ACS FOOD SCIENCE & TECHNOLOGY

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Article

Designing Fortified Rice-Based Analogues for the Elderly Population

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Cite This: *ACS Food Sci. Technol.* 2026, 6, 732−745

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ABSTRACT: Population aging is a growing global phenomenon, and Latin America has a habit of consuming rice with beans that present adequate amino acid balance. Vitamin D and magnesium are essential nutrients at this stage of life. However, deficiencies are common due to low intake and limited sun exposure. Food fortification by using rice analogue emerges as an effective solution to resolve these nutritional deficiencies. This study aimed to obtain a rice analogue made from rice and cowpea enriched with vitamin D and magnesium using cooking and cold extrusion. Rice flour (RF), bean flour (BF), extruded rice flour (ERF), and emulsified extruded rice flour (EERF) were obtained by milling and thermoplastic extrusion and used in the pasta-making process. The analogues (T1, T2, T4, T7, $T7^+$, T8, T9, $T9^+$) showed 7.29−7.91 mm length, 2.49−2.76 mm width, and an L/W average ratio in the range of 2.64−3.10, thus classified as long grain. The cooking time was shorter (6−12 min) than that of control rice (14 min), presenting weight increases of 59−152 and 5.08−15.95% soluble solid loss. The TPA texture profile revealed 8.72−46.48 N hardness, 2.50−12.94 of chewiness, and 2.78−26.65 gumminess, comparable to those of polished rice. The color measurements showed a whiteness index (W.I.) of 83.06−90.45, similar to the control (93.19). Regarding pasting properties, analogues showed 477.33−683.66 cP peak viscosity (PV), 47.33−399 cP breakdown viscosity (BDV), and 474−1762 cP setback viscosity (SBV). As expected, all analogues showed a decrease in gelatinization parameters compared to the control (polished rice) since extruded flours were used in the eight formulations. The retrogradation tendency of analogues is an important differentiating advantage, since Brazilian consumers prefer loose rice. In addition, the gelatinization temperature measured by differential calorimetry varied from 78.79 to 86.04 °C, which is similar to that of polished rice (83.01 °C). In terms of proximate composition, the rice analogues with addition of the vitamin−mineral mix showed 7.65% proteins, 0.81% dietary fiber, and 187.14 J/g energy value/50 g portion. Finally, the analogues ($T7^+$, $T9^+$) are classified as "rich in vitamin D and magnesium", as they provide a final concentration of 4.53 μ/100g of vitamin D, 278 mg/100g of Mg, and for up to 4 months, there was no significant difference in vitamin D loss ($p < 0.05$). Furthermore, the $T9^+$ formulation is the most similar to the control (polished rice) in terms of hardness (8.72 and 10.26 N), cohesiveness (0.48 and 0.44), and gumminess (4.21 and 4.51), and the technological qualities of the analogues were improved by the addition of EERF.

KEYWORDS: *beans, vitamin D, magnesium, extrusion, dysphagia-friendly food, aging*

1. INTRODUCTION

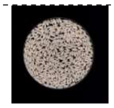
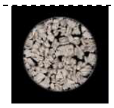
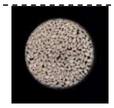
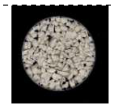
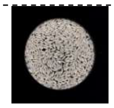
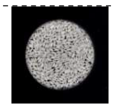
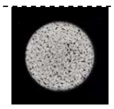
Population aging is a global and demographic phenomenon of the 21st century, and one of the challenges is the health and well-being promotion of population aged 60 years or over.[1] The world population over 60 years has now reached 1.2 billion people, with a projection of 2.1 billion by 2050.[2] In Brazil, 16.6% of the population is over 60 years old, and it will reach 29.3% by 2050.[3]

Members of *Generation X* (Gen X), who are currently in their mid-40s-to-late 50s (born between around 1965 and 1979), are earlier adopters of the new approach to healthy aging that includes products that will help them thrive in their diverse lifestyles for decades to come. Products addressed to these consumers must consider nutritional, physical, mental, and emotional requirements, by seeking to innovate in formulations that properly attend these issues.[4] Literature reports the development of novel products and/or ingredients as cassava-based rice analogues[5] and saffron nanoencapsulation[6] in order to enhance the bioactive potential, expanding the production of fortified foods addressed to elderly people.

Vitamin D, which is a fat-soluble vitamin, is the main regulator of serum calcium and phosphorus homeostasis. It also participates in cell differentiation and proliferation and has effects on the responses of the immune and nervous systems.[7] Vitamin D belongs to the calciferol group, with its two main compounds denoted as vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The differences between the D_2 and D_3 forms do not affect metabolism, and both of them present identical responses with the same efficiency.[8]

Received: October 21, 2025
Revised: December 17, 2025
Accepted: December 18, 2025
Published: January 7, 2026

Table 1. Formulations and Visual Appearance of the Rice Analogues

Sample	RF (%)	ERF (%)	EERF (%)	BF (%)	CSL (%)	Mix (%)	Analogue formation	Dry product
T1	50	50	-	5	0.5	-	Yes	
T2	50	50	-	10	0.5	-	Yes	
T3	50	50	-	5	-	-	No	
T4	50	50	-	10	-	-	Yes	
T5	50	50	-	-	0.5	-	No	
T6	50	50	-	-	1	-	No	
T7	50	-	50	5	-	-	Yes	
T8	50	-	50	10	-	-	Yes	
T9	50	-	50	-	-	-	Yes	
T7⁺	50	-	50	5	-	2	Yes	
T9⁺	50	-	50	-		2	Yes	

RF, raw rice flour; ERF, extruded rice flour; EERF, emulsified extruded rice flour; BF, raw bean flour; CSL, calcium stearoyl-2-lactyllactate; MIX, Mg + Vit.D; $^{+}$mix addition.

Approximately 90% of vitamin D is produced in the skin through sun exposure, and 10% is obtained through diet, via sources such as UV-exposed mushrooms, eggs, and high-fat fish. Its production occurs in the skin, varying according to age, ethnicity, pigmentation, sun exposure, and use of sunscreen. Elderly people are subject to an elevated risk of vitamin D deficiency due to limited exposure to sunlight.[9] Vitamin D deficiency is prevalent worldwide, and the absence of food fortification with vitamin D leads to low dietary intake.[10]

Magnesium is primarily involved in the development and maintenance of bones, but it also performs several essential biological functions, such as regulating metabolic and biochemical reactions, participating in protein synthesis, and playing a key role in nerve and muscle function, heart rate,

https://doi.org/10.1021/acsfoodscitech.5c01076
ACS Food Sci. Technol. 2026, 6, 732−745

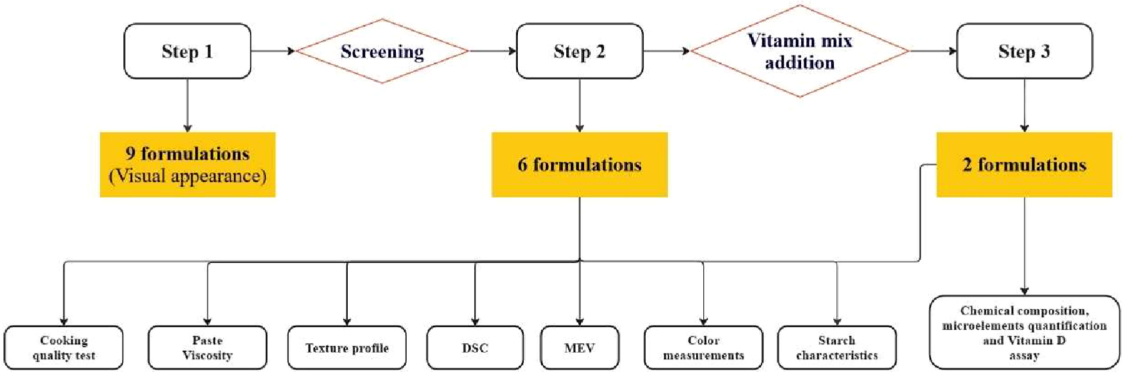


Figure 1. Sequential experiment strategy for screening the formulations.

muscle contraction, and bone health.[11,12] Inadequate intake of this mineral has been reported in several parts of the world.[13−18]

Magnesium and vitamin D supplementation in the elderly is often recommended due to the high prevalence of nutritional deficiencies in this age group associated with physiological changes associated with aging. Vitamin D plays a fundamental role in calcium homeostasis and bone mineralization, being crucial for the prevention of osteopenia, osteoporosis, and fractures.[19] Magnesium, in turn, is a cofactor in more than 300 enzymatic reactions, including the activation of vitamin D, in addition to directly participating in bone formation.[20] Deficiency of these micronutrients is associated with loss of muscle mass, increased risk of falls, and impaired neuromuscular function.[21]

Food fortification is the process of adding vitamins and minerals to foods while they are being processed to increase their nutritional value. Therefore, food fortification is an efficient and viable option to supplement different people groups with underachieved requirements due to inadequate dietary intake.[22] According to the technical regulation on the requirements for food enrichment, RDC No. 714 (July 1, 2022) of the Brazilian Ministry of Health, the definition of enriched foods is those that contain the addition of one or more nutrients for the purpose of reinforcing their nutritional value or preventing or correcting nutritional deficiencies and must appear on the label as "Enriched with" or "Fortified with". In addition, the value of the added nutrient must be considered per 100 mL or 100 g of the ready-to-eat product.[23]

Rice (*Oryza sativa* (L.)) is a cereal with potential for the development of fortified foods since it represents one of the main foods consumed in Brazil and Asia.[24] Approximately 10 million tons are produced in Brazil and 800 million tons worldwide.[25] Some intrinsic characteristics of rice are related to its wide acceptance by most consumers, including being *gluten-free, hypoallergenic, easily digestible, and mildly flavored, having small granules, being white in color,* and having considerable *freeze−thaw stability* compared to pulse starches.[26] Rice analogue is a substitute for rice grains as it is shaped like rice obtained from different ingredient mixtures, presenting properties similar to those of rice and delivering health benefits. Fortified rice analogue can provide the daily nutrient, vitamin, and mineral requirements of senior citizens.[27]

Extrusion emerges as a technology that can be used in order to formulate fortified foods such as rice analogue. Both cold extrusion and extrusion cooking processes involve passing a mixture of flours, additives, and water through a single- or twin-screw extruder. Extrusion cooking involves temperatures above 70 °C in contrast to cold extrusion, which does not use heating except the heat generated during the process itself.[28,29] In addition, extrusion cooking is a process where the mechanical action of screws is combined with external heat to knead continuously the material, leading to starch conversion, protein denaturation, and enzyme inactivation, thus forming new textures and structures.[30] In this process, changes occur in the structures of the materials, including phase transition, molecular degradation, and rearrangement of molecular structures, attributed to the effects of heat, shear, and pressure generated by the heat extruder.[31]

Some legume flours, such as bean flour (BF), can be incorporated into the preparation of analogues to increase the fiber content, quantity and quality of proteins, and micronutrients.[27] Cowpea (*Vigna unguiculata*) is a good option because it is a nutritious legume that provides proteins, fibers, vitamins, and minerals.[32] In addition, it is also rich in essential amino acids such as lysine, leucine, and tyrosine.[33] When combined with rice, it results in a high-biological-value protein that is important for the elderly. Furthermore, this combination aligns with the dietary and cultural habits of this population.

Emulsifiers are also important ingredients in the preparation of analogues that are often added to the flour mixture to reduce the loss of mass solids in the water during cooking and increase the uniformity, thickness, and shine of the dough.[34] Besides, emulsifiers also improve the texture of the final product.[35] Thus, the present study was carried out aiming the development of rice analogues enriched with vitamin D and magnesium, using rice flour, cowpea flour, emulsifier, and vitamin mineral mix, through extrusion techniques, evaluating the physicochemical properties and nutritional quality.

2. MATERIALS AND METHODS

2.1. Material

Polished white rice was purchased from a local market (Rio de Janeiro, Brazil). Cowpea bean (*Vigna unguiculata* (L.) Walp.) was kindly donated by Granfino (Nova Iguaçu, Brazil). The vitamin−mineral mix formulated with maltodextrin, magnesium sulfate monohydrate, and cholecalciferol was donated by DSM-Firmenich (Ames, USA), and the emulsifier calcium stearoyl-2-lactyllactate (CSL) (Grindsted CSL P 2522) was donated by Danisco Brasil Ltd. (Cotia, Brazil). The cholecalciferol chromatographic standard used to determine vitamin D was purchased from Merck/Sigma-Aldrich (code C9756−5G, Darmstadt, Germany).

https://doi.org/10.1021/acsfoodscitech.5c01076
ACS Food Sci. Technol. 2026, 6, 732−745

2.2. Experimental Strategy and Formulation Selection

The strategy adopted in order to carry out the experiments was divided into stages: (i) preparation of the matrix with analogue formulations (Table 1); (ii) selection of the six formulations based on the best analogue formation (T1, T2, T4, T7, T8, T9); (iii) cooking test of selected formulations; (iv) selection of two best formulations ($T7^+$, $T9^+$) in terms of the cooking test for vitamin mix addition; (v) evaluation of the physical and chemical properties of the formulations (T1, T2, T4, T7, $T7^+$, T8, T9, $T7^+$) and determination of the proximal composition, vitamin D, and microelements of the formulations ($T7^+$, $T9^+$) (Figure 1).

2.3. Preparation of Raw Flours

Raw rice flour (RF) was prepared by using a Lab Mill 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm sieve. Raw BF was prepared using a 3600 disk mill (Perten Instruments AB, Huddinge, Sweden) with a 0.6 mm gap between disks and then in a Lab Mill 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden). The flours were packaged and stored in a refrigerator at 4 °C for further processing.

2.4. Preparation of Extruded Flours

Extruded rice flour (ERF) was prepared using RF, and emulsified extruded rice flour (EERF) was prepared using RF and 0.5% emulsifier, which were previously mixed for 15 min using a TE-201/05 homogenizing agitator (Tecnal, São Paulo, Brazil) before being subjected to extrusion processing. The flours were processed in an Evolum HT25 twin-screw corotating extruder (Clextral Inc., Firminy, France), with a length/diameter ratio of 40:1, equipped with a 6 mm diameter circular die at a screw speed of 250 rpm. The feed rate was adjusted to 5.3 kg/h, with the assistance of a loss-in-weight gravimetric feeder model GRMD15 (Schenck Process, Darmstadt, Germany). The 10 heating zones were regulated at temperatures of 25, 25, 40, 50, 90, 100, 100, 90, 70, and 70 °C, from the feeding zone to the exit die. The water flow rate (2.3 L/h) was controlled by injecting potable water between the first and second zones of the extruder through a port with an internal diameter of 5.25 mm using a plunger metering pump Super K PP 6.35 (DKM Clextral Inc., Firminy, France). Finally, the extruded material was dried at 40 °C for 16 h in a food dehydrator (DMS-G. E, Macanuda, Joinville, Brazil), cooled to room temperature, ground in a 3600 disk mill (Perten Instruments AB, Huddinge, Sweden) with 0.6 mm diameter to reduce it into smaller pieces and then in a Lab Mill 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) to obtain flours, and stored in polyethylene plastic bags for later use.

2.5. Rice Analogue Preparation

The rice analogues were prepared with a mixture of RF, BF, EERF, emulsifier, and vitamin−mineral mix according to Table 1. Each mixture flour was added with 33% cold water and homogenized continuously for 5 min in a MSA planetary mixer (Guangzhou Panyu Jianye Imp. & Exp. Trading Co. Ltd., Guangzhou, China). The analogues were formatted using a pasta production machine (Pastaia 2, Italvisa, Tatuí-SP, Brazil) coupled to a *risoni* die.

A target concentration of magnesium of 126 mg/kg and vitamin D of 4.5 μg/kg was defined considering a magnesium daily reference value (DRV) of 420 mg and vitamin D of 15 μg.[36] In order to achieve the desired concentration, 2% of the vitamin−mineral mix was added to the T7 and T9 formulations. All analogues were dried in an oven (Fabbe-Primar Ind., São Paulo, Brazil) with hot-air circulation at 40 °C for 16 h and then ground in a Lab Mill 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) fit with a 0.8 mm sieve aperture obtaining a flour and packaged in metallized plastic and kept refrigerated at 4 °C for subsequent analyses.

2.6. Physical Properties

2.6.1. Paste Viscosity. The paste viscosity profiles of the analogue flours were measured with a Rapid Visco Analyzer (RVA) series 4 (Newport Scientific Pty. Ltd., Warriewood, Australia) running at 160 rpm at an initial temperature of 25 °C.[37] 3 g of the sample was adjusted to 14% of water (wet basis) and mixed with 25 mL of distilled water in an RVA aluminum container. The properties evaluated were paste temperature (Tp), peak viscosity (PV), setback (SBV), breakdown (BDV), and final viscosity (FV). The viscosity values were recorded in centipoises (cP). All measurements were carried out in triplicates.

2.6.2. Texture Analysis. Texture profile analysis (TPA) of the cooked rice analogue was conducted to evaluate parameters such as hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience, determined according to the definition provided in ref 38. The texture of the samples was measured using a TA XT Plus Exponent texture analyzer, version 6.1.11.0 (Stable Micro Systems, Surrey, UK), equipped with a load cell of 5 kg. For each analogue sample, 20 grains were randomly selected, which were analyzed individually, totaling 20 repetitions. Each grain was positioned symmetrically on the table and compressed with a 25 mm aluminum probe with 75% sample compression. The pretest, test, and post-test speeds were fixed at 5 mm/s, and the compression time was 5 s between cycles.[39] The results correspond to the averages of these runs.

2.6.3. Differential Scanning Calorimetry. Thermal properties of the analogue flours were determined in duplicate by differential scanning calorimetry (DSC) using a DSC Q200 (TA Instruments, New Castle, USA). The onset (T_o), peak (T_p), and completion (T_c) gelatinization temperatures, as well as the calorimetric enthalpy (ΔH) values, were calculated based on the thermograms using Advantage software version 5 (TA Instruments, New Castle, USA). Approximately 2−3 mg aliquots of the analogue flour with a known moisture content were added with deionized water in a 1:2 ratio (analogue/water) and were placed in an aluminum pan. The pans were sealed and stored at room temperature for at least 2 h before analysis. An empty pan was used as a reference. The scanning occurred in the range of 5−110 °C at a rate of 10 °C/min.[40]

2.6.4. Color Measurements. Colorimetric measurements of the analogue flours were performed using a CR-400 portable colorimeter (Konica Minolta, Tokyo, Japan) in at least five replicates. The equipment was standardized with a white block (Y = 93.01; x = 0.3139; y = 0.3200) using illuminant D65 and a 2° observer. The color parameters were recorded by measuring the values of lightness (L^*), yellow intensity (b^*), and red intensity (a^*). The whiteness index (W.I.) was calculated using formula 1.[41]

$$\text{W. I.} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

2.7. Morphological Characteristics

The microstructure of rice was observed using a Benchtop TM 3000 scanning electron microscope (Hitachi, Tokyo, Japan), which operated in an evacuated chamber at 15 kV acceleration. Rice grains were dried in a desiccator with anhydrous calcium chloride ($CaCl_2$) for 15 days and then fixed with double-sided tape on an aluminum stub. Images were captured at 30× and 100× magnification.[42]

2.8. Cooking Quality Test

The cooking quality test was performed according to the 16−50 method.[43] The cooking time was determined using aliquots of 10 g of pasta cooked in 140 mL of boiling water. The optimum cooking time was determined by compressing the sample of the cooked product every 1 min between two glass plates until the central nucleus disappeared. The percentage of weight increase (AP) of the cooked product was determined by the ratio between 10 g of raw rice and its weight after cooking, using the optimum cooking time of each sample (formula 2). The values were expressed as a percentage. The loss of soluble solids in the cooking water was determined by removing 25 mL of the cooking water and placing it in an oven with air circulation at 105 °C for 16 h. The solids were weighed, and the loss of soluble solids (SS %) was calculated (formula 3).

$$\text{AP}(\%) = \left({}^{\text{PF}}\!/_{\text{PI}} \times 100 \right) - 100 \quad (2)$$

https://doi.org/10.1021/acsfoodscitech.5c01076
ACS Food Sci. Technol. 2026, 6, 732−745

$$\mathrm{SS}(\%) = \left({\mathrm{PR}(\mathrm{g}) \times V_{\mathrm{c}}(\mathrm{mL})}\Big/{\mathrm{PA}(\mathrm{g}) \times V_{\mathrm{a}}(\mathrm{mL})} \right) \times 100 \quad (3)$$

2.9. Analogue Dimensions

The dimensions (length and width) of the analogues were measured using a 150 mm digital caliper model 316119 (MTX, Moscow, Russia). The weight of each grain was determined using a Shimadzu analytical balance model AUX 320 (Shimadzu Corporation, Kyoto, Japan).

2.10. Chemical Characterization

2.10.1. Chemical Composition. The chemical composition of the analogues T7$^+$, T9$^+$ with the addition of the vitamin−mineral mix and the control was performed according to the AOAC methods.[44] The protein content was calculated using method 2001.11 (factor of 6.25) with modification, ash (method 923.03), total dietary fiber (method 991.43), and moisture (method 925.09), and the carbohydrate content was calculated by the difference. The fat content was determined according to method Am 5−04.[45]

2.10.2. Starch Characteristics. The amylose content (AM) of the raw analogue was determined using a K-AMYL kit (Megazyme International, Bray, Ireland) following the method 79−13. The resistant starch content (RS) was determined using a K-RSTAR kit (Megazyme International, Bray, Ireland) according to methods 2002.02 and 32−40.01.[46]

2.10.3. Microelement Quantification. The quantification of microelements (magnesium, sodium, potassium, calcium phosphorus, manganese, iron, zinc, copper) was performed according to the methods 990.10 and 999.08, respectively.[47] For sample digestion, cavity microwave mineralization was performed using model MARS5 (CEM, Matthews, USA) with a maximum power of 1600 W, a heating ramp of 20 min up to 180 °C, and a plateau of 180 °C for 20 min. After digestion, quantification was performed in an inductively coupled plasma optical emission spectrometer (ICP-OES) model Optima 2100DV (PerkinElmer, Shelton, USA), with a cyclonic nebulization chamber and concentric nebulizer, with sequential optics and dual-view torch visualization.

2.10.4. Vitamin D Assay. To determine vitamin D contents, formulations T7$^+$ and T9$^+$ were packaged in metallic plastic bags and stored at room temperature during 0, 4, and 6 months. The analogues were ground in a Lab Mill 3100 hammer mill (Perten Instruments AB, Huddinge, Sweden) equipped with a 0.8 mm sieve to obtain a fine flour. Then, samples were extracted for 15 min with 0.1 N HCl and 95% ethanol at 55 °C, and the extracts were subjected to liquid−liquid extraction with hexane. The hexane extract was evaporated in a rotary evaporator, and the solid residue was dissolved in isopropanol. Vitamin D was separated in UPLC Acquity HClass (Waters Corporation, Milford, USA) with a C18 column model Acquity UPCL Beh C18 1.7 μm (Waters Corporation, Wexford, Ireland) followed by gradient elution of methanol and water acidified with formic acid (0.1%). Detection was by triple-quadrupole mass spectrometry with a Xevo TQD (Waters Corporation, Milford, USA) with electron spray ionization in positive mode (ESI+). Quantification was performed using the chromatogram of the mass transition at 385−259 m/z. To confirm the presence of vitamin D_3, the transitions 385 > 367, 385 > 259, 385 > 255, and 385 > 241 were used. Only the peak eluting close to 4.49 min (cholecalciferol peak) was considered, and the peak 4.23−4.35 min was discarded for quantification. The vitamin D_3 standard used was the reference C9756−5G (Sigma-Aldrich, St. Louis, USA). The retention time (RT) of vitamin D_3 was 4.49 min. Three concentration points were injected in the working range of 100−300 ng/mL.

2.11. Statistical Analysis

All results were expressed as mean ± standard deviation (SD). Statistical analysis was performed with one-way ANOVA, followed by Tukey's HSD test ($p < 0.05$) using XLSTAT software version 2023.2.0 (Lumivero, Denver, USA). Principal component analysis (PCA), hierarchical cluster analysis (HCA), and radar chart analyses were performed with XLSTAT (Lumivero, Denver, USA).

3. RESULTS AND DISCUSSION

3.1. Physical Properties

3.1.1. Pasting Viscosity Properties. The Rapid Visco Analyzer (RVA) paste properties are used to measure the viscosity of food products such as pastes, flour, starches, and proteins. This technique is essential in the food industry as it allows the quality of both raw materials and final products to be assessed.

Pasting curves of the rice analogue samples exhibited a similar profile (Figure 2), showing a tendency for retro-

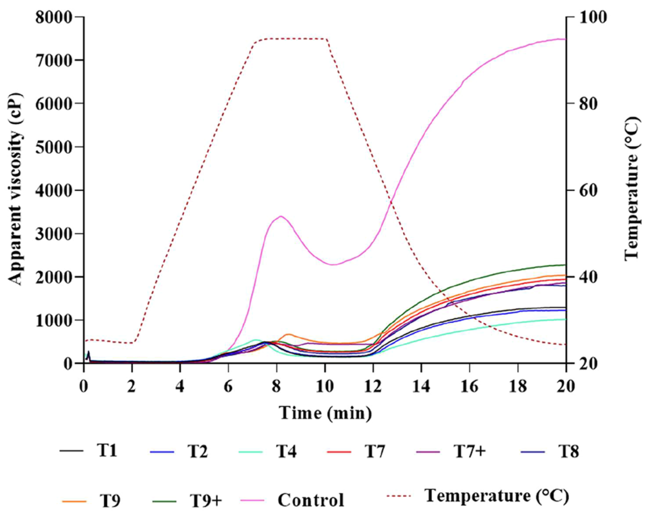


Figure 2. Paste viscosity properties of rice analogues. T1 = (50% RF, 50% ERF, 5% BF, 0.5% CSL); T2 = (50% RF, 50% ERF, 10% BF, 0.5% CSL); T4 = (50% RF, 50% ERF, 10% BF); T7 = (50% RF, 50% EERF, 5% BF, 0.5% CSL); T7$^+$ = (50%, 50% EERF, 5% BF, 0.5% CSL, 2% Mix), T8 = (50% RF, 50% EERF, 10% BF, 0.5% CSL); T9 = (50% RF, 50% EERF, 0.5% CSL); T9$^+$ = (50%, 50% EERF, 0.5% CSL, 2% Mix).

gradation during cooling, while the control (polished white rice) sample was distinguished among all of them. The pasting temperature, peak viscosity, breakdown viscosity, final viscosity, and setback of rice analogues ranged from 57.20−68.16 °C, 454.66−3352.33, 47.44−1073.33, 1018.33−7492.66, and 474.00−4140.33 cP, respectively (Table S1). The addition of different concentrations of BF causes changes in the paste properties, especially in the PT, FV, and SBV values ($p < 0.05$) (Formulation T4). This result was already expected, since the proteins present in the bean interact with the starch, changing the properties of the paste.[48]

The addition of the vitamin−mineral mix caused a reduction in all pasting properties of formulation T7$^+$ and showed the lowest value (454.66 cP) of peak viscosity (PV) and breakdown (BDV) (47.33 cP) ($p < 0.05$). In contrast, the addition of the vitamin−mineral mix in formulation T9$^+$ (without BF) led to an increase in parameters related to the cooling stage (BDV, SBV, and FV) ($p < 0.05$).

3.1.2. Thermal Properties. DSC is a useful thermal analysis method to study the thermal changes, particularly gelatinization (endothermic thermal event), that occur during the cooking process of starch granules, and the results are summarized in Table 2. The onset temperatures (T_o), peak temperatures (T_p), and conclusion temperature (T_c) ranged from 60.7 to 66.2 °C, 68.1 to 73.8 °C, and 78.8 to 87.8 °C,

https://doi.org/10.1021/acsfoodscitech.5c01076
ACS Food Sci. Technol. 2026, 6, 732−745

Table 2. Thermal Properties and Color Characteristics of Rice Analogues

	DSC parameters				color measurements			
samples	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	L^*	a^*	b	W.I.
T1	62.31 ± 0.98^a	70.17 ± 0.89^a	84.73 ± 1.87^a	2.34 ± 0.21^{bc}	89.67 ± 0.20^e	0.00 ± 0.00^e	9.14 ± 0.22^d	86.21 ± 0.28^e
T2	62.34 ± 1.06^a	69.94 ± 0.40^a	82.95 ± 2.36^{ab}	1.47 ± 0.49^c	87.28 ± 0.13^g	0.52 ± 0.04^b	10.75 ± 0.08^b	83.34 ± 0.12^h
T4	66.2 ± 4.75^a	73.8 ± 3.90^a	86.04 ± 2.15^a	1.79 ± 0.66^c	88.30 ± 0.04^f	0.58 ± 0.01^a	9.96 ± 0.15^c	84.62 ± 0.12^g
T7	63.83 ± 0.35^a	70.35 ± 0.39^a	78.79 ± 0.00^b	2.46 ± 0.24^{bc}	89.54 ± 0.09^e	-0.23 ± 0.02^h	10.11 ± 0.08^c	85.44 ± 0.11^f
T7$^+$	65.48 ± 1.01^a	72.28 ± 0.95^a	87.81 ± 1.07^a	5.28 ± 0.03^b	90.76 ± 0.25^d	0.26 ± 0.03^c	7.18 ± 0.20^e	88.29 ± 0.32^d
T8	65.61 ± 0.67^a	72.04 ± 0.34^a	83.21 ± 0.15^{ab}	2.79 ± 0.14^{bc}	87.44 ± 0.12^g	0.15 ± 0.04^d	11.36 ± 0.12^a	83.06 ± 0.16^h
T9	65.07 ± 2.88^a	71.92 ± 2.04^a	82.91 ± 0.43^{ab}	3.90 ± 1.52^{bc}	92.52 ± 0.17^b	-0.26 ± 0.11^h	5.92 ± 0.14^f	90.45 ± 0.22^b
T9$^+$	60.75 ± 0.62^a	68.49 ± 0.19^a	83.21 ± 0.71^{ab}	3.91 ± 1.94^{bc}	91.98 ± 0.11^c	-0.17 ± 0.01^g	6.20 ± 0.80^f	89.86 ± 0.14^c
control	61.22 ± 0.29^a	68.1 ± 0.25^a	83.01 ± 0.57^{ab}	8.92 ± 0.15^a	95.58 ± 0.05^a	-0.06 ± 0.02^f	5.18 ± 0.07^g	93.19 ± 0.09^a

Note: Values are mean ($n = 3$) $\pm$ standard deviation. Data with same letters in the same column did not differ significantly by the Tukey test ($p < 0.05$). T_0 = temperature onset; T_p = peak temperature; T_c = conclusion temperature; ΔH = enthalpy; and W.I. = whiteness index; T1 = 50% RF, 50% ERF, 5% BF, 0.5% CSL; T2 = 50% RF, 50% ERF, 10% BF, 0.5% CSL; T4 = 50% RF, 50% ERF, 10% BF; T7 = 50% RF, 50% EERF, 5% BF, 0.5% CSL; T7$^+$ = 50%, 50% EERF, 5% BF, 0.5% CSL, 2% mix, T8 = 50% RF, 50% EERF, 10% BF, 0.5% CSL; T9 = 50% RF, 50% EERF, 0.5% CSL; T9$^+$ = 50%, 50% EERF, 0.5% CSL, 2% mix.

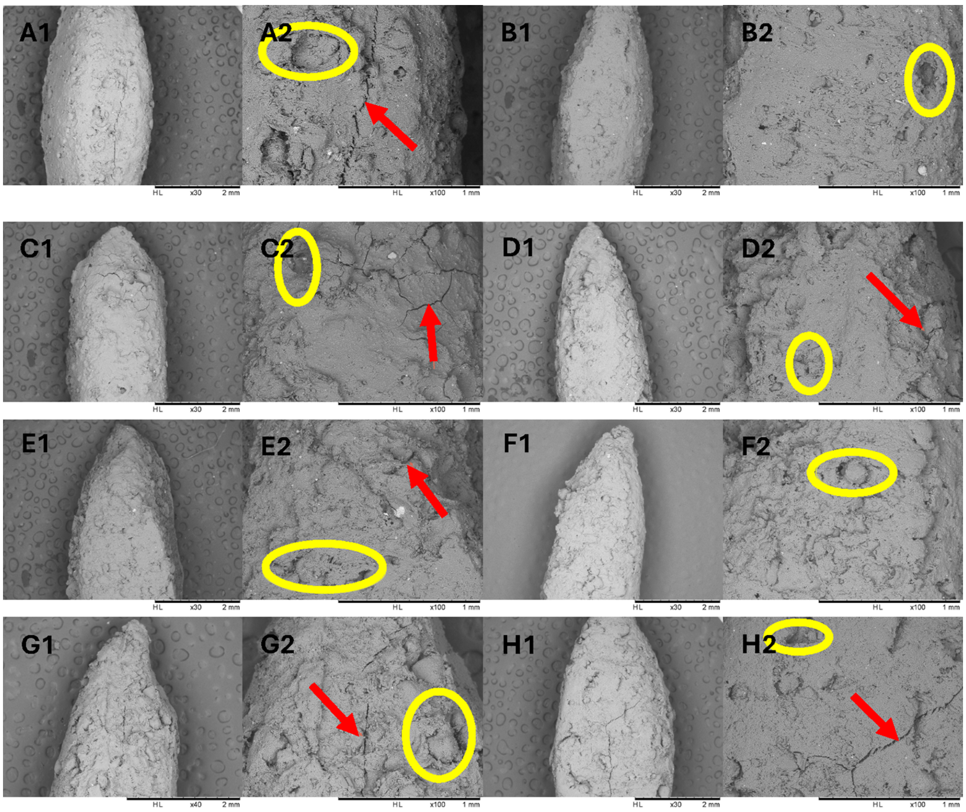


Figure 3. SEM images of rice analogues: (A) T1, (B) T2, (C) T4, (D) T7, (E) T8, (F) T9, (G) T7$^+$, and (H) T9$^+$. A1−H1 are whole grain longitudinal section images at 30× magnification; A2−F2 are whole grain longitudinal section images at 100× magnification. Yellow oval shape: rough and irregular surface; red arrow: cracks.

respectively; however, statistical difference was not observed concerning T_o and T_p ($p < 0.05$).

Particularly, in the case of the T7$^+$ formulation, the vitamin−mineral mix addition led to a large increase in enthalpy (2.46−5.28 J/g), probably due to the presence of the lipid containing vitamin D, which would act as a lubricant, reducing starch breakdown and also forming the V-type amylose complex.[49] Nevertheless, all analogues presented a lower enthalpy (ΔH) compared to that of the control as expected, suggesting that less energy was required to disrupt the internal bonds of amylopectin molecules considering that all formulations used at least 50% of extruded flour. It is well-known that extrusion cooking causes starch degradation, physically modifying the granules, resulting in a reduction in ΔH.[50] Wang et al. also observed this reduction in the enthalpy of rice analogues using broken indica rice flour and purple sweet potato powder (90:10).[51] These authors attributed this reduction to the hydrogen bonds between starch molecules that were broken, suggesting that less energy was needed to disrupt the internal bonds of starch molecules.

3.1.3. Color Measurement. The color of food products has a significant impact on the purchase intention and consumer acceptance. The color measurements of the analogues are presented in Table 2. The L^* value ranged

https://doi.org/10.1021/acsfoodscitech.5c01076
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from 87.28 to 92.52, and it was observed that all measurements presented significant statistical difference ($p < 0.05$). It was observed that the L^* value decreased in the samples with 10% BF addition (T2, T4, T8), which can be attributed to the composition of the flour due to the colored pigments present in the grain pericarp.[52] In contrast, the combined effect of a low BF content on the formulation and the addition of the vitamin–mineral mix (T7, T7$^+$, T9, and T9$^+$) led to L^* values similar to the control (95.58). In fact, the W.I. values of these fortified samples (88.29 and 89.86) are closer to the control value (93.19).

3.1.4. Scanning Electron Microscopy. Scanning electron microscopy (SEM) images (Figure 3) showed that all rice analogues had irregular, rough, and porous surfaces. The rough and porous surface may be due to heterogeneous mixing during processing, in addition to the shear at the die exit and drying conditions after the extrusion process. As observed in the SEM micrographs, the surfaces are not compact, as indicated by a yellow oval and a red arrow. This suggests that the process may not provide sufficient cohesion and structure to form a smooth and compact grain. In fact, Liu et al. suggest that prolonged mixing may improve the appearance of the analogues.[5] Cracks and irregular pores were observed in the longitudinal section of all of the analogues, which may be due to the drying conditions and retrogradation during cooling. A more compact analogue improves the texture and prevents the loss of solid components during cooking. The cracks were suggested to be channels for water penetration during cooking.[53] No differences were observed in the rice analogues with the addition of a vitamin mixture.

3.2. Texture Profile Analysis

TPA is a method widely used in food science to measure textural characteristics. The procedure consists of compressing a small sample of food twice with a specific probe, simulating the movement of a bite. From the force–time curve generated, several textural parameters are extracted, which present good correlation to the sensory perception of these attributes.[54]

The texture profiles of rice analogues are shown in Figure 4 and Table S2. Considering that the hardness of food increases

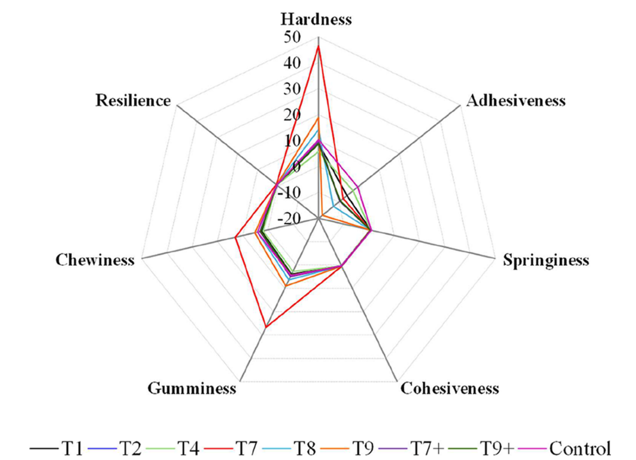

Figure 4. TPA profile of rice analogues. T1 = (50% RF, 50% ERF, 5% BF, 0.5% CSL); T2 = (50% RF, 50% ERF, 10% BF, 0.5% CSL); T4 = (50% RF, 50% ERF, 10% BF); T7 = (50% RF, 50% EERF, 5% BF, 0.5% CSL); T7$^+$ = (50%, 50% EERF, 5% BF, 0.5% CSL, 2% mix); T8 = (50% RF, 50% EERF, 10% BF, 0.5% CSL); T9 = (50% RF, 50% EERF, 0.5% CSL); T9$^+$ = (50%, 50% EERF, 0.5% CSL, 2% mix).

the number of chews and the duration of processing, soft foods are the most suitable and preferred by elderly consumers due to the physiological conditions of the oral cavity such as tooth loss and reduction of chewing function.[55] Findings showed that the hardness of the analogues ranged from 5.71 to 46.48 N, and formulation T7 presented the highest value, i.e., 4.5 times superior to the control ($p < 0.05$). Thus, the formulations T1, T2, T4, T7$^+$, and T9$^+$ are softer than the control and represent a good option for the target consumer. The addition of vitamin–mineral mix led to a decrease in hardness, gumminess, and chewiness in formulations 7$^+$ and 9$^+$, with values comparable to the control values ($p < 0.05$), i.e., very similar to those of polished rice, which is the traditional form of consumption.

The palatability of cooked rice is one of the factors that affects its quality. Hardness is the most important evaluation parameter, since high values can interfere with its sensory acceptance, and it is an important human sensory perception.[56,29] Our results agree with Budi et al. who found similar hardness values (16.77–42.74 N) in a rice analogue using yellow corn flour, corn starch, and glycerol monostearate.[57]

As previously mentioned, aging can lead to physiological changes in the oral digestive system, such as tooth loss, reduced saliva, decreased flexibility of the tongue and masticatory muscles, and dysphagia.[55] These changes lead to increased swallowing difficulties and the risk of aspiration of food residues.[58] Food cohesion refers to the ability to hold food particles together to form a cluster. It has been reported that foods with low cohesive strength are more likely to break apart during swallowing and can cause choking.[59] Furthermore, foods with high cohesion are difficult to pass through the pharynx due to high resistance to stretching, thus increasing the risk of swallowing. The analogues showed cohesion results similar to those of the control (0.44–0.57), demonstrating that analogues may be indicated for the elderly as a substitute of polished white rice, which is traditionally consumed.

Based on Table S2, the adhesiveness of the analogue ranged from −2.93 to −16.4 g.s., which was lower than that of the control (−0.65 g.s), which is an interesting characteristic in terms of mastication. The adhesiveness refers to the stickiness of rice during decompression or the energy required to pull the compression plunger away from the rice.[60] It was observed that the stickiness of milled rice has a negative correlation to the amylose content, which may be attributed to its high value, which tends to leach out gelatinized starch granules and form a coated surface on the rice grain, thus contributing to the reduced stickiness.[61]

3.3. Analogue Dimensions

The dimensions of the rice analogues are given in Table 3. The length and width of the analogues ranged between 7.29–7.91 and 2.48–2.83 mm, respectively. It was observed that there was no significant difference in the length and width among the analogues, except for T9$^+$ in the case of length. According to the Brazilian food regulation 02/2012 (Brazil, 2012), if the result of dividing the length by the width is ≥2.75, the grain will be considered as fine-long class; if the result of dividing the length by the width is less than 2.75, the grain will be considered as long class.[62] The average L/W ratio of the analogues ranged from 2.64 to 3.10, which classifies the grains as long and fine. The dimensions obtained for the analogues were larger than those of the control (6.69 × 2.02 mm, L ×

https://doi.org/10.1021/acsfoodscitech.5c01076
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Table 3. Grain Dimensions, Cooking Quality, and Starch Characteristics of Rice Analogues

samples	length (mm)	width (mm)	weight (mg/grain)	length width ratio (mm)	cooking time (min)	gruel solid loss (%)	water uptake ratio (%)	amylose (%)	resistant Starch (%)
T1	7.63 ± 0.27^{abc}	2.76 ± 0.25^{ab}	30.48 ± 0.00^{a}	2.76 ± 0.02^{c}	09.00 ± 0.00^{c}	5.54 ± 0.38^{b}	125.21 ± 11.55^{bcd}	22.31 ± 1.19^{bc}	0.63 ± 0.03^{e}
T2	7.29 ± 0.26^{b}	2.54 ± 0.13^{bc}	20.14 ± 0.00^{bc}	2.87 ± 0.17^{bc}	08.00 ± 0.00^{cd}	8.13 ± 2.02^{b}	152.67 ± 12.59^{ab}	21.99 ± 0.41^{bc}	1.29 ± 0.08^{c}
T4	7.48 ± 0.28^{bc}	2.83 ± 0.23^{a}	29.64 ± 0.00^{a}	2.64 ± 0.24^{c}	12.00 ± 0.00^{b}	9.33 ± 4.34^{ab}	137.93 ± 0.23^{abc}	24.13 ± 0.18^{ab}	1.01 ± 0.01^{d}
T7	7.57 ± 0.25^{abc}	2.48 ± 0.15^{c}	14.89 ± 0.00^{c}	3.05 ± 0.20^{ab}	06.00 ± 0.15^{d}	5.44 ± 3.94^{b}	59.41 ± 1.02^{f}	22.54 ± 0.10^{bc}	1.56 ± 0.00^{b}
T7^{+}	7.86 ± 0.01^{a}	2.75 ± 0.16^{ab}	29.79 ± 0.00^{a}	2.86 ± 0.18^{bc}	09.00 ± 0.04^{c}	15.95 ± 1.98^{a}	103.72 ± 16.78^{cd}	19.08 ± 0.32^{c}	0.59 ± 0.04^{e}
T8	7.74 ± 0.16^{ab}	2.49 ± 0.16^{c}	17.93 ± 0.00^{c}	3.10 ± 0.22^{ab}	07.00 ± 0.09^{d}	6.15 ± 1.77^{b}	101.5 ± 10.59^{de}	24.87 ± 0.36^{ab}	0.50 ± 0.07^{e}
T9	7.73 ± 0.24^{ab}	2.62 ± 0.23^{abc}	24.02 ± 0.01^{b}	2.95 ± 0.22^{b}	06.30 ± 0.02^{e}	5.08 ± 0.69^{b}	67.74 ± 4.64^{ef}	23.76 ± 2.22^{ab}	0.89 ± 0.04^{d}
T9^{+}	7.91 ± 0.12^{a}	2.73 ± 0.18^{abc}	29.79 ± 0.00^{a}	2.90 ± 0.03^{b}	06.30 ± 0.00^{e}	11.41 ± 1.90^{ab}	96.94 ± 6.88^{de}	21.78 ± 0.05^{bc}	0.46 ± 0.03^{e}
control	6.69 ± 0.38^{c}	2.02 ± 0.10^{d}	16.90 ± 0.00^{c}	3.31 ± 0.25^{a}	14.00 ± 0.02^{a}	5.31 ± 0.66^{b}	167.37 ± 3.82^{a}	26.55 ± 0.35^{a}	3.89 ± 0.00^{a}

Note: Values are mean (n = 3) ± standard deviation. Data with the same letters in the same column did not differ significantly by the Tukey test ($p < 0.05$). T1 = (50% RF, 50% ERF, 5% BF, 0.5% CSL); T2 = (50% RF, 50% ERF, 10% BF, 0.5% CSL); T4 = (50% RF, 50% ERF, 10% BF); T7 = (50% RF, 50% EERF, 5% BF, 0.5% CSL); T7^{+} = (50%, 50% EERF, 5% BF, 0.5% CSL, 2% Mix), T8 = (50% RF, 50% EERF, 10% BF, 0.5% CSL); T9 = (50% RF, 50% EERF, 0.5% CSL); T9^{+} = (50%, 50% EERF, 0.5% CSL, 2% Mix).

W), notably in the case of width, whose values reach up to 73% higher than that of the control.

Cruz and Khush reported that rice with lengths of 6.61−7.50 mm can be considered as *long rice* and those longer than 7.50 as *very long grain*.[63] Thus, the rice analogues T2 and T4 are classified under the long grain category and the analogues T1, T7, T7^{+}, T8, T9, and T9^{+} under the very long grain category. The larger size of the rice analogue is responsible for the variation in grain weight compared to the control (16.90 mg/grain), and the weight ranged from 14.89 to 30.48 mg/grain.

3.4. Cooking Characteristics

Cooking characteristics are important parameters for the quality of the rice analogue. The optimum cooking time, loss of soluble solids, and weight increase of the analogues are presented in Table 3. It was observed that the analogue did not disintegrate and maintained its shape after being cooked (Figure S1b). The cooking time of the analogues varied between 6 (T7) and 12 min (T4). All samples showed a shorter cooking time when compared to the control (14 min); this means a reduction up to 14% in time, which is a convenient quality to simplify the elderly's routine. The reduced optimum cooking time is related to the 50% increase in extruded flour. The extrusion process causes starch breakdown, which is important for reducing the cooking time.[64,65] Our findings were consistent with the RVA results, in which formulations using extruded flours presented a decrease in pasting parameters compared to the control (polished rice) (Figure 2).

After cooking, all samples tended to undergo an increase in their weight between 59.41 (T7) and 152.67% (T2), but the water uptake of the control (167%) was higher compared to that of the analogues. The weight increase is an important parameter to evaluate the yield of the rice analogue. The weight increase in rice occurs due to the high water retention capacity during cooking.[66] Kraithong and Rawdkuen reported that better water absorption can contribute to a softer texture of rice pasta.[67]

Soluble solids indicate the content of solid components found in cooking water. The soluble solid loss values of the analogues ranged from 5.08 (T9) to 15.95% (T7^{+}). The solid loss of the analogue was, in general, greater than that of the control. In fact, the presence of dietary fiber may increase solid losses due to the disruption of the starch matrix, which can lead to greater starch leaching during cooking. Similar results of increased solid loss with the addition of dietary fiber were observed in the analogue rice prepared with defatted soy flour.[68]

3.5. Chemical Characterization

3.5.1. Chemical Composition. The chemical compositions of the analogues are presented in Table 4. These formulations produced rice analogues with macronutrient compositions providing 8.03 and 7.66 g of protein, 0.69 and 0.79 g of lipids, and 86.13 and 84.13 g of carbohydrates, together with ash values of 1.68 and 1.76 g. This proportion can be considered ideal to achieve a desirable balance of nutrients in the rice analogue, providing protein and fat, in addition to offering a substantial amount of carbohydrates. In general, it is observed that the addition of beans did not contribute to an increase in macronutrients.

Moisture is one of the important parameters that affects the shelf life of the rice analogue. The moisture content should be well controlled through processing, such as drying, to ensure a

Table 4. Chemical Composition, Vitamin D Quantification, and Microelement Profile of Rice Analogues

composition	$T7^+$	$T9^+$	control
moisture (g/100g)	2.67 ± 0.28^b	4.46 ± 0.38^b	8.05 ± 0.75^a
ash (g/100g)	1.67 ± 0.02^a	1.75 ± 0.03^a	0.30 ± 0.01^b
protein (g/100g)	8.03 ± 0.21^a	7.65 ± 0.13^a	7.65 ± 0.13^a
lipid (g/100g)	0.68 ± 0.13^a	0.79 ± 0.28^a	0.51 ± 0.04^a
carbohydrates (g/100g)	86.13 ± 0.35^a	84.13 ± 0.52^{ab}	82.92 ± 0.95^b
fiber dietary (g/100g)	0.81 ± 0.02^b	1.20 ± 0.01^a	0.57 ± 0.01^c
vitamin D (μg/100g) (0 months)	4.53 ± 0.54^b	8.52 ± 1.20^a	0.00 ± 0.00^c
vitamin D (μg/100g) (4 months)	4.48 ± 0.29^b	8.67 ± 0.07^a	0.00 ± 0.00^c
vitamin D (μg/100g) (6 months)	1.83 ± 0.31^b	5.17 ± 0.03^a	0.00 ± 0.00^c
Mg (mg/100g)	286.80 ± 7.57^a	278.25 ± 1.25^b	4.30 ± 0.10^c
Na (mg/kg)	11.84 ± 0.07^a	11.12 ± 0.94^{ab}	8.39 ± 0.96^b
K (mg/kg)	396.17 ± 6.51^b	662.03 ± 6.12^a	325.42 ± 1.56^c
Ca (mg/kg)	323.96 ± 2.79^a	316.63 ± 0.06^b	8.20 ± 0.15^c
P (mg/kg)	543.21 ± 0.26^b	635.88 ± 15.51^a	400.98 ± 4.22^c
Mn (mg/kg)	5.96 ± 0.02^b	6.06 ± 0.16^b	6.62 ± 0.02^a
Fe (mg/kg)	4.18 ± 0.24^b	4.88 ± 0.00^b	0.00 ± 0.00^c
Zn (mg/kg)	9.43 ± 0.19^b	10.55 ± 0.32^a	6.57 ± 0.04^c
Cu (mg/kg)	5.82 ± 0.21^a	6.13 ± 0.96^a	1.40 ± 0.02^b

Note: Values are mean (n = 3) ± standard deviation. Data with the same letters in the same lines did not differ significantly by the Tukey test ($p <$ 0.05). $T7^+$ = 50%, 50% EERF, 5% BF, 0.5% CSL, 2% mix; $T9^+$ = 50%, 50% EERF, 0.5% CSL, 2% mix.

longer shelf life of the rice analogue. Reducing the moisture content is effective in preventing fungal growth and insect proliferation.[69] The moisture content was low in both formulations, ranging from 2.67 to 4.47%. The ash contents in formulations $T7^+$ (1.68) and $T9^+$ (1.76) were higher than that found in the control (0.31). Higher ash values indicate that the rice was successfully enriched and are related to the content of microelements (Table 4) found in the samples. The $T7^+$ analogue has the highest protein content (8.03 g/100 g) but does not show statistical difference ($p < 0.05$). The addition of BF to the analogues was associated with a higher protein content.[70]

The analogue with the highest lipid content (0.79%) is $T9^+$, which does not contain beans. Handayani et al. (2024) reported a lipid content between 2.02 and 3.13% in rice analogues using cowpea flour addition. In addition, these authors using also skimmed milk powder in formulations that can contribute to the high values found.[70] In general, the analogues ($T7^+$ and $T9^+$) contained more significant amounts than the control, which is due to the supplementation of BF and emulsifier.

The highest carbohydrate content (86.13 g) was observed in the $T7^+$ sample, which was formulated using BF. Handayani et al. reported that the carbohydrate content in analogues with beans varied between 81.03 and 84.06.[70] The carbohydrate content found in the formulations $T7^+$ and $T9^+$ is higher than that of the control rice (82.92). This difference in carbohydrate may have been influenced by the addition of the vitamin mix, which is composed of maltodextrin (glucose polymers). A higher fiber content was observed in the $T7^+$ (0.81%) and $T9^+$ (1.20%) analogues compared to the control 0.57%. Fiber plays an important role in improving the overall gut health; therefore, a higher fiber content in the analogue is nutritionally favorable. According to Kanetro et al. (2017), the cowpea analogue has a lower glycemic index than conventional rice.[71]

3.5.2. Starch Characteristics. The amylose content substantially influences both the cooking quality and the sensorial characteristics of rice, whereas resistant starch has an impact on carbohydrate metabolism in human health and disease. The amylose content and the resistant starch content are presented in Table 3. The amylose content ranged from 19.08 to 24.87%, which is comparable to that of the control (26.55%). The amylose content is categorized as low (15–22%), intermediate (23–26%), high (27–30%), and very high (>30%); thus, the rice analogues can be classified as having an intermediate amylose content, which tends to be softer and stickier.[72] Furthermore, the higher the amylose content, the greater the retrogradation tendency that leads to an increase in resistant starch.[73] Regarding resistant starch, analogues showed values ranging from 0.47 to 1.56% below that of the control (3.89%), as expected.[74] Resistant starch is an important functional ingredient in food that is not digested in the small intestine, which provides it with several health benefits. It can be used to improve the nutritional quality of foods and is considered a form of dietary fiber due to its capacity to reduce the glycemic index of foods and increasing satiety after meals, making it an ally in promoting a more balanced and healthy diet.

3.5.3. Microelements. Minerals are micronutrients that play important roles in various systems of the human body, energy metabolism, and the immune system, such as (i) bone and teeth health, (ii) relaxation and contraction of muscles, (iii) nerve functioning, (iv) blood clotting and blood pressure regulation, (v) good balance in body fluids, and (vi) hormone components among others.[75] The sodium, potassium, magnesium, calcium, manganese, iron, zinc, copper, and phosphorus contents of rice analogues are presented in Table 4.

The magnesium found in the $T7^+$ formulation was 286.80 mg/100g, and in the $T9^+$ formulation, it was 278.25 mg/100g. According to the Brazilian Food Composition Table, polished white rice showed 29.5 mg of magnesium.[76] The values found in the analogues are in agreement with the labeling "rich", which corresponds at least 30% of the VDR, i.e., provides at least 126 mg/100g of magnesium per day considering both fortified formulations (Annex II of IN 75/2020).[36] Given these results, it would be important to conduct an in vitro digestion analysis as the next step to evaluate the bioavailability of

https://doi.org/10.1021/acsfoodscitech.5c01076
ACS Food Sci. Technol. 2026, 6, 732−745

magnesium in elderly individuals, such as the INFOGEST model.

It was observed that the original rice contained 8.20 mg of calcium, while the rice analogues presented high calcium values (323.96 and 316.63 mg/kg), probably due to the use of an emulsifier (calcium stearoyl-2-lactyl lactate).

Iron (4.18 and 4.88 mg/kg) and zinc (9.43 and 10.55 mg/kg) values are higher than those found in the control. Iron and zinc are considered trace elements and have specific functions in the human body. Foods containing these trace elements can help reduce the incidence of iron deficiency anemia and strengthen the immune system.[11]

3.5.4. Vitamin D Quantification.

Determining vitamin D levels in foods is essential for assessing the dietary vitamin D intake and correlating it with overall health. Vitamin D deficiency in elderly patients is common and a risk factor for various diseases including osteoporosis, sarcopenia, cardiovascular disease, cancer, and even mortality.[77] The vitamin D quantification is presented in Table 4.

At zero time, the vitamin D contents found in formulations T7$^+$ and T9$^+$ were 4.54 and 8.52 μg/100g, respectively. After 4 months of keeping at room temperature, the results found were 4.48 and 8.67 μg/100g for T7$^+$ and T9$^+$ formulations, respectively. After 6 months, there was a 144% reduction in the vitamin D content for sample T7$^+$ and 64% for sample T9$^+$, showing that, up to 4 months, there was no significant difference in vitamin D loss during storage at room temperature ($p < 0.05$).

The stability of vitamin D is a challenge due to its instability and heterogeneous distribution in food. It could be affected by some factors such as the food matrix, oxidation during storage, and poor retention during heat processing such as extrusion.[78] Considering that T7$^+$ and T9$^+$ were stored under identical conditions, the more pronounced decline in the vitamin D content in T7$^+$ after 6 months may be attributed to the presence of BF. The incorporation of raw beans likely introduced oxidoreductive enzymes (lipoxygenase), pro-oxidant compounds (polyunsaturated fatty acids), and phenolic (flavonoids) substances that can promote oxidative degradation or isomerization of the vitamin.[79−81]

In addition, modifications in the matrix composition and lipid distribution may have reduced the protective micro-environment surrounding vitamin D, thereby increasing its susceptibility to oxidation.[82,83] According to the Brazilian Food Composition Table,[76] raw polished white rice naturally does not contain vitamin D. The values found in the analogues are in accordance with the labeling "rich", which corresponds to at least 30% of the RDA, i.e., provides at least 4.5 μg/100g of vitamin D per day for both formulations until 4 months and T9$^+$ even after 6 months (Annex II of IN 75/2020).[36]

3.6. Pairwise Correlation Coefficient (r) and PCA

Regarding exploring the relationship between pasting properties, thermal and texture profile, cooking quality, grain dimensions, and amylose and resistant starch contents of rice analogues, a correlation matrix was calculated (Figure 5A). The correlogram indicates a strong positive relationship between resistant starch with peak viscosity ($r = 0.92$), final viscosity ($r = 0.89$), setback ($r = 0.83$), breakdown ($r = 0.89$), springiness ($r = 0.72$), enthalpy ($r = 0.71$), and amylose content ($r = 0.61$).

Amylose can easily form double helices after cooking and show a high level of retrogradation.[65] The positive correlation

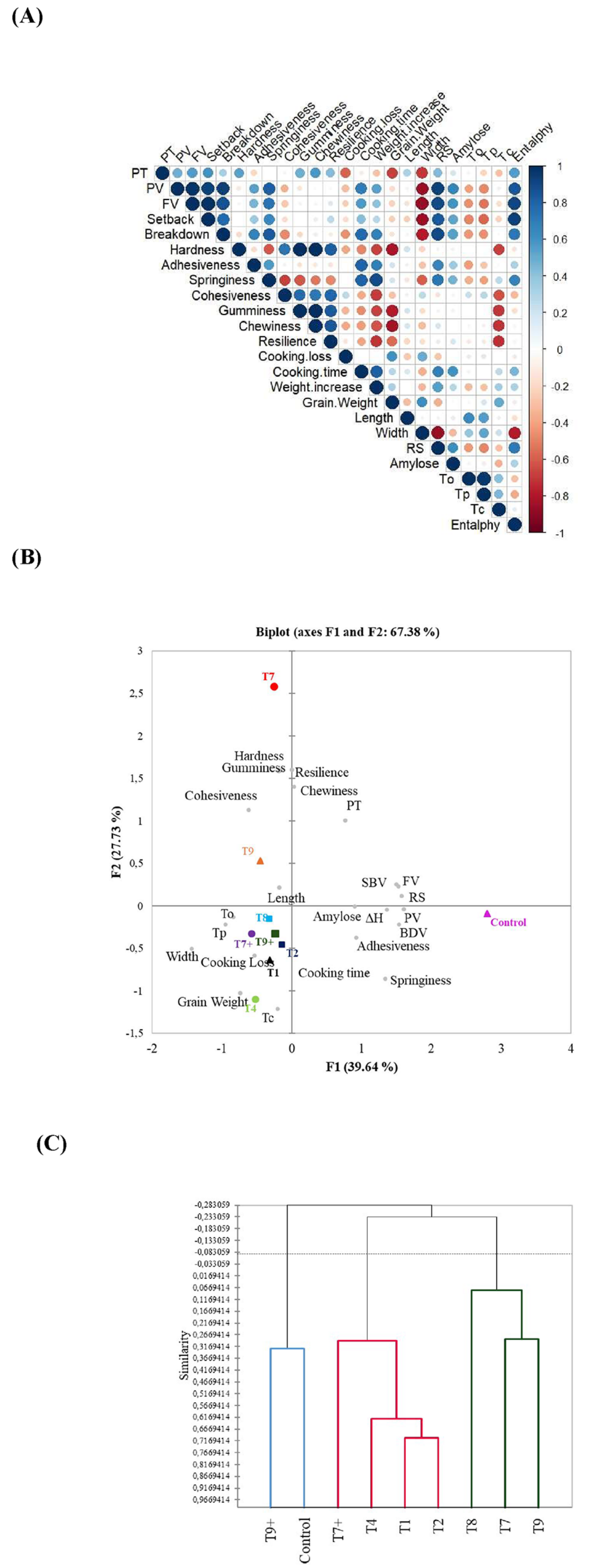

Figure 5. Pairwise correlation coefficient (r) for pasting, thermal, texture, grain dimension, and starch properties (A), biplot from PCA (B), and HCA by the principal components of PCA (C). PT = pasting temperature; PV = peak viscosity; FV = final viscosity; BDV = breakdown viscosity; SBV = setback viscosity; T_o = onset temperature; T_p = peak temperature; T_c = conclusion temperature; TS = total starch; and RS = resistant starch.

https://doi.org/10.1021/acsfoodscitech.5c01076
ACS Food Sci. Technol. 2026, 6, 732−745

with the amylose content and RS can be explained by the fact that the amylose content is a strong predictor of RS.[84] RS has a strong impact on enthalpy and the peak melting temperature, since higher levels of RS present a high degree of crystallinity and more stabilization of double helical structures.[85]

A strong negative correlation was found between the width, all parameters of pasting properties (PT: $r = -0.70$; PV: $r = -0.85$; BDV: $r = -0.86$, SBV: $r = -0.85$; FV: $r = -0.78$), and enthalpy ($r = -0.78$). A negative correlation was also observed between grain weight and hardness ($r = -0.81$), gumminess ($r = -0.78$), and chewiness ($r = -0.83$). Regarding the hardness and dimensions of the grains, our results are in contrast to those found in the literature.[86] These authors attribute the strong positive correlation between the hardness, surface area ($r = 0.91$), and volume ($r = 0.93$) of the rice to the compressive force required to break the rice grain in terms of their higher surface area and volume.

Biplot PCA was applied to evaluate the relationship among the 22 variables related to rice analogue characteristics (Figure 5B). The parameters of the close vectors are positive and correlated. PCA1 and PCA2 explained 67.38% of the total variance among four quality characteristics of rice analogues (physical, texture profile, cooking properties, and starch). PCA analysis evidenced the differences between the rice analogues and the control (polished rice). Dim 1 is described by hardness, gumminess, resilience, chewiness, pasting temperature, length, setback, final viscosity, and resistant starch. The other variables described by Dim 2 are amylose, thermal profile, cooking loss, cooking time, springiness, adhesiveness, pasting viscosity, and breakdown grain weight and width. HCA of the principal components of PCA was performed to better categorize similar parameters of rice analogues in groups (Figure 5C). HCA formed three sample groups according to their similarities: Cluster 1 ($T9^+$, control); Cluster 2 ($T7^+$, T4, T1, and T2), and Cluster 3 (T8, T7, and T9).

Our results showed that different formulations of rice analogues had positive effects on physical properties and starch characteristics. The technological qualities of the analogues were improved by using extrusion technology in the preparation of the flours, such as the reduction of cooking time. The T9+ formulation presented similar characteristics in terms of cooking loss and hardness, which are important parameters for the sensory acceptance of an elderly consumer. Therefore, it can be concluded that the formulations, specially T9+ containing 50:50 (RF:EERF), have the potential to be used as a rice analogue with adequate nutritional, technological, and texture characteristics in order to be a useful alternative for the food industry. Digestibility and bioavailability could be considered in further studies, as well as shelf life and consumer acceptability, in order to include rice analogues at the industrial scale.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.5c01076.

> Visual aspects of the $T9^+$ rice analogue before and after cooking (Figure S1); physical properties of rice analogues (Table S1); and texture parameters (TPA) of analogues of rice (Table S2)(PDF)

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Funding

The Article Processing Charge for the publication of this research was funded by the Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES), Brazil (ROR identifier: 00x0ma614). This research was financially supported by the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) (Processes no 140245/2020−0, 140214/2023−2, 161815/2024-3) and the Fundação Carlos Chagas

Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (Faperj) (Processes: E-26/210.022/2020, E-26/201.000/2021 and E-26/201.030/2021).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

AM,amylose content; ANOVA,analysis of variance; BDV,breakdown viscosity; BF,raw bean flour; CSL,calcium stearoyl-2-lactyllactate; CIE,International Commission on Illumination; DRV,daily reference value; DSC,differential scanning calorimetry; EERF,emulsified extruded rice flour; ERF,extruded rice flour; FV,final viscosity; PCA,principal component analysis; PV,peak viscosity; RF,raw rice flour; RS,resistant starch; RVA,rapid visco analyzer; SBV,setback; SEM,scanning electron microscopy; SS,soluble solids; Tc,conclusion temperature; To,onset temperature; Tp,paste temperature; TPA,texture profile analysis; ΔH,enthalpy

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