$$

where: WAI is the water absorption index, WSI is the water solubility index.

2.4.2. Oil retention capacity

The oil retention capacity (ORC) was determined [27]. For the analysis, 2.5 g of the sample was weighed into 50 mL Falcon® tubes, and 5 mL of soybean oil at 25 °C was added. The tubes were intermittently homogenized and then centrifuged at 2200 rpm for 15 min. The supernatant was removed by inverting the tube, and the result was expressed as g of oil retained per g of sample. The calculations were performed according to Eq. 6.

$$ORC = \frac{oil\ absorbed\ by\ the\ sample\ (g)}{dry\ sample\ weight (g)} \tag{6}$$

where: ORC is the oil retention capacity in g oil/g sample.

2.4.3. Pasting properties of extruded flour, rice and rice mixed with seaweed, and paste hardness

The pasting properties were analyzed using a Rapid Visco-Analyzer (RVA-4500 model from Perten Instruments, Warriewood, Australia) with the software Thermocline for Windows version 3.17.5.515 to determine the pasting properties of the samples using the analysis program *Extrusion 1* [46]. A sample of 3.5 g (adjusted to a 14 % moisture basis) and 25 mL of distilled water were placed in aluminum containers specific to the equipment and attached to the RVA through a stirring propeller, where they were agitated throughout the experiment (20 min). All determinations were performed in triplicate, and the evaluated parameters were cold peak (cP), hold peak (cP), breakdown (cP), final peak at 25 °C (cP), setback (cP) and time peak (min).

For hardness, the pastes resulting from the RVA analysis were kept in the same aluminum cup, covered with PVC film, and stored at 7 °C for 24 h, after the samples were kept at room temperature for about 1 h before the analysis, and the hardness of the cooled paste was determined using a texture analyzer TA-XTplusC 650H texture analyzer (Stable Micro Systems, Haslemere, GBR) with a 50 kg load cell, using a 25 mm diameter acrylic cylindrical probe. The analysis was performed in triplicate, and the results were expressed in Newtons (N).

2.4.4. Differential scanning calorimetry

Thermal properties were determined using Differential Scanning Calorimetry (DSC) (Mettler Toledo, DSC1, Schwerzenbach, Switzerland). 2.5 mg of seaweed samples (R, R + SD, R + SL, and R + SC) were collected using a metal microspatula, and about 7.5 g of distilled water were weighed into Al crucibles (40 μL). The crucibles were sealed with a lid and individually taken to the equipment for analysis. The samples were heated from 35 to 120 °C at a heating rate of 10 °C/min under an inert N2 atmosphere at a flow rate of 80 mL/min.

Table 2
Techno-functional properties of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) extrudates.[1]

Properties	R	R + SD	R + SL	R + SC
Extrusion process				
Torque (%)	49.55 ± 0.71^{a}	50.66 ± 1.41^{a}	47.66 ± 0.71^{a}	38.00 ± 4.95^{b}
SME (W.h/kg)[2]	146.26 ± 3.02^{a}	151.42 ± 4.29^{a}	123.46 ± 5.62^{b}	119.60 ± 1.40^{b}
Extruded products				
Moisture (%)	5.35 ± 0.05^{d}	7.27 ± 0.21^{a}	6.05 ± 0.03^{c}	6.67 ± 0.15^{b}
Protein (g/100 g)[3]	8.87 ± 0.04^{b}	9.47 ± 0.32^{a}	9.43 ± 0.11^{a}	9.34 ± 0.12^{a}
Expansion index	2.79 ± 0.27^{a}	2.56 ± 0.18^{bc}	2.63 ± 0.11^{ab}	2.43 ± 0.08^{c}
Bulk density (g/cm^{-3})	0.46 ± 0.08^{a}	0.40 ± 0.04^{b}	0.40 ± 0.03^{b}	0.45 ± 0.03^{a}
Hardness (N)	21.85 ± 11.76^{b}	60.15 ± 10.52^{a}	30.33 ± 11.71^{b}	25.34 ± 11.87^{b}
Cross-section				
Porosity (%)	39.02 ± 18.75^{ns}	40.54 ± 9.47^{ns}	44.36 ± 12.11^{ns}	31.96 ± 16.14^{ns}
Area (mm^{2})	135.56 ± 10.86^{a}	99.14 ± 4.73^{b}	104.87 ± 8.90^{b}	87.48 ± 1.95^{c}
Perimeter (mm)	44.10 ± 1.88^{a}	37.80 ± 1.18^{c}	39.45 ± 1.60^{b}	35.85 ± 0.81^{d}
Circularity	0.87 ± 0.02^{ns}	0.87 ± 0.02^{ns}	0.84 ± 0.04^{ns}	0.85 ± 0.02^{ns}
Diameter (mm)	13.22 ± 0.58^{a}	11.31 ± 0.31^{c}	11.80 ± 0.51^{b}	10.71 ± 0.40^{d}

[1] The results are presented as mean ± standard deviation. Means followed by different letters in the same row differ significantly from each other according to the Scott-Knott multiple comparison test ($p < 0.05$). ns = not significant. [2]SME = specific mechanical energy. [3]The protein content was calculated considering the ready-to-eat sample (6 % moisture).

2.4.5. X-ray diffraction

X-ray diffraction data were collected using an Empyrean diffractometer (Panalytical) with CuKα radiation (λ = 1.54056 Å), operating at an acceleration voltage of 40 kV and a current of 40 mA. Measurements were performed over an angular range of 5° to 60° (2θ) with steps of 0.01313° and a time of 300 s per step. X-ray photon detection was carried out using a PIXcel3D-Medipix3 1 × 1 area detector.

2.4.6. Antioxidant capacity and bioactive compounds

The extruded flour samples were solubilized with methanol (1 mg/mL) and subjected to analysis of DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS+ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging capacity, FRAP (Ferric Reducing Antioxidant Power), and total phenol content (TPC), based on methodologies with slight modifications for microplate reading [28].

For the DPPH assay, 10 μL of the sample was added to 190 μL of the DPPH solution (100 μM) and incubated in the dark for 30 min. The absorbance was then read using a microplate reader at 515 nm (Epoch 2, Agilent BioTek, Santa Clara, CA, USA). A Trolox standard curve ranging from 100 to 2000 μM was prepared ($y = -0.0004x + 0.7353$, $R^2 = 0.9965$). In the ABTS+ assay, the sample was added to the ABTS+ solution at a 1:10 ratio (*v*/v) and incubated in the dark for 6 min. The absorbance was then read using a microplate reader at 725 nm (Epoch 2, Agilent BioTek, Santa Clara, CA, USA). A Trolox standard curve ranging from 125 to 2000 μM was prepared ($y = -0.0003x + 0.7344$, $R^2 = 0.9997$). The results of the radical scavenging capacity assays were expressed in μM Trolox Equivalents.

The FRAP assay was performed using the previously prepared FRAP reagent by adding acetate buffer (0.3 M, pH = 3.6), TPTZ (2,4,6-tripyridyl-*s*-triazine) solution (10 mM), and $FeCl_3 \cdot 6H_2O$ (20 mM) in a 10:1:1 ratio, according to the method of Pulido et al. [47]. In a microplate, a 9 μL aliquot of the sample was mixed with 27 μL of ultrapure water and added to 270 μL of the FRAP reagent. The microplate was incubated for 30 min at 37 °C, and the absorbance was measured at 595 nm using a microplate reader (Epoch 2, Agilent BioTek, Santa Clara, CA, USA). The results were obtained using the regression equation from the standard curve of ferrous sulfate ranging from 250 to 2000 μM ($y = 0.0009x - 0.1664$, $R^2 = 0.9900$) and expressed in μM Fe (II)/g of extract.

The TPC of seaweed was determined using a modified Folin-Ciocalteu method [29]. For the assay, the sample was added to a 1:1 reaction mixture of Folin-Ciocalteu reagent and sodium bicarbonate (6 %), kept in the dark for 90 min, and then analyzed using a microplate reader at 750 nm (Epoch 2, Agilent BioTek, Santa Clara, CA, USA). A gallic acid standard curve ranging from 31.2 to 1000 μg/mL was prepared ($y = 0.0029x + 0.2373$, $R^2 = 0.9938$), and the results were expressed in mg of gallic acid equivalents per gram of sample (mg GAE/g).

2.5. Color of the formulations, extrudates, and pre-gelatinized flours

The color analysis of the extrudates was performed by two methods: computer image and by colorimeter:

- Firstly, it was conducted through computer images, adapting the protocol detailed by Ayustaningwarno et al. [30] with modifications to suit extruded products described below: The extruded (12 pieces from each trial) were placed on a white background under a 5400 K lightbox. Images were captured using a 24.2 MP (megapixels) color digital camera (Sony α6000 with 16–50 mm lens, f/3.5–5.6, at 50 mm), mounted 25 cm from the samples, set to ISO-100, with an aperture of f/6.3, and an exposure time of 1/80s. Color calibration, white balance adjustment, and background removal were performed in Adobe Photoshop 2020, and the resulting images were saved in TIFF format. The saved images were then processed using ImageJ software, supplemented by the Color Inspector 3D plugin. The region of interest was outlined with the Color Threshold tool.
- Second, color was also determined using the HunterLab UltraScan PRO colorimeter (Hunter Associate Laboratory Inc. Reston, USA) operating with illuminant D 65 and a 10° observation angle. The CIELab system (L^*, a^*, and b^*) was used, where the variable L^* indicates lightness, distinguishing light colors from dark ones (where: 0 – black and 100 – white), and chromaticity a^* and b^* provide color information (where: - a^* represents the direction toward green and + a^* toward red, and - b^* represents the direction toward blue and + b^* toward yellow). The values L^*, a^*, and b^* were converted to RGB using the Nix Color Sensor program. The color difference (ΔE) between the samples was calculated using Eq. 7, where "0" indicates the values of the extrudates made solely from R.

$$\Delta E = \sqrt{(L1^* - L2^*)^2 + (a1^* - a2^*)^2 + (b1^* - b2^*)^2} \quad (7)$$

where: ΔE is the color difference, $L1^*$, $a1^*$, $b1^*$ are the values of the reference sample (in this case, the R sample), and $L2^*$, $a2^*$, $b2^*$ are the values of the evaluated sample.

2.6. Statistical analysis

The analysis of variance (ANOVA) of the means was performed using

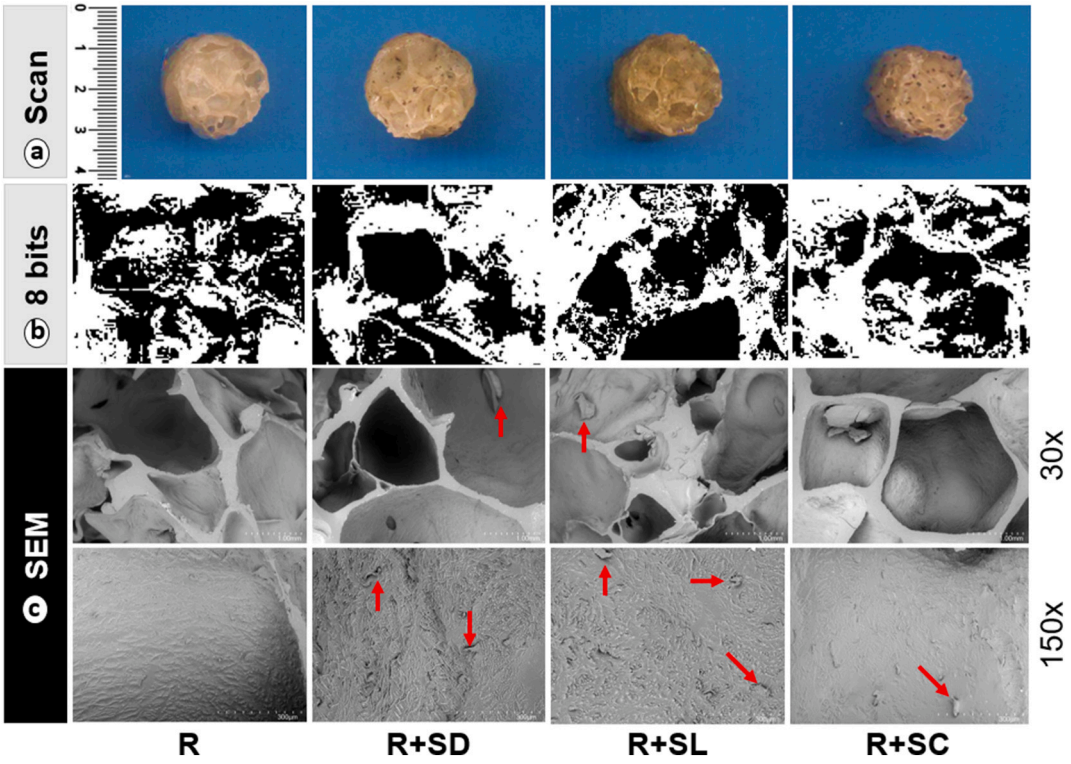

Fig. 3. Cross-sectional images digitized and presented with scale in cm (A), 8-bits[1] porosity area (B), and SEM captures at 30× (C) and 1500× (D) magnifications of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) extruded products. Red arrows highlight cell wall details, emphasizing irregular points on the wall.
Where: [1]bits are the basic unit of digital information, with 8 bits representing that each pixel can have 256 different values (2^8). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Sisvar software version 5.6 (Federal University of Lavras, Lavras, MG, Brazil) with a significance level of 95 %. When significant, the *Scott-Knott* test was used to determine statistical differences between the means ($p \leq 0.05$).

3. Results and discussion

3.1. Development process of rice and seaweed flour extrudates

The composition of SD, SL, and SC seaweed was determined in a previous study by Tagliapietra et al. [6] and presented, on a dry basis (db), 15.47, 16.81, and 13.65 % protein; 3.32, 3.98, and 2.38 % lipids; 17.60, 13.04, and 19.05 % ash; 72.5, 78.42, and 77.91 % dietary fiber, respectively. R was analyzed for this study and presented, on a db, 8.6 % protein, 0.31 % ash, 1.55 % lipids, and 78.6 % available carbohydrates.

Studies by Gressler et al. [31] and Lorenzo et al. [32] indicated that seaweed proteins have more balanced amino acid profiles, including significant amounts of essential amino acids such as leucine, lysine, and threonine, which may contribute to improving the biological value of rice protein, whose limiting amino acids are lysine and tryptophan [33].

Regarding the extrusion process, Torque and SME were analyzed, as shown in Table 2. A significant decrease in torque was observed only in the process containing R + SC. It is likely that factors such as fiber composition or the specific interaction between the constituents of the commercial seaweed and the rice matrix contributed to this effect. The SME values (Table 2) of R and R + SD extrudates showed no significant differences. However, R + SL and R + SC exhibited a significant decrease, as these seaweeds had the highest fiber concentrations in common. Although the seaweed substitution was only 1.5 %, the mineral (ash) and fiber contents may have impacted the reduction in torque and SME, suggesting that SL and SC may be advantageous for industrial-scale processing, as they could lead to lower energy consumption in the process. The results of Sampaio et al. [34] support this study regarding the effects of fibers and minerals on the thermoplastic extrusion process.

3.2. Physicochemical characterization of rice and seaweed flour extrudates

Regarding the characteristics of the extrudates (Table 2), R showed the lowest moisture and protein contents, which was expected since seaweed contained higher amounts of protein and fiber than rice. Therefore, fiber and protein may have contributed to moisture retention during the dehydration stage of the extruded products.

The EI (Table 2) was higher in R and R + SL, while EI and bulk density were higher in the extrudates R and R + SL and R and R + SC, respectively. The hardness of the extrudates was highest for R + SD. Thus, it can be stated that differences in composition, origin, and drying methods of the seaweeds affected the physicochemical properties of the rice extrudates, highlighting the importance of the ingredient production process for its application in thermoplastic extrusion.

The addition of seaweed to food products often increases the hardness of the food matrix due to its significant content of structural fibers, especially cellulose [35], which contribute to the formation of more rigid structures, resulting in more compact products with greater resistance to deformation, in other words, harder products. In addition to cellulose, brown seaweed contains alginate, which increases dough cohesion and contributes to a firmer texture [36]. Mamat et al. [37] demonstrated that the inclusion of seaweed flour (2–8 %) increased the firmness of bread crumb, attributing this to the formation of thicker cell walls and the water retention capacity of the seaweed hydrocolloid polysaccharides.

The data analysis for cross-section (Table 2) showed that porosity and circularity did not present significant differences between R and the other extrudates containing R and seaweed. However, regarding area,

Table 3
Water Absorption Index (WAI), Water Solubility Index (WSI) and Oil Retention Capacity (ORC) of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) of pre-gelatinized flours.[1]

Properties	R	R + SD	R + SL	R + SC
WAI	6.86 ± 0.24^{b}	6.79 ± 0.14^{b}	7.43 ± 0.11^{a}	6.73 ± 0.38^{b}
WSI	21.61 ± 0.92^{b}	24.95 ± 0.14^{a}	22.63 ± 0.83^{b}	21.13 ± 1.38^{b}
ORC	1.67 ± 0.07^{a}	0.88 ± 0.06^{b}	0.84 ± 0.05^{b}	0.82 ± 0.02^{b}

[1] The results are presented as mean ± standard deviation. Means followed by different letters in the same row differ significantly from each other according to the Scott-Knott multiple comparison test ($p < 0.05$).

perimeter, and diameter, R exhibited the highest values, indicating that seaweed flour, regardless of origin and drying process, contributed to producing extrudates with a smaller area and more heterogeneous structure.

The microstructure of the extrudates showed a large area (white part of the 8-bit image) and low porosity (bold part of the 8-bit image), without statistical significance, as demonstrated in Fig. 3, which presents photos of the extrudates, the 8-bit image, and the SEM.

Overall, the extrudates appear visually similar, with relatively thin, irregular, and continuous walls, without differentiation between macronutrients such as starch and proteins. R had a more homogeneous and smoother surface compared to R + SD, R + SL, and R + SC, which exhibited small roughness and some more heterogeneous points. The presence of visible rough areas in the formulations containing seaweed may be associated with the structural characteristics of the raw material, such as the presence of fibers, proteins, and minerals, which hinder the formation of a continuous and expanded starch matrix during the extrusion process.

3.3. Techno-functional characterization of pre-gelatinized rice and seaweed flours

Thermoplastic extrusion is highly versatile, as it can produce not only expanded products but also pre-gelatinized flours, which are obtained after drying the extrudates and milling them into flour. These flours can be used as thickeners in soups, porridges, and sauces, making the properties of WSI, WSI and ORC important for guiding their application.

The techno-functional properties WSI, WAI, and ORC of the pre-gelatinized flours are presented in Table 3. Regarding WAI, the highest value was observed for R + SL, which was significantly higher than the other samples. The higher WAI in R + SL is probably associated with the preservation of structure and porosity during the freeze-drying process. Only R + SD showed a significant increase in WSI. R exhibited the highest ORC value while all formulations containing seaweed showed marked reductions. The decrease in ORC indicates that the addition of seaweed reduced the system's affinity for the oil phase. This may be related to the higher presence of fibers, which compete for binding sites with lipids, and to the relative reduction of starch derived from rice.

Table 4 presents the pasting properties of the formulations and the pre-gelatinized flours obtained by extrusion, including R and R combined with SD, SL, and SC. Pasting properties allow the evaluation of processing impacts and how effectively they modify starch [48]. For the formulations (Table 4), there were no significant differences in RVA values for cold peak, hold peak, peak time, and peak area between R and the other flours containing S, indicating that the paste behavior was predominantly influenced by the gelatinization of rice starch.

All pre-gelatinized flours (Table 4) showed initial viscosity when dispersed in water at room temperature, which is characteristic of already gelatinized ingredients. As expected for this type of material, a profile without the typical gelatinization peak of native starches was observed, along with a tendency for viscosity reduction throughout the analysis, possibly due to starch polymer fragmentation during extrusion.

O gráfico obtido por RVA pode ser encontrado no Material Suplementar 2 (RVA curves). The extrusion process alters the structure of macromolecules such as starches and proteins due to the high temperatures and pressures involved [38].

Fig. 4 presents the DSC thermogram, showing the thermal transition curves of the materials. R contains starch, whose gelatinization is a critical factor in extrusion. The analysis results demonstrated that the initial transition temperature (T_o onset) of sample R was 58.86 °C, with a

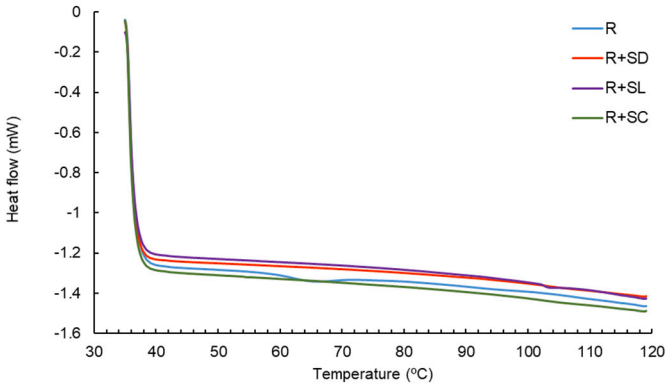

Fig. 4. DSC thermograms of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) of pre-gelatinized flours.

Table 4
Paste properties by Rapid Vysco Analyzer, and hardness of gel of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) of pre-gelatinized flours.[1]

Samples	Cold peak (cP)	Raw peak (cP)	Breakdown (cP)	Final peak (cP)	Setback (cP)	Peak time (min)	Hardness of the cooled paste (N)
Formulation							
R	82.00 ± 1.73^{ns1}	4975.33 ± 6.47^{a}	1266.33 ± 9.48^{a}	9873.33 ± 9.30^{a}	6164.33 ± 1.42^{a}	8.24 ± 0.04^{ns}	9.13 ± 0.06^{a}
R + SD	78.66 ± 6.17^{ns}	4437.00 ± 2.86^{b}	796.33 ± 2.61^{b}	9087.00 ± 4.67^{c}	5446.33 ± 4.67^{b}	8.15 ± 0,10^{ns}	7.99 ± 0.31^{b}
R + SL	63.00 ± 5.08^{ns}	5040.33 ± 7.14^{a}	1361.00 ± 1.50^{a}	10,003.66 ± 2.77^{a}	6324.33 ± 2.77^{a}	8.09 ± 0,03^{ns}	8.81 ± 0.14^{a}
R + SC	67.33 ± 5.81^{ns}	4922.00 ± 8.04^{a}	1109.00 ± 3.64^{a}	9643.33 ± 4.04^{b}	5830.33 ± 4.04^{b}	8.06 ± 0,07^{ns}	8.47 ± 0.54^{b}
Pregelatinized flours							
R	1123.33 ± 9.81^{b}	1027.00 ± 3.51^{b}	905.33 ± 7.75^{b}	644.66 ± 5.50^{b}	523.00 ± 5.50^{b}	2.07 ± 0,00^{ns}	2.12 ± 0.20^{ns}
R + SD	1314.66 ± 5.48^{a}	1269.00 ± 7.33^{a}	1148.33 ± 5.35^{b}	641.66 ± 2.55^{b}	521.00 ± 2.55^{b}	2.11 ± 0.03^{ns}	1.63 ± 0.08^{ns}
R + SL	1280.33 ± 2.35^{a}	1179.00 ± 7.35^{a}	991.66 ± 2.15^{a}	822.66 ± 2.03^{a}	635.33 ± 2.03^{a}	2.35 ± 0.34^{ns}	2.18 ± 0.17^{ns}
R + SC	1128.00 ± 7.83^{b}	945.33 ± 9,72^{b}	777.66 ± 2.16^{a}	802.00 ± 8.59^{a}	634.33 ± 8.59^{a}	2.17 ± 0.08^{ns}	1.94 ± 0.41^{ns}

[1] Results are presented as mean ± standard deviation. Means followed by different letters in the same column differ significantly from each other by the *Scott-Knott* multiple comparisons test ($p < 0.05$), where formulations were compared with each other and extrudates were also compared with each other separately. ns = not significant.

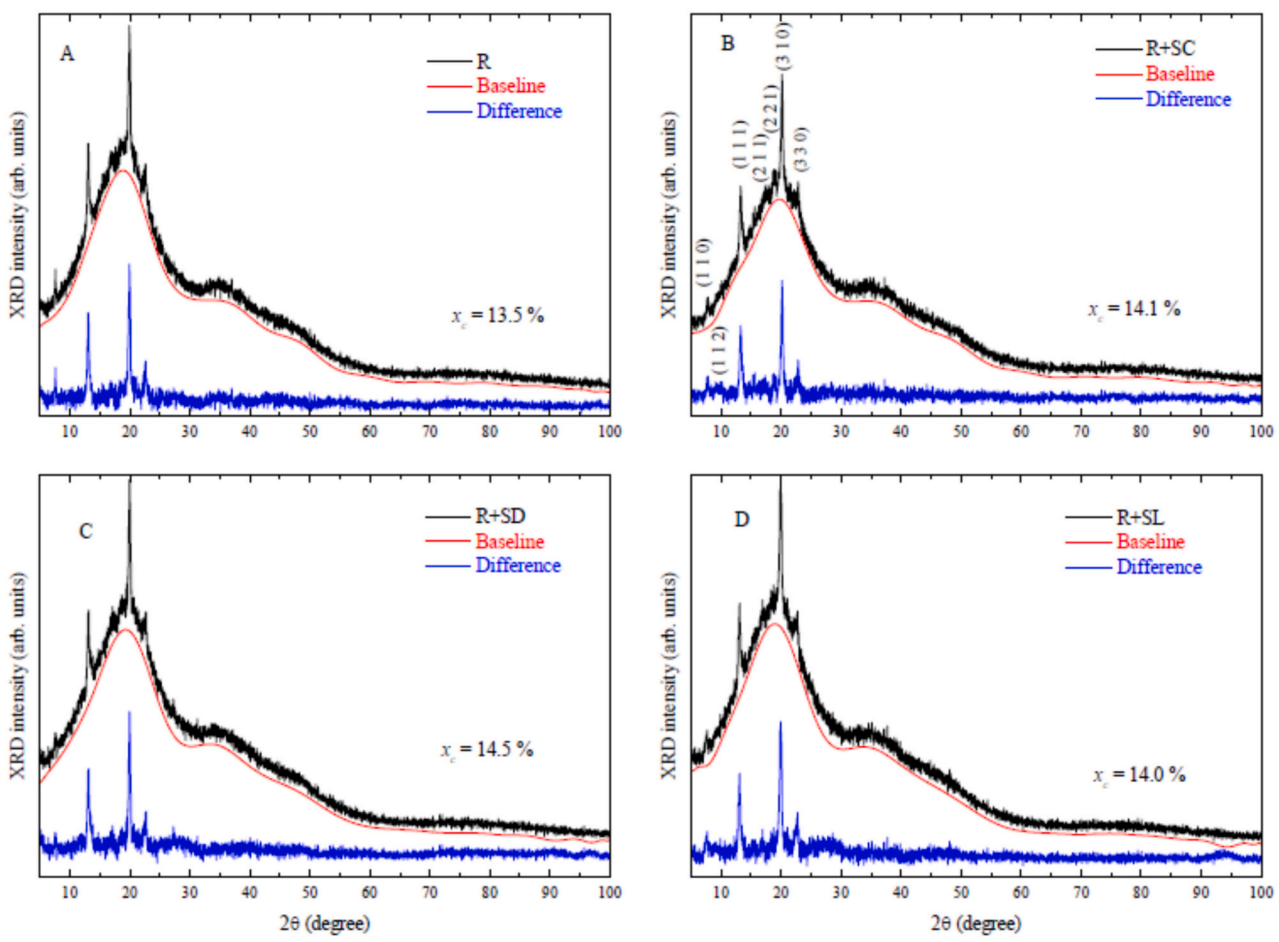

Fig. 5. X-ray diffraction patterns of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC).

peak at 65.2 °C. The recorded enthalpy value (ΔH = 1.13 J/g) corresponds to an endothermic process, as expected for starch gelatinization. It is noted that the software setting (exothermic up) resulted in the graphical representation of the peak pointing upwards, but the physical phenomenon corresponds to heat absorption.

It was expected that the presence of seaweed, due to its different composition from starch, would alter the starch structure and/or modify its thermal properties. In the graph (Fig. 4), it can be observed that no peaks were detected in the seaweed-containing samples (R + SD, R + SL, and R + SC), and they exhibited similar behavior. These findings may be related to the significant amount of dietary fibers and minerals, whose thermal resistance may be higher than the temperature used in the DSC analysis, which was up to 130 °C. Future studies should include the thermal analysis of the raw mixtures, which may help to clarify whether the components of *S. filipendula* interfere with the thermal transition of intact starch granules.

The results of the techno-functional properties indicate that it is possible to obtain pre-gelatinized R with 1.5 % substitution by seaweed flours, resulting in ingredients with initial viscosity and functional properties that may be useful as stabilizers or bodying agents in ready-to-eat foods. However, due to the loss of swelling capacity and the absence of a viscosity peak during heating, these flours are not suitable as traditional thickeners that require thermal gelatinization during food preparation. Furthermore, future studies can be conducted to adjust the properties of these ingredients as needed for different products, ranging from baked goods to salad dressings.

The results of the X-ray diffraction patterns are shown in Fig. 5 for R, R + SD, R + SL, and R + SC flours. Previous studies have demonstrated that X-ray diffraction revealed the presence of type I cellulose, KCl crystals, and traces of SiO_2 in commercial *Sargassum* [6]. However, in the case of the pre-gelatinized flours containing seaweed, no evident signals of these phases were detected, possibly due to the overlap of intense diffuse scattering, associated with amorphous material, and narrow crystalline peaks located at 2θ ≈ 7.6°, 13.2°, 19.9°, and 22.7°.

The diffuse scattering was modeled using Chebyshev polynomials (red lines in Fig. 5), representing the amorphous fraction of the material. The remaining crystalline phase was identified as type V starch, with an orthorhombic structure and space group $P2_1\ 2_1\ 2_1$. To determine the relative crystalline (Xc), the ratio between the areas corresponding to the crystalline and amorphous phases was calculated, following the methodology described by Pinto et al. [39]. The obtained values indicate that, in all samples analyzed, crystallinity was approximately 14 % type V starch and 86 % amorphous phase.

To highlight the peaks associated with the crystalline phase, the experimental diffractograms were subtracted from the diffuse scattering, resulting in the blue profiles presented in Fig. 5. In Fig. 5B, the Miller planes attributed to the narrowest and most evident peaks, such as (1 1 2), (2 1 1), (2 2 1), and (3 3 0), are highlighted, as well as other more subtle ones that become clearer in the difference between the curves.

The extrusion process may play an important role in the preferential orientation of crystals, due to the stretching of fibers and molecules during processing. This crystallographic texture effect was observed in all flours, including the control (Fig. 5A), reinforcing the influence of extrusion on the structural organization of the analyzed materials.

Table 5 presents the results of antioxidant capacity (DPPH, ABTS,

Table 5
Antioxidant capacity and bioactive compounds of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) of pre-gelatinized flours.[1]

Samples	Antioxidant capacity			Bioactive compounds
	DPPH[2] (μmol TE/g)	ABTS[3] (μmol TE/g)	FRAP[4] (μmol TE/g)	TPC[5] (mg GAE/g)
R	118.08 ± 5.77[c5]	301.11 ± 10.72[a]	337.85 ± 2.57[d]	nd[6]
R + SD	98.08 ± 5.20[d]	194.44 ± 11.71[c]	356.37 ± 3.57[b]	nd
R + SL	147.25 ± 6.61[a]	166.67 ± 8.82[d]	347.85 ± 2.80[c]	nd
R + SC	128.08 ± 5.20[b]	264.44 ± 11.71[b]	366.37 ± 3.90[a]	nd

[1] The results are presented as mean ± standard deviation. Means followed by different letters in the same row differ significantly from each other according to the *Scott-Knott* multiple comparison test ($p < 0.05$).
[2] DPPH = 2,2-diphenyl-1-picrylhydrazyl.
[3] ABTS = 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).
[4] FRAP = Ferric Reducing Antioxidant Power.
[5] TPC = Total Phenol Content.
[6] nd = not detected.

FRAP) and total phenolic content (TPC) of pre-gelatinized R with seaweeds. The R + SL flour exhibited the highest DPPH radical scavenging capacity, which can be attributed to the effective preservation of antioxidant compounds during the lyophilization process [21,40]. The R flour showed the highest ABTS value, while the R + SC flour demonstrated the highest ferric reducing power (FRAP). Total phenolics were not detected (nd) in any of the flours, indicating that the phenolic concentration was below the detection limit of the method used. These variations may be linked to the origin of the seaweed, dehydration conditions, and the thermal processing by extrusion, which could result from the combination of several factors.

3.4. Color of the formulations, extrudates, and pre-gelatinized flours

Fig. 6 shows the color analysis by computational imaging of the R, R + SD, R + SL, and R + SC formulations (Fig. 6A), as well as their extrudates (Fig. 6B) and pre-gelatinized flours (Fig. 6C).

The formulations (Fig. 6A) showed visible differences in color distribution. The R formulation exhibited greater uniformity and a light-yellow tone, which is typical of flour derived from polished white rice.

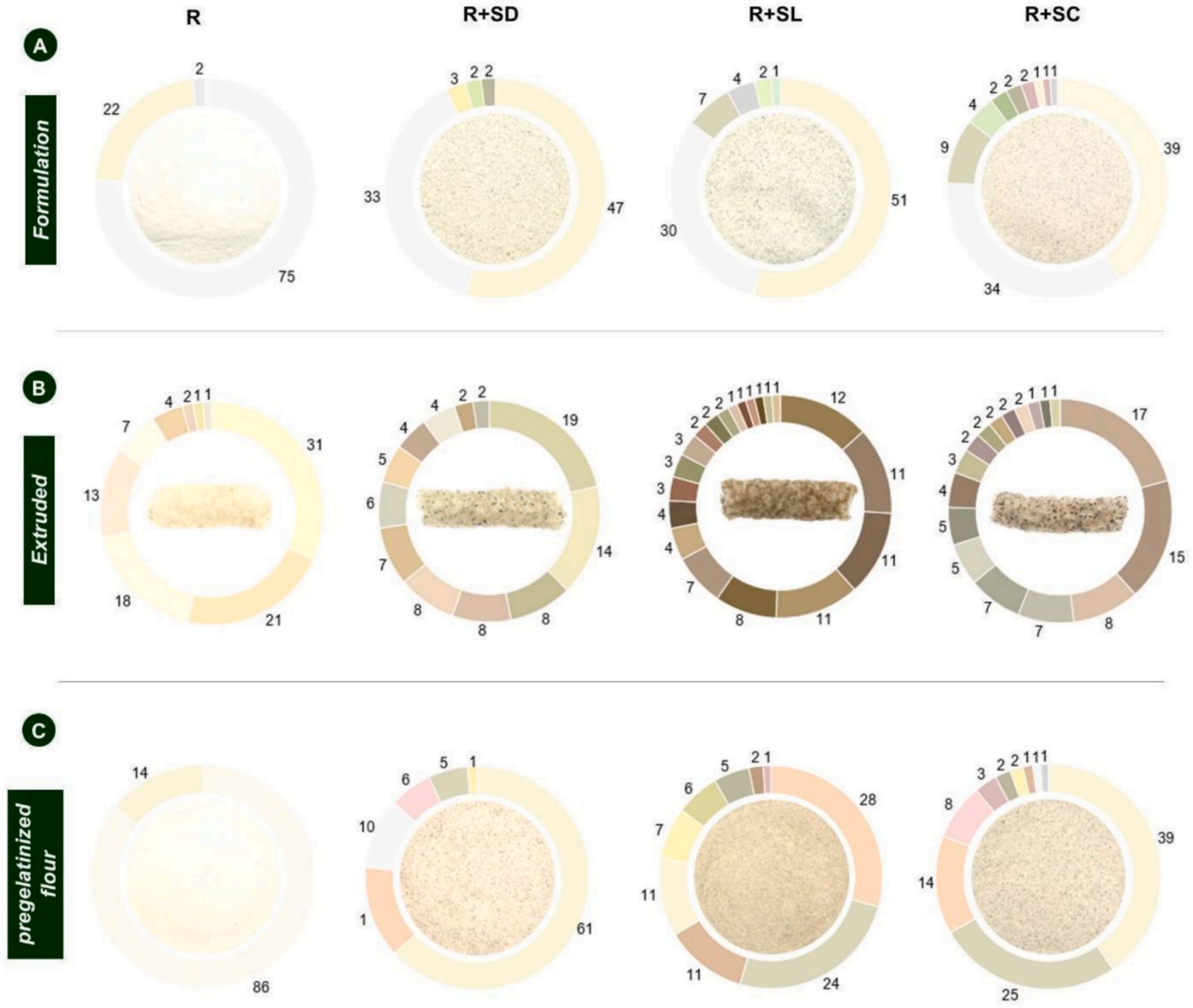

Fig. 6. Color obtained by imaging of rice flour (R) and rice flour substituted of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC) where A are the formulations before extrusion, B and C are after extrusion, B are the extruded products, and C are pregelatinized flours.

Table 6
Color obtained by the colorimeter and expressed by the CIELab System of the formulations before extrusion, the extruded products and pregelatinized flour of rice flour (R) and rice flour added of *Sargassum filipendula* oven drying (R + SD) and lyophilized (R + SL) and commercially *Sargassum* sp. (R + SC).[1]

Samples	R	R + SD	R + SL	R + SC
Formulation flours				
*L**	94.33 ± 0.03^{a}	85.58 ± 0.22^{b}	83.21 ± 0.17^{c}	83.15 ± 2.38^{c}
*a**	−0.38 ± 0.07^{b}	−2.09 ± 0.06^{d}	−1.58 ± 0.08^{c}	+0.83 ± 0.02^{a}
*b**	+4.45 ± 0.66^{c}	+10.51 ± 0.09^{b}	+12.32 ± 0.09^{a}	+9.86 ± 0.28^{b}
Extruded products				
*L**	57.69 ± 2.25^{a}	48.43 ± 1.58^{b}	35.31 ± 0.94^{d}	40.24 ± 1.56^{c}
*a**	+0.17 ± 0.04^{d}	+0.54 ± 2.57^{c}	+1.98 ± 0.13^{a}	+0.96 ± 0.03^{b}
*b**	+11.03 ± 0.63^{b}	+10.71 ± 0.10^{b}	+15.39 ± 0.38^{a}	+8.64 ± 0.12^{c}
Pre-gelatinized flours				
*L**	89.89 ± 0,12^{a}	81.60 ± 0,77^{b}	76.97 ± 0.11^{d}	80.20 ± 0.23^{c}
*a**	+0.17 ± 0.02^{d}	+0.26 ± 0.52^{c}	+0.69 ± 0.05^{b}	+1.03 ± 0.18^{a}
*b**	+9.51 ± 0.02^{c}	+12.57 ± 0.52^{b}	+14.26 ± 0.05^{a}	+9.83 ± 0.18^{c}

[1] The results are presented as mean ± standard deviation. Means followed by different letters in the same line differ significantly by the *Scott-Knott* multiple mean comparison test ($p < 0.05$).

On the other hand, the R + SD, R + SL, and R + SC formulations displayed greater chromatic diversity, with an increase in darker shades.

In the extrudates (Fig. 6B), the R extrudate had a predominantly white color with little variation, whereas the R + SD, R + SL, and R + SC extrudates exhibited a predominantly brown color. Notably, in R + SL, light brown and greenish tones appeared. Since chlorophyll is more sensitive to heat and is present in relatively lower concentrations than fucoxanthin, lyophilized seaweed formulations tend to present greener hues, as demonstrated in studies by Park et al. [41] and Zhao et al. [42]. The drying method significantly affects the color of seaweed flours, with lyophilization preserving the original color of raw materials, resulting in a lighter and more yellowish appearance [6,40].

In the pre-gelatinized flours (Fig. 6C), the R flour had a white color, with 86 % of its area in the lightest shade, whereas the R + SD, R + SL, and R + SC flours exhibited greater chromatic variation, ranging from slightly yellowish tones to light beige shades. Thus, image analysis using ImageJ software proved to be efficient in distinguishing colors at the three processing stages: in the formulations as ingredients, in the extrudates, and in the pre-gelatinized flours.

Analyzing the color using the CIELab system (Table 6), significant variations in *L**, *a**, and *b** values were observed among the raw flours. The *L* * was highest for R (94.33), indicating a lighter color. The addition of *S. filipendula* significantly reduced the *L** in the raw R + SD, R + SL, and R + SC flours. In the extrudates, *R + SL* had the highest *L** (48.73), indicating a lighter color compared to the other seaweed-containing samples (R + SD and R + SC), which was also confirmed in the computational image analysis (Fig. 6B).

For the pre-gelatinized flours (Table 6), the addition of seaweed flour to R resulted in darker shades, reducing *L** and significantly increasing the tendency toward yellow *(+b*)* and red *(+a*)*. This color distinction between the formulations and the R-only flour highlights the influence of seaweed incorporation. Given the complexity of color reactions that may have formed, this analysis, combined with image analysis, can guide future studies on the main color modifications occurring due to the processing steps of the product.

4. Conclusion

The choice of seaweed drying method is important to optimize the functional and bioactive properties of the final products, with lyophilization showing greater effectiveness in preserving antioxidant compounds. Additionally, the incorporation of *S. filipendula* influenced physicochemical parameters such as protein content and extrudate hardness, as well as structural and color characteristics. The formulations also showed variations in techno-functional properties, such as water absorption capacity and paste formation, as observed in the RVA and texture analyses. This study demonstrated that there is a promising field of research to deepen the understanding of the interactions between rice and seaweed flours, with notable modifications observed during processing, in both the extrudates and pre-gelatinized flours. Factors such as seaweed composition, dehydration method, and extrusion conditions can influence the results obtained. The most relevant aspect is the feasibility of developing new rice- and seaweed-based products, promoting their consumption and expanding the market for ingredients with functional potential and positive impact on coastal populations.

Additionally, this research contributes to promoting sustainable food development and improving nutritional quality by exploring alternative and nutritious sources such as seaweeds. Future research directions include optimizing the proportion between seaweed and rice, as well as exploring other processing technologies that may enhance the advantages of this combination, increasing its functional value and applicability in the food market.

CRediT authorship contribution statement

Bruna Lago Tagliapietra: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gustavo do Nascimento Costa:** Methodology, Investigation. **Rebeca Salvador-Reyes:** Writing – original draft, Methodology, Investigation. **Elisabeth Harumi Nabeshima:** Writing – review & editing, Methodology, Conceptualization. **Jaqueline de Araújo Bezerra:** Methodology, Investigation. **Josiana Moreira Mar:** Methodology, Investigation. **Edgar Aparecido Sanches:** Supervision. **Maria Teresa Pedrosa Silva Clerici:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation. **Camila Costa Pinto:** Writing – original draft, Methodology, Investigation. **Sergio Michielon de Souza:** Writing – original draft, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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