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Dual thermal valorization of jackfruit seeds for nutritionally tailored plant-based milk alternative

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ABSTRACT

Jackfruit (*Artocarpus heterophyllus*) seeds, often underutilized after fruit processing, are rich in starch, protein, and bioactive compounds, making them a promising candidate for producing non-dairy beverage formulations. This study evaluates the impact of dual thermal processing (roasting and repeated boiling) on the yield and nutritional composition, antinutritional factors, physicochemical, and functional properties of jackfruit seed-based milk alternatives (JSBMA). Optimized roasting conditions (72 °C, 34.36 min) based on response surface methodology increased JSBMA extraction yield by 13.7 %, improved its whiteness, and decreased pH, indicating acid formation and non-enzymatic browning. The optimal roasting significantly reduced antinutritional compounds (phytates and tannins), improved protein digestibility, and enhanced viscosity of JSBMA. Meanwhile, sequential boiling of the roasted dregs produced three additional extracts (JSBMA 2–4), each with declining protein, fat, and carbohydrate content. JSBMA 2 offered a favorable balance of nutrition, physical stability, and low glycemic index. JSBMA 1 and JSBMA 2 showed smaller particle sizes and better dispersion (higher stability coefficient), whereas JSBMA 3 and JSBMA 4 showed greater heterogeneity and sedimentation. Overall, the proposed processing strategy supports development of jackfruit seed-based milk alternatives with tailored composition and functionality.

1. Introduction

Lactose-free, vegan, and environmentally friendly alternatives to dairy milk are currently in high demand due to the expanding global market for plant-based beverages (Sethi et al., 2016; Brahma and Ray, 2023). Plant-based milk alternatives (PBMAs) are gaining popularity as protein-rich and functional beverages (McClements, 2020). Most of the conventional plant-based milks are derived from soy, oats, or almonds; however, these beverage systems are often associated with allergenicity, high glycemic index, and low protein content (Shkembi and Huppertz, 2023; Mäkinen et al., 2015; Yao et al., 2022). To meet rising demand sustainably, there is growing interest in utilizing underexplored plant resources (e.g., tropical seeds), as novel sources for PBMA development (Saeed et al., 2025).

Jackfruit (*Artocarpus heterophyllus* Lam) seeds offer a promising and sustainable alternative to conventional seeds for PBMA developments. These seeds, often underutilized after fruit processing, are rich in starch, protein, dietary fiber, and minerals (Swami et al., 2012). However, challenges such as the presence of antinutrients (phytates, tannins), off-flavors, and inconsistent functional properties limit their direct use in beverage applications. Several thermal and mechanical processing methods, including roasting and boiling, have shown promise in reducing these limitations and improving the nutritional and functional properties of seed-based milk beverages (Alajaji and El-Adawy, 2006).

The novelty of this study focuses on how sequential thermal processing influences the compositional profile of jackfruit seed-based milk alternatives. Unlike previous studies that have only examined simple pasteurization or single-process methods (Hartati, 2022), the combined

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effect of sequential heating steps on beverage composition remains less clearly defined. In this study, we evaluate a dual thermal strategy (optimized roasting followed by repeated boiling extractions) and quantify how proximate composition, sugars, amino acids, and key antinutritional factors change across successive extracts (JSBMA 1–4). These compositional shifts are then related to selected physicochemical/functional attributes relevant to beverage quality. The findings support the development of nutritionally tailored, plant-based milk alternatives from jackfruit seeds.

Accordingly, this study focuses on quantitative compositional characterization of jackfruit seed-based milk alternative produced after roasting and sequential aqueous extraction. Specifically, we quantify changes in proximate composition, major sugars, amino acid profile, and key antinutritional factors in the unroasted and roasted extracts, as well as across successive extracts (JSBMA 1–4) obtained by repeated boiling of the residual seed matrix. Physicochemical and selected functional indicators (e.g., pH, colour, viscosity and stability indices) are reported only to contextualize how compositional shifts may influence beverage-relevant properties.

2. Materials and methods

2.1. Raw material collection and preparation

Fully matured jackfruit (*Artocarpus heterophyllus* Lam, variety J33) was procured from Selangor Fruit Valley, Malaysia. The seeds were manually separated, washed, peeled to remove the brown outer seed coat, and sliced into uniform pieces for consistent heat exposure. Damaged or germinated seeds were discarded. The prepared seeds were stored at 4 °C and used within 24 h.

2.2. Processing design and preparation of JSBMA extracts

2.2.1. Roasting treatment and optimization

Jackfruit seed slices were roasted in a forced-convection oven (Copens Scientific, Malaysia). To optimize the roasting conditions, a Central Composite Design (CCD) based on Response Surface Methodology (RSM) was employed. Two independent variables included roasting temperature (70–153.36 °C) and roasting time (10–34.36 min). The response variables analyzed included extraction yield, macronutrient composition (protein, fat, fiber, ash, carbohydrate), pH, colour (whitening index), antinutritional factors (phytates, oxalates, tannins), sugar profile, and amino acid content. The CCD consisted of 13 experimental runs, including five centre points, designed to evaluate the linear, quadratic, and interaction effects of the roasting variables. The full design matrix with coded variables is provided in Supplementary Table S1.

Following roasting, the seeds were boiled at 100 °C for 2–3 min to soften the matrix, blended for 1 min, homogenized at 18,000 rpm for 10 min, and filtered through muslin cloth to obtain the plant-based milk extract (JSBMA 1). All extracts were stored at 4 °C prior to analyses.

2.2.2. Repeated boiling extraction of dregs (JSBMA 2–4)

The solid residue (dregs) remaining after the first extraction was reconstituted with water in a 1:3 (w/v) ratio and subjected to repeated boiling at 100 °C for 10, 15, and 20 min using a thermostatic water bath. After boiling, the mixture was filtered again to obtain JSBMA 2. This cycle was repeated twice to produce JSBMA 3 and JSBMA 4, respectively. All extracts were stored at 4 °C for further analysis.

2.3. Quantitative compositional analyses

2.3.1. Proximate composition of JSBMA

Protein was measured by the Kjeldahl method (conversion factor of 6.25). Crude fat was determined via Soxhlet extraction with petroleum ether, and crude fibre was analyzed via acid-alkali digestion. Standard AOAC (2005) methods were used to determine moisture and ash content. Total carbohydrates were calculated by difference:

$$\text{Carbohydrates (\%)} = 100 - (\text{moisture} + \text{ash} + \text{fat} + \text{protein} + \text{fiber})$$

2.3.2. Sugar profile

Glucose, fructose, and sucrose were quantified on a dry basis using the K-SUFRG kit (Megazyme, Ireland) following the manufacturer's protocol. Samples were clarified with Carrez I and II solutions, treated with NaOH, filtered, and measured using a UV–visible spectrophotometer (Epoch, Biotek).

2.3.3. Amino acid profile

Amino acid composition was determined using an automatic amino acid analyzer (Aracus, MembraPure GmbH, Germany) based on ion-exchange chromatography with post-column ninhydrin derivatization. Absorbance was measured at 570 nm for most amino acids and 440 nm for proline and hydroxyproline. Quantification was performed using amino acid standards, and results were expressed as g amino acid/100 g protein.

2.3.4. Antinutritional factors

Phytate content was determined using the Wade reagent method as described by Latta and Erskine (1980). A 0.5 g dried sample was extracted in 2.4 % HCl, centrifuged at 3000 rpm, and the supernatant reacted with Wade reagent. Absorbance was measured at 500 nm, and values were expressed as mg phytic acid/100 g using a standard curve. Total tannin content was estimated using the Folin-Ciocalteu (FC) assay, as described by Haile and Kang (2019). The extract was reacted with FC reagent and 35 % sodium carbonate, incubated for 30 min, and the absorbance was measured at 700 nm. Results were reported as mg tannic acid equivalent (TAE) per 100 g.

Oxalate content was analyzed by permanganate titration (Day and Underwood, 1986). One gram of sample was acid-extracted in 1.5 M H_2SO_4, filtered, and titrated with 0.1 M $KMnO_4$ at 80–90 °C until a persistent pink colour appeared. Results were reported in mg oxalate/100 g.

2.4. Physicochemical and functional properties

2.4.1. Extraction yield

A portion (10 mL) of each JSBMA was transferred into a weighing dish and dried at 105 °C in a drying oven until the weight of the sample remained constant. The extraction yield was expressed as:

Extraction yield (g/g) = Weight of extracted solids / Initial sample weight

2.4.2. pH and color (Whitening Index)

pH was measured using a calibrated digital pH meter (Mettler Toledo FiveEasy Plus). Whiteness Index (WI) was derived from colour parameters (L*, a*, b*) using a Minolta Chroma Meter (CR-410). With the obtained data, the whiteness index (WI) was calculated according to Lohman and Hartel (1994). Three measurements were taken from the triplications.

$$WI = 100 - \sqrt{(100 - L^*)2 + a^*2 + b^*2} \quad (1)$$

2.4.3. Physical stability, viscosity, and particle size

Apparent viscosity was measured using a rotational viscometer (NDJ-5S) with rotor 0 at 60 rpm and 25 °C (Shimoyamada et al., 2019). Centrifugal Sedimentation Rate (CSR) was measured by centrifuging 5 mL of milk at 4192.5 × g for 10 min. The CSR was calculated as:

$$w_1 = ((m_2\text{-}m_1)/m_0)\ x100 \quad (2)$$

In the above formula, w_1 is the centrifugal sedimentation rate, %; m_0 is the sample mass, mg; m_1 is the mass of the centrifuge tube, mg; and m_2 represents the mass of the centrifuge tube after discarding the supernatant, mg.

Particle size distribution and average diameter were measured using a laser diffraction analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK) equipped with a Hydro 2000MU unit (DKSH Technology, Malaysia). Distilled water (RI = 1.33) was the dispersion medium, and particle refractive indices were set at 1.76 (real) and 0.05 (imaginary). Particle size was reported as volume-weighted mean diameter (D[4,3]). A refractive index of 1.342 was used for cow milk (Singh et al., 1997).

The stability coefficient was measured by using the method described in (Zhang et al., 2017). The absorbance of the sample before and after centrifugation was measured at 785 nm with a UV–Visible spectrophotometer (UV-1280, Shimadzu Corporation, Kyoto, Japan). The stability coefficient was calculated as follows:

$$R = A_2/A_1 \quad (3)$$

In the above formula, R is the stability coefficient; A_1 denotes the absorbance of the sample before centrifugation; and A_2 is the absorbance of the supernatant after centrifugation.

According to the particle size, viscosity, and density of JSBMA, the sedimentation velocity was calculated as follows:

$$v = g(\rho_1 - \rho_2) \quad d_2/18\eta \quad (4)$$

In the above formula, v is the sedimentation velocity, nm/s; g is the gravitational acceleration, 9.80 m/s2; ρ_1 represents the particle density (density of all particles except water in JSBMA), g/cm3; ρ_2 denotes the water density, g/cm3; d refers to the particle diameter, cm; 18 is the conversion coefficient; and η is the viscosity of water, Pa·s.

2.4.4. Rheological behavior

Rheological measurements were carried out at 25 °C using a controlled-stress rheometer (Haake RheoStress 600, Thermo Electron Corporation, Germany). Samples were allowed to equilibrate on the plate for 1 min before measurement. Steady shear tests were conducted over a shear rate range of 1–100 s^{-1} to investigate the flow behavior of JSBMA after repeated boiling. The flow curves were modeled using the Ostwald–de Waele (Power Law) equation to determine flow consistency and flow behavior indices, as described by Steffe (1996).

2.4.5. In vitro starch digestibility and estimated glycemic index

For in vitro starch digestion, samples were pretreated with pepsin (gastric phase) and then incubated with α-amylase (intestinal phase). Aliquots were taken at 0, 30, 60, 90, 120, and 180 min. Glucose content was measured using the GOPOD, D-glucose assay. The results were used to calculate the Rate of Starch Hydrolysis (RSH), the Hydrolysis Index (HI), and the Estimated Glycemic Index (eGI) according to Graça et al. (2020) and Hakimah et al. (2020).

2.5. Statistical analysis (and RSM model)

All experiments were conducted in triplicate, and results are presented as mean ± SD. Analysis of variance (ANOVA) was used to determine significant differences ($p < 0.05$). For roasting optimization, a second-order polynomial regression model was fitted to each response:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \quad (5)$$

where x_1 is roasting temperature and x_2 is roasting time. Model adequacy was evaluated using R^2, adjusted R^2, and lack-of-fit tests. Optimization, regression, and response surface plots were generated using Minitab v19 (Minitab Inc., USA). The design matrix is provided in Supplementary Table S1.

3. Results and discussions

3.1. Effect of roasting temperature and time on yield of JSBMA

As indicated in Table 1, roasting conditions significantly influenced the extraction of nutritional solids in JSBMA, with roasting temperature emerging as the most influential factor. Because yield reflects the amount of soluble nutrients transferred from the seed matrix into the beverage, changes in yield directly impact the overall nutritional density of the plant-based milk alternative. The unroasted sample showed a low yield (0.54 ± 0.0 g/g), corresponding to limited release of proteins, carbohydrates, and soluble minerals.

Roasting enhanced nutrient extractability, with the average yield increasing to 0.61 ± 0.02 g/g after optimization. The highest individual yield (0.66 ± 0.001 g/g; Supplementary Table 2) was achieved at lower roasting temperatures (≤70 °C) coupled with longer roasting times (≥34 min). These conditions appear to preserve moisture and macromolecular integrity, facilitating better extractability. In contrast, higher temperatures (≥111 °C), particularly when combined with prolonged roasting, led to a marked decrease in yield, likely due to moisture loss, protein denaturation, and reduced solubility of macromolecules.

Statistical analysis confirmed these trends. As shown in the ANOVA results (Table 2), roasting temperature had a statistically significant effect on the yield ($p < 0.05$). In comparison, the effects of roasting time and its interaction with temperature were not statistically significant.

Total tannins are measured in mgTAE per 100 g, mg phytic acid equivalents and Oxalate in mg/100 g. All values are presented as mean ± standard deviation. TAE stands for Tannic Acid Equivalents, and PAE stands for Phytic Acid Equivalents. (J et al., 2013; Alozie and Udofia, 2015; Maria and Victoria, 2018; Nowshin et al., 2018).

The fitted quadratic model demonstrated strong explanatory power with an R^2 of 80.01 %, indicating a good fit between the model and observed data. The corresponding response surface plot (Fig. 1) visually reinforces this pattern, showing a consistent decrease in temperatures across all time levels. To support these findings, the original raw data used in the response surface regression model have been provided in Supplementary Table S2, which includes values for roasting temperature, roasting time, and corresponding extraction yields.

Additionally, the unroasted JSBMA sample exhibited a low yield (0.54 ± 0.01 g/ g), attributed to its limited soluble solids content. This value is notably lower than those typically reported for other plant-based beverage alternatives to milk (Table 1) (Tunde Akintunde and Akintunde, 2002), highlighting the role of controlled roasting in enhancing extractability in JSBMA.

3.2. Quantitative compositional profile after roasting

The compositional dataset (Table 1) indicates that roasting was associated with measurable shifts in proximate composition of JSBMA, consistent with enhanced extractability of seed constituents. Protein increased from 1.55 ± 1.21 % (unroasted) to 1.80 ± 0.01 % (after roasting), while fat remained low (0.40 ± 0.05 % to 0.46 ± 0.07 %). Dietary fiber increased from 0.36 ± 0.23 % to 0.71 ± 0.61 %, and ash increased from 0.03 ± 0.09 % to 0.09 ± 0.011 %. Carbohydrates decreased from 2.57 ± 1.01 % to 2.14 ± 0.12a%. These patterns suggest that roasting may preferentially enhance recovery of proteinaceous and fiber-associated fractions while also altering carbohydrate availability and partitioning.

When positioned against comparator plant-based beverages (Table 1), roasted JSBMA remained lower in protein than soybean milk (3.05–3.38 %) but aligned with the upper end of reported almond milk protein ranges (0.7–1.6 %). This is relevant for positioning JSBMA as a nutritionally meaningful seed-derived PBMA while retaining a low-fat profile. Overall, the quantitative proximate profile supports the use of jackfruit seed as a viable compositional base for PBMA development, with roasting acting as a pre-treatment to improve nutrient transfer into

Table 1
Physico-chemical, nutritional composition, and antinutritional factors of jackfruit seed-based milk alternative prepared from unroasted and roasted seeds.

Categories	Nutrient	Jackfruit seed-based milk	Almond milk	Soybean milk	JSBMA after roasting
Physicochemical analysis	Yield (g/g)	0.54 ± 0.01	0.53–0.56	0.59–0.84	0.61 ± 0.02
	pH	5.90 ± 0.64	6.53–6.93	6.50–6.58	5.40 ± 0.14
	Whitening index	73.97 ± 1.89	-	-	76.83 ± 1.12
Proximate composition %	Protein	1.55 ± 1.21	0.7–1.6	3.05–3.38	1.80 ± 0.01
	Fat	0.40 ± 0.05	1.6–2.3	2.04–2.26	0.46 ± 0.07
	Fiber	0.36 ± 0.23	0.11–0.28	0.05–0.12	0.71 ± 0.61
	Ash	0.03 ± 0.09	0.12–0.29	0.8–1.0	0.09 ± 0.011
	Moisture	95.57 ± 0.03	94–96	89–92	96.94 ± 0.21
	Carbohydrates	2.57 ± 1.01	0.33–2.77	2.5–3.1	2.14 ± 0.12[a]
Amino acids (g/100 g)	Leucine	4.3 ± 0.04	3.5	11.71	4.5 ± 0.07
	Lysine	3.9 ± 0.87	1.0	10.68	4.2 ± 0.1
	Isoleucine	3.9 ± 0.12	1.7	10.65	3.8 ± 0.05
	Valine	5.4 ± 0.06	2.0	9.38	5.4 ± 0.03
	Phenylalanine	4.6 ± 0.12	2.69	5.58	4.9 ± 0.23
	Methionine	0.2 ± 0.23	0.49	8.15	0.2 ± 1.21
	Proline	3.1 ± 0.56	-	8.14	3.5 ± 1.10
	Arginine	1.6 ± 0.54	-	13.31	1.7 ± 2.11
	Tyrosine	3.2 ± 0.08	-	12.69	3.4 ± 0.1
	Histidine	1.1 ± 1.06	-	9.16	1.0 ± 0.05
	Cysteine	0.1 ± 0.02	-	8.90	0.1 ± 0.34
	Alanine	2.7 ± 0.06	-	4.47	3.6 ± 1.21
	Glutamic acid	6.3 ± 0.05	-	9.96	6.3 ± 0.03
	Glycine	4.3 ± 1.05	-	6.63	4.5 ± 1.20
	Threonine	2.2 ± 0.03	1.6	10.49	2.6 ± 0.32
	Serine	1.4 ± 1.22	-	4.64	1.6 ± 1.13
Sugars (g/100 g)	Glucose	0.32 ± 0.05	-	-	0.31 ± 0.007
	Fructose	1.17 ± 1.20	-	-	1.15 ± 0.43
	Sucrose	1.27 ± 1.04	-	-	1.34 ± 0.05
Antinutritional component	Tannins	15.57 ± 0.19	0.10	< 0.1	12.69 ± 1.30
	Phytates	0.20 ± 0.02	1.5–3	23.35	0.18 ± 0.01
	Oxalate	18.90 ± 1.88	0.08	< 0.1	12.12 ± 0.35

Table 2
Analysis of variance for different responses of JSBMA.

Source	DF	Yield	pH	WI	Phytate	Oxalate	Tannins	Glucose	Fructose	Sucrose
Model	5	0.173119*	3.00348*	380.555*	0.00494*	79.6336*	228.938*	0.03640*	0.02639*	0.16089*
Linear	2	0.145367*	2.33448*	269.691*	0.00405*	64.3135*	227.538*	0.03207*	0.02372*	0.13868*
X_1	1	0.13802*	2.18407*	235.752*	0.00370*	44.9634*	224.090*	0.26667*	0.02042*	0.13202*
X_2	1	0.00735*	0.15042	33.99*	0.00035*	19.3501*	3.447	0.00540*	0.00282*	0.00666
Quadratic	2	0.02415	0.63837*	52.341*	0.00075*	7.1833*	0.053	0.00962	0.00341*	0.014113
X_1^2	1	0.021104	0.51068*	44.898*	0.00069*	4.7279*	0.0221	0.00050	0.00208*	0.00154
X_2^2	1	0.00018	0.00338	0.001	0.00001	0.3850	0.012	0.00003	0.00015	0.01407*
Interaction	1	0.00360	0.03062	58.522*	0.00014*	8.1368*	1.348	0.00360*	0.00023	0.00810*
X_1 x X_2	1	0.00360	0.03062	58.522*	0.00014*	8.1368*	1.348	0.00360*	0.00026	0.00810*
Lack of Fit	3	0.591	0.400	0.204	0.496	0.06	0.445	0.314	0.126	0.238
R^2	-	80.01	91.85	97.89	96.80	97.47	92.54	96.54	93.41	93.07

X_1: Roasting temperature, X_2: Roasting time, WI: Whitening index, DF: degree of freedom, * Significant at $p < 0.05$

the beverage phase.

3.3. Effect of roasting on sugar composition of JSBMA

The ANOVA results (Table 2) demonstrated that roasting temperature and time significantly influenced the sugar profile of jackfruit seed-based milk alternative (JSBMA). The primary sugars identified were sucrose, fructose, and glucose. In the unroasted sample, sucrose was the most abundant (1.27 ± 1.04 g/100 g), followed by fructose (1.17 ± 1.12 g/100 g) and glucose (0.32 ± 0.05 g/100 g). These sugar distributions were similar to those commonly seen in seed-based systems (Tesfay et al., 2016).

At lower roasting temperatures (70–111 °C), sucrose content initially increased, likely due to cell wall softening and improved extractability of soluble sugars. However, at higher temperatures (120–153.36 °C), sucrose levels significantly declined. This reduction may be attributed to thermal hydrolysis and sugar degradation during heat treatment, as previously reported (Yaylayan and Kaminsky, 1998; Goff, 2017; Zhang et al., 2020a). While caramelization typically initiates around 160 °C, recent studies have shown that sugar degradation, interconversion, or early-stage caramel-like reactions can occur at slightly lower temperatures, especially in moist food systems or under prolonged heating (Wang et al., 2015). Therefore, the observed reductions in sucrose and glucose can be linked to both hydrolytic breakdown and limited early-stage caramelization during roasting.

Statistical modelling further confirmed the significant impact of roasting parameters. Sucrose content showed a strong dependence on roasting temperature ($R^2 = 93.07$ %), with a significant quadratic effect of time, suggesting a dynamic balance between sugar release and degradation. Glucose levels declined sharply (−65.62 %) with increasing roasting intensity, likely due to its high reactivity in Maillard-type reactions. This trend was supported by a high coefficient of determination ($R^2 = 96.54$ %) and a significant interaction between time and temperature (Guo et al., 2022). In contrast, fructose was more thermally stable, exhibiting only a 12.82 % reduction. Nevertheless, its content was still significantly influenced by both linear and quadratic terms ($R^2 = 93.41$ %). All regression models exhibited a non-significant lack of fit, confirming their reliability in predicting roasting-induced changes in

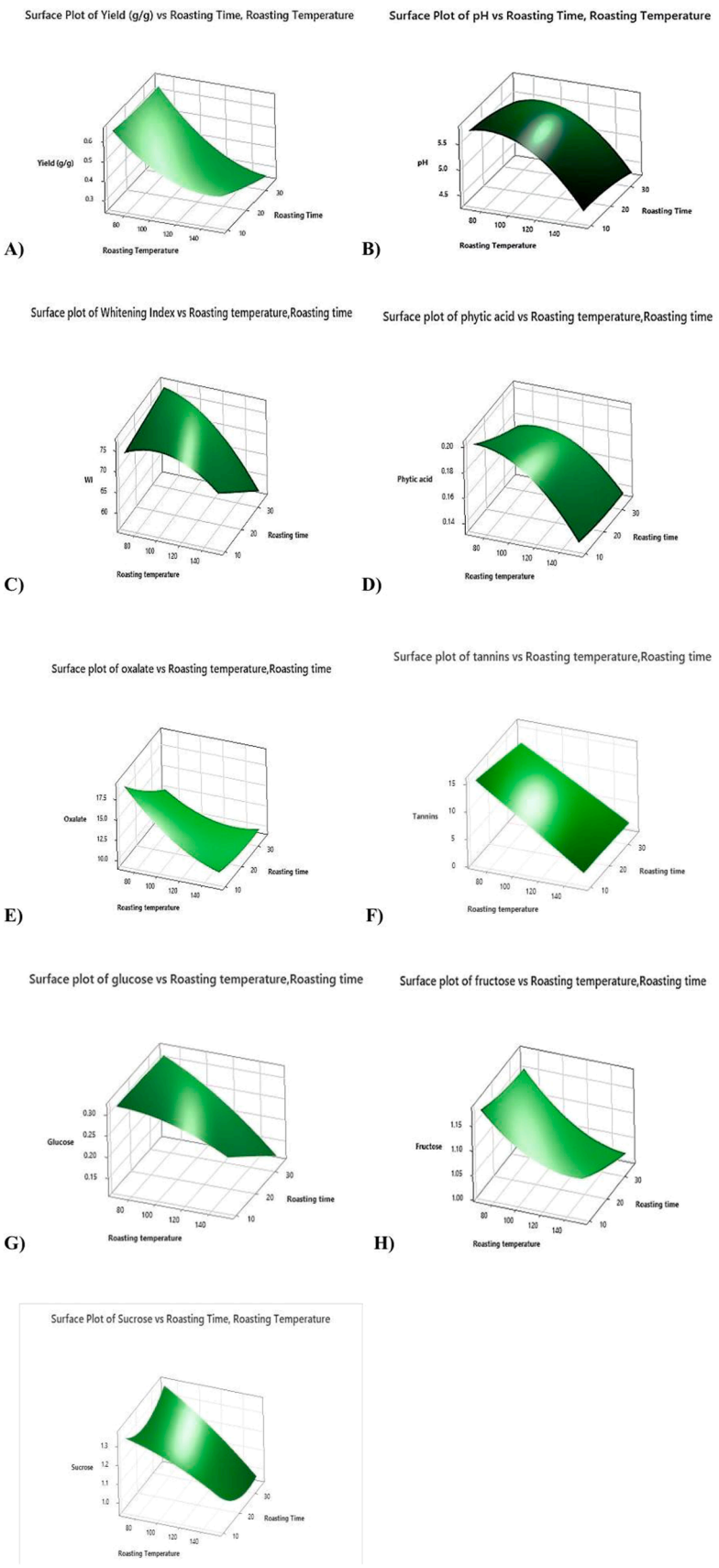

Fig. 1. Response surface plots showing the effects of roasting temperature and time on (A) extraction yield, (B) pH, (C) whiteness index (WI), (D) phytic acid, (E) oxalate, (F) tannins, (G) glucose, (H) fructose, and (I) sucrose content of jackfruit seed-based milk analogues (JSBMA).

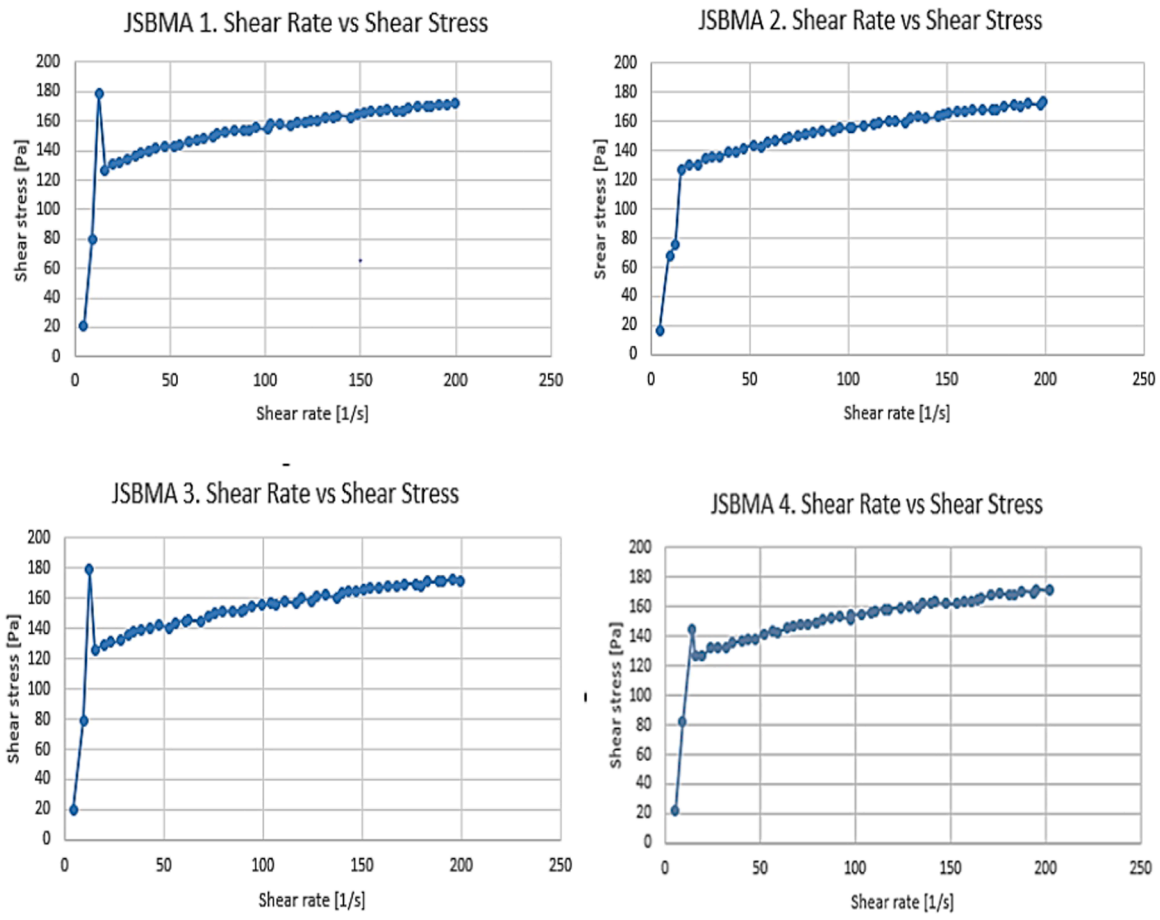

Fig. 2. Flow behaviour of jackfruit seed-based milk analogues (JSBMA) fitted with the power law model, demonstrating shear-thinning properties.

sugar composition.

3.4. Amino acid profile as an indicator of protein nutritional quality under roasting

The amino acid composition of jackfruit seed-based milk (JSBMA) reflects a nutritionally valuable profile, with both essential and non-essential amino acids contributing to its quality. Among the essential amino acids, leucine, lysine, isoleucine, valine, phenylalanine, methionine, threonine, and histidine were all detected in appreciable amounts. Optimized roasting (72 °C for 34.36 min) slightly enhanced their concentrations, notably leucine (from 4.3 to 4.54 mg/100 g), lysine (3.9–4.2), and threonine (2.2–2.6), likely due to increased protein solubility and unfolding that improves amino acid extractability (Nosworthy and House, 2017).

However, high-temperature roasting (153.36 °C) degraded heat-sensitive amino acids, such as valine and lysine, possibly via Maillard reactions and thermal decomposition (Ma et al., 2011; Zhang et al., 2021).

Among non-essential amino acids, a significant increase was observed in alanine (from 2.7 to 3.6 mg/100 g, +33.3 %), proline (3.1–3.5, +12.9 %), and cysteine (0.1–0.2, +100 %), indicating enhanced availability post-roasting. Notably, glutamic acid (6.3 mg/100 g) and glycine (4.3) remained stable, suggesting resistance to moderate thermal conditions. Overall, ANOVA confirmed no significant losses in total amino acid content across roasting conditions, reinforcing the thermal resilience of jackfruit seed proteins. These findings suggest that controlled roasting can improve the nutritional profile of JSBMA by enhancing the availability of both essential and non-essential amino acids while minimizing thermal degradation.

3.5. Reduction of antinutritional factors under roasting

Jackfruit seeds are a nutrient-rich resource, and their utilization in food applications is limited due to the presence of antinutritional compounds such as phytic acid, oxalates, and tannins, which are known to reduce mineral bioavailability and protein digestibility (Swami et al., 2012). The initial concentrations of these compounds in unroasted jackfruit seed-based milk alternative (JSBMA), as shown in Table 1, were phytic acid: 0.20 ± 0.02 mg/100 g, oxalates: 18.90 ± 1.88 mg/100 g, and tannins: 15.57 ± 0.19 mg/100 g. These compounds are known to chelate minerals such as calcium, iron, and zinc, reducing their absorption (Hassan et al., 2011).

Roasting significantly reduced all three antinutritional components, particularly at higher temperatures and longer durations. Phytic acid content decreased by 34.63 %, reaching 0.13 ± 0.04 mg/100 g at 153.36°C, confirming that thermal treatment breaks down phytates and enhances mineral availability (Noor Fadilah et al., 2021; Sewwandi et al., 2022). Oxalate levels dropped by 49.52 % to 9.54 mg/100 g under higher-temperature conditions, reducing the risk of calcium oxalate crystal formation (Bossi et al., 2024). Tannin content was the most affected, decreasing by 91.2 % to1.37 mg/100 g, which may improve protein digestibility and reduce astringency (Mbah et al., 2012).

ANOVA (Table 2) confirmed that phytic acid was significantly affected by both roasting temperature and time, including their interaction, while the quadratic term for time was not significant, suggesting limited benefit from extending roasting time beyond a certain point. Oxalate showed significant linear and quadratic effects of both factors with a strong interaction, indicating a non-linear, synergistic reduction pattern. In contrast, tannin reduction was primarily temperature-driven, with time and interaction terms not significant.

Although Codex Alimentarius and the U.S. FDA have not set official limits for these antinutritional factors, proposed thresholds in the literature include phytic acid > 300 mg/100 g (reduced mineral absorption), oxalate > 250 mg/100 g (kidney stone risk), and tannins > 100 mg/100 g (reduced protein and micronutrient bioavailability) (Sotelo et al., 2010; Lazarte et al., 2015; Lee et al., 2015). In this study, both unroasted and roasted JSBMA contained levels far below these values, indicating nutritional safety and supporting roasting as an effective approach to improving the beverage's nutritional and functional quality.

3.6. Physicochemical attributes reflecting roasting-driven compositional modification

The pH of unroasted JSBMA was 5.90 ± 0.64 (Table 1), slightly acidic and comparable to other plant-based milks like soy and almond (pH 6.0–7.0) (Kundu et al., 2018). Increasing roasting temperature significantly reduced pH from 5.90 ± 0.68–4.40 ± 0.89 across 70–153.36 °C, suggesting enhanced acid formation through thermal degradation. In contrast, roasting time (10–34.36 min) did not have a significant effect as confirmed by ANOVA (Table 2), and further supported by the original data provided in Supplementary Table S2. The quadratic term for temperature was substantial, indicating a non-linear decrease in pH more pronounced beyond 120 °C, while the time for the quadratic term remained non-significant. These results confirm that temperature is the primary factor affecting pH, consistent with observations by Saunders and Jervis (1966), who linked acidification to protein and sugar oxidation during roasting.

Colour changes, expressed as the Whitening Index (WI), also reflected the thermal severity of roasting. The Whitening Index (WI) of unroasted JSBMA was 73.97 ± 1.89 (Table 1), reflecting bright visual appeal. WI declined from 76.78 ± 2.11 at 70 °C to 61.30 ± 4.12 at 153.36 °C, indicating melanoidin formation via Maillard and caramelization reactions. ANOVA results (Table 2) showed roasting temperature significantly affected WI; roasting time and their interaction were also significant, although the quadratic effect of time was not. The model showed a strong fit with a non-significant lack of fit. These physicochemical shifts also carry nutritional relevance. The reduction in pH and WI reflects the breakdown of carbohydrates and polyphenolic structures, processes that can enhance digestibility and modify antioxidant behaviour (Sun et al., 2022). The formation of Maillard reaction products may further influence antioxidant capacity and mineral-binding characteristics, contributing to the overall nutritional profile of JSBMA.

3.7. Interpretation of surface plots of JSBMA

The surface plots in Fig. 1 revealed significant relationships between roasting parameters (time and temperature) and the product physicochemical properties. As roasting intensity increased, several consistent trends emerged. Product yield (A) decreased linearly with roasting intensity, consistent with expectations of moisture loss and pyrolytic mass reduction. The pH (B) decreased progressively with temperature, indicating an increase in acidity. This trend is characteristic of thermal degradation processes, where organic acids form as byproducts of Maillard reactions and carbohydrate breakdown. The WI (C) declined sharply under more intense roasting conditions, reflecting pronounced browning. This is attributable to the formation of melanoidins and other polymeric pigments generated during non-enzymatic browning reactions.

Antinutritional compounds, phytic acid (D), oxalates (E), and tannins (F) showed progressive declines, indicating thermal degradation. This reduction may improve mineral bioavailability in the final product. Glucose (G) and fructose (H) exhibited a biphasic response: initial increases at moderate roasting conditions (likely due to sucrose hydrolysis) followed by declines at higher temperatures as these reducing sugars were further degraded or incorporated into Maillard products. Sucrose content (I) diminished significantly with elevated roasting intensity, suggesting thermal decomposition or participation in browning reactions.

3.8. Model verification at optimized roasting condition

Roasting-induced changes in both nutritional and antinutritional properties were evident across all measured responses. Compared with the unroasted control, roasting at the optimized condition (72 °C for 34.36 min, as shown in Table 1) significantly improved multiple quality attributes. The yield increased from 0.54 ± 0.01–0.61 ± 0.02 g/g (+13.7 %), likely due to enhanced extractability resulting from cell wall disruption and protein denaturation. The whiteness index (WI) rose from 73.97 ± 1.89–76.83 ± 1.12 (+3.8 %), indicating a brighter, more appealing product colour. A pH reduction from 5.90 to 5.42 (−8.1 %) was observed, which may reflect the formation of organic acids during Maillard reactions and other thermal pathways.

Roasting also reduced antinutritional compounds, with phytates decreasing by 13.0 %, oxalates by 34.7 %, and tannins by 19.1 %, supporting improved nutritional quality. For the sugar profile, sucrose increased by 5.51 %, potentially reflecting partial breakdown of complex polysaccharides during roasting. In contrast, glucose and fructose remained relatively stable, showing only minor decreases (−3.44 % and −1.71 %, respectively), consistent with limited involvement of these reducing sugars in early-stage Maillard reactions under the relatively mild roasting condition.

Model adequacy was confirmed by validation at 72 °C for 34.36 min. In Supplementary Table S3, predicted and actual values for yield, pH, WI, antinutrients, and sugars were closely aligned, with error margins < 10 % and no significant differences ($p > 0.05$), confirming robustness of the RSM model. Thereafter, roasted jackfruit seed dregs were subjected to repeated boiling for additional extracts to evaluate compositional retention across sequential fractions.

3.9. Compositional changes across sequential extracts during repeated boiling

The proximate composition of all JSBMA following roasting and successive boiling cycles is presented in Table 3. JSBMA 1, extracted without boiling, exhibited the highest nutritional content, with protein at 1.80 ± 0.01 %, carbohydrates at 2.14 ± 0.12 %, fat at 0.46 ± 0.07 %, and dietary fiber at 0.71 ± 0.61 %. These values reflect the initial yield from roasted jackfruit seed powder, which contains the most extractable soluble compounds.

Subsequent extractions through boiling (JSBMA 2–4) showed a statistically significant reduction in protein content ($p < 0.05$), likely due to leaching, thermal denaturation, and matrix saturation. According to post hoc Tukey tests, JSBMA 1 had significantly higher protein than all boiled samples, while no significant differences were observed among JSBMA 2, 3, and 4. The progressive nutrient decline aligns with findings by Fan et al. (2023), who reported similar reductions with repeated aqueous extractions in seed-based formulations. Increased boiling time (10–20 min) further influenced nutrient recovery. While JSBMA 2 (10 min) retained moderate values, JSBMA 3 and 4 exhibited marginal levels of protein, fat, and carbohydrates.

Minor increases in ash and fiber in later samples suggest enhanced extraction of non-soluble minerals and polysaccharides. However, extended boiling for 15 or 20 min may compromise thermolabile components and reduce the overall extractable yield. Thus, a 10-minute boil appears optimal for balancing nutrient recovery with preservation.

3.10. Functional properties of JSBMA after repeated boiling: viscosity, stability, rheology, and particle size

The physical properties of JSBMA 1–4 were evaluated for viscosity, stability, sedimentation rate, sedimentation velocity, and density, as

Table 3
Nutritional composition of JSBMA under different boiling times and extraction cycles.

Boiling Time	Milk Sample	Protein %	Fat%	Ash %	Dietary fiber %	Carbohydrates %
0 min	JSBMA 1	1.80 ± 0.01^{a}	0.46 ± 0.07^{a}	0.09 ± 0.011^{a}	0.71 ± 0.61^{a}	2.14 ± 0.12^{a}
10 min	JSBMA 2	0.77 ± 0.031^{b}	0.36 ± 0.01^{b}	0.032 ± 0.015^{b}	0.08 ± 0.019^{b}	0.47 ± 0.02^{b}
	JSBMA 3	0.29 ± 0.040^{c}	0.11 ± 0.011^{c}	0.013 ± 0.03^{c}	0.012 ± 0.03^{b}	0.2 ± 0.003 ^{cd}
	JSBMA 4	0.07 ± 0.031^{d}	0.036 ± 0.01^{d}	0.003 ± 0.004^{c}	0.01 ± 0.047^{b}	0.07 ± 0.05^{d}
15 min	JSBMA 2	0.76 ± 0.041^{b}	0.34 ± 0.016^{b}	0.030 ± 0.006^{b}	0.09 ± 0.034^{b}	0.49 ± 0.023^{b}
	JSBMA 3	0.22 ± 0.007^{c}	0.12 ± 0.025^{c}	0.013 ± 0.003^{c}	0.013 ± 0.046^{b}	0.18 ± 0.014 ^{cd}
	JSBMA 4	0.07 ± 0.033^{d}	0.04 ± 0.016^{d}	0.004 ± 0.03^{c}	0.008 ± 0.03^{b}	0.072 ± 0.01^{d}
20 min	JSBMA 2	0.67 ± 0.05^{b}	0.28 ± 0.04^{b}	0.030 ± 0.05^{b}	0.076 ± 0.05^{b}	0.5 ± 0.03^{bc}
	JSBMA 3	0.19 ± 0.05^{c}	0.09 ± 0.005^{c}	0.014 ± 0.011^{c}	0.014 ± 0.003^{b}	0.18 ± 0.017 ^{cd}
	JSBMA 4	0.05 ± 0.02^{d}	0.01 ± 0.014^{d}	0.004 ± 0.04^{c}	0.01 ± 0.10^{b}	0.07 ± 0.012^{d}

Values are presented as mean ± SD (n = 3) significant differences ($p < 0.05$)

shown in Table 4. In general, plant-based drinks tend to be physically less stable than cow's milk. These parameters are critical in evaluating the colloidal stability and consumer acceptability of plant-based beverages.

Viscosity, a key quality attribute, was highest in JSBMA 1 (9.50 ± 0.16 mPa·s), likely due to its richer starch-protein matrix. It decreased progressively in JSBMA 2 (6.27 ± 0.20 mPa·s), JSBMA 3 (4.13 ± 0.10 mPa·s), and JSBMA 4 (3.93 ± 0.11 mPa·s), indicating thinning effects from repeated boiling. Compared to commercial alternatives, JSBMA 1 aligns with soymilk (8–12 mPa·s), while JSBMA 3 and JSBMA 4 resemble thinner almond milks (Jeske et al., 2017; Manzoor et al., 2021).

Stability coefficient peaked in JSBMA 2 (0.723 ± 0.0004), followed by JSBMA 1 (0.694 ± 0.0004), suggesting better dispersion in early extracts. JSBMA 3 and 4 exhibited poor stability (<0.04), likely due to prolonged heating-induced matrix degradation. Sedimentation rate was highest in JSBMA 1 (1.494 ± 0.04 %) and declined across JSBMA 2–4, suggesting improved suspension stability with repeated extraction. Conversely, sedimentation velocity increased from JSBMA 1 (16.3 ± 0.06 nm/s) to JSBMA 4 (33.7 ± 1.06 nm/s), indicating faster settling due to reduced viscosity and matrix cohesion.

Density values (1.0019–1.025 g/cm^3) remained within the typical range for plant-based milks (Chalupa-Krebzdak et al., 2018) and showed no significant variation after boiling. Estimated particle density varied slightly across JSBMAs, but high variability suggests uncertainty due to matrix breakdown or reaggregation.

Stability coefficient was highest in JSBMA 2 (0.723 ± 0.0004) and decreased markedly in later extracts, indicating that compositional depletion and cumulative heating may weaken matrix interactions responsible for dispersion stability. Particle size distribution data (Table 4) also supported this: broader spans and larger D90 values in JSBMA 2–4 suggest aggregation and reduced colloidal uniformity, consistent with reports that heating promotes protein–polysaccharide interactions and phase separation in plant-based systems (Jeske et al., 2017; Benjakul et al., 2016). As larger particles settle faster (Salvia-Trujillo et al., 2013), the observed PSD differences provide a structural basis for sedimentation behavior.

Rheologically, all JSBMAs showed pseudoplastic behavior (n < 1), consistent with macromolecule-rich dispersions (Jeske et al., 2017; Silva et al., 2019; Fiorda et al., 2013). The higher consistency index in earlier extracts aligns with their higher compositional density, while later extracts reflect weakened structuring as solids are depleted. Together, these outcomes reinforce that quantitative compositional dilution across extraction cycles explains the technological behavior, allowing the manuscript to remain composition-centered while retaining functional relevance.

Table 4
Physical, rheological, and particle size properties of JSBMAs prepared through repeated boiling cycles.

Property	JSBMA 1	JSBMA 2	JSBMA 3	JSBMA 4
Viscosity (mPa·s)	9.50 ± 0.16^{a}	6.27 ± 0.20^{b}	4.13 ± 0.10^{c}	3.93 ± 0.11^{c}
Stability Coefficient	0.694 ± 0.0004^{b}	0.723 ± 0.0004^{a}	0.0377 ± 0.0003^{c}	0.0026 ± 0.002^{d}
Sedimentation Rate (%)	1.494 ± 0.04^{a}	1.102 ± 0.017^{b}	0.497 ± 0.025^{c}	0.220 ± 0.02^{d}
Sedimentation Velocity (nm/s)	16.3 ± 0.06^{d}	29.3 ± 1.2^{c}	33.5 ± 0.09^{ab}	33.7 ± 1.06^{a}
Density (g/cm^3)	1.022 ± 0.1^{a}	1.025 ± 0.16^{a}	1.0019 ± 0.05^{a}	1.0023 ± 0.05^{a}
Estimated particle density (g/cm^3)	1.28 ± 0.02^{a}	1.81 ± 1.10^{a}	1.84 ± 1.06^{a}	1.76 ± 1.04^{a}
K (Pa·s^n)	0.46 ± 0.02	0.42 ± 0.08	0.39 ± 0.02	0.40 ± 0.05
N	0.51 ± 0.01	0.45 ± 0.05	0.50 ± 0.01	0.42 ± 0.03
R^2	0.98	0.98	0.99	0.98
D50 (μm)	0.59 ± 0.01^{b}	0.41 ± 0.013^{c}	0.63 ± 0.01^{a}	0.58 ± 0.008^{b}
D90 (μm)	4.08 ± 0.03^{d}	5.96 ± 0.04^{c}	9.43 ± 0.06^{a}	8.10 ± 0.014^{b}
D[4,3] (μm)	1.71 ± 0.03^{d}	2.14 ± 0.08^{c}	2.84 ± 0.05^{a}	2.63 ± 0.01^{b}
Span	2.58 ± 0.07^{d}	4.93 ± 0.06^{a}	4.22 ± 0.02^{c}	4.42 ± 0.04^{b}

Values are presented as mean ± SD (n = 3) significant differences ($p < 0.05$). n = flow index

3.11. In vitro digestibility and estimated glycemic Index (eGI)

The estimated glycemic index (eGI) and glycemic load (eGL) values of the JSBMA samples and commercial almond milk are presented in Table 5. A clear decreasing trend was observed in both eGI and eGL from JSBMA 1 through JSBMA 4, indicating a progressive reduction in post-prandial glycemic potential with each successive extraction cycle. JSBMA 1, prepared from the first boiling of roasted jackfruit seed slurry, exhibited the highest eGI (46.26 ± 0.21) and eGL (0.639 ± 0.014), which can be attributed to its higher content of readily digestible starch. In contrast, JSBMA 2, 3, and 4, obtained through repeated boiling of the residual seed matrix, demonstrated significantly reduced glycemic indices (43.62 ± 0.51, 41.70 ± 0.52, and 40.62 ± 0.29, respectively) and correspondingly lower glycemic loads (0.200 ± 0.013, 0.093 ± 0.016, and 0.020 ± 0.031, respectively).

These observations were further supported by in vitro starch hydrolysis curves (Fig. 3), which showed that white bread hydrolyzed

Table 5
Estimated glycemic index (eGI) and glycemic load (eGL) of JSBMA samples.

Sample	eGI (Mean ± SD)	eGL (Mean ± SD)
JSBMA 1	46.26 ± 0.21^{a}	0.639 ± 0.014^{a}
JSBMA 2	43.62 ± 0.51^{b}	0.200 ± 0.031^{ab}
JSBMA 3	41.70 ± 0.52^{c}	0.093 ± 0.016^{b}
JSBMA 4	40.62 ± 0.29 ^{cd}	0.019 ± 0.013^{c}
Almond milk	40.13 ± 0.60^{d}	0.159 ± 0.010^{bc}

Values are expressed as mean ± standard deviation (n = 3). P < 0.05

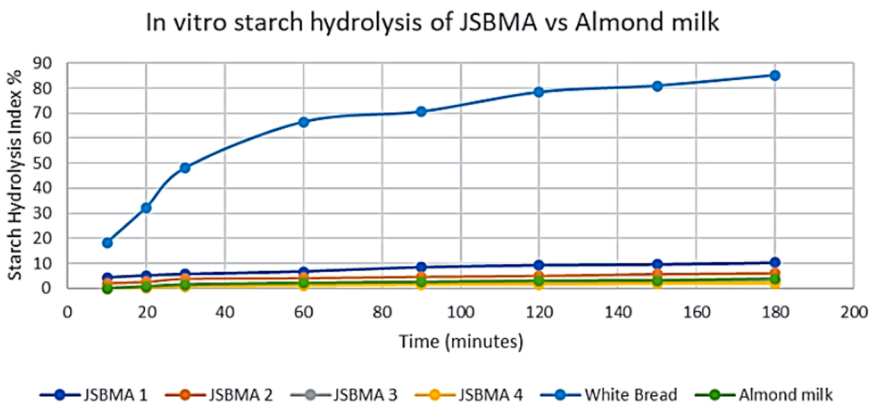

Fig. 3. In vitro starch hydrolysis profile of JSBMA during simulated digestion, indicating glucose release over time and estimated glycemic response.

rapidly (85 % by 180 min), confirming its high glycemic index. In contrast, all JSBMA samples showed significantly lower hydrolysis rates, with JSBMA 1 peaking around 10 % and JSBMA 4 remaining below 5 %, indicating an increasing resistance to enzymatic digestion with successive extractions. Almond milk, used as a low-GI control, maintained minimal hydrolysis throughout, closely paralleling the behavior of JSBMA 4.

The mechanisms that explained this glycemic decline were the thermal degradation and progressive depletion of gelatinized starch due to repeated boiling, and the formation of resistant starch (RS3) through starch retrogradation during successive heating and cooling cycles. Retrograded starch is less susceptible to enzymatic hydrolysis, thereby reducing glucose release upon digestion (Wang et al., 2015). This is supported by our in vitro glucose release data, which showed a significant decrease from 1.40 mg/g in JSBMA 1–0.072 mg/g in JSBMA 4. These findings are consistent with prior research indicating that thermal treatment alters starch crystallinity and digestibility (Chung et al., 2011; Mahasukhonthachat et al., 2010; Lu et al., 2023).

Furthermore, the naturally occurring resistant starch and dietary fiber in jackfruit seeds (Swami et al., 2012) may contribute to the attenuation of glycemic response. Particle size also played a role, with JSBMA 4 exhibiting larger, more aggregated particles that likely hindered enzymatic accessibility and diffusion. Notably, when compared to commercial almond milk, JSBMA 4 exhibited comparable or even superior glycemic performance, positioning it as a promising low-GI, sustainable, and cost-effective plant-based beverage for individuals requiring glycemic control.

4. Conclusions

This study demonstrates that jackfruit seeds can be converted into a functional plant-based milk alternative using an optimized dual-thermal strategy that combines roasting and sequential extraction. Roasting at 72 °C for 34.36 min improved extraction yield and product quality while reducing key antinutritional factors in the primary extract (JSBMA 1). Subsequent boiling of the roasted residues produced additional fractions (JSBMA 2–4) with progressively reduced macronutrient levels and distinct physicochemical behavior. Overall, JSBMA 2 offered the most favorable balance between nutritional contribution and beverage-relevant performance (colloidal stability and lower estimated glycemic response), supporting its potential as a functional beverage base. A key limitation is the reduced nutrient density and stability of later extracts (JSBMA 3–4), which may limit standalone use without formulation support. Future work should focus on sensory acceptance, storage stability, and in vivo/clinical validation to enable product optimization and commercialization.

CRediT authorship contribution statement

Amna Saeed: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Noranizan Mohd Adzahan:** Writing – review & editing, Supervision, Resources, Methodology. **Farooq Anwar:** Writing – review & editing, Validation, Supervision, Methodology. **Norhasnida Zawawi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Wan Zunairah Wan Ibadullah:** Writing – review & editing, Validation, Supervision, Methodology.

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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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2025.108836.

Data availability

Data will be made available on request.

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