

Effect of debranching enzyme hydrolysis and microwave treatments on the resistant starch enrichment of breadfruit

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Abstract: Breadfruit's substantial carbohydrate content makes it a viable starch source, specifically resistant starch (RS) that helps prevent chronic diseases. This study investigated the effects of enzyme hydrolysis and microwave treatment (MT) on enriching type III RS in breadfruit. It also determined its structural and functional properties, including swelling power, solubility index, water absorption capacity, oil binding capacity, and syneresis. MT at $30 \text{ W} \cdot \text{g}^{-1}$ for 3 min resulted in the highest RS content of 74.8%, significantly surpassing pullulanase hydrolysis ($1.0 \text{ U} \cdot \text{g}^{-1}$ dry basis for 12 h) at 17.3% RS. The breadfruit starch granules exhibited a regular shape, approximately $7.9 \mu\text{m}$ in length, whereas modified granules were less than $11 \mu\text{m}$, along with observable deformation in their structural shape. In conclusion, the study demonstrates the efficacy of MT for enhancing RS content in breadfruit, highlighting its potential as a healthy functional ingredient and starch substitute.

Keywords: functional properties; microwave irradiation; pullulanase debranching; treatment method

Breadfruit (*Artocarpus altilis*) boasts historical medicinal use and serves as a traditional, nutrient-rich starch source. Rich in carbohydrates, fibre, vitamins, and minerals, it's gaining interest in formulations. Research mainly focuses on its starch properties, with initial findings indicating human tolerance and the absence of anti-nutrients (Ijarotimi and Aroge 2005). Yet, industrial utilisation, especially for resistant starch (RS) that aids metabolic and colonic health, remains limited. Among RS types, RS type III (RS3) stands out for thermal stability and preserved nutritional functionality. However, integrating breadfruit-derived RS in-

dustrially is hindered by an incomplete understanding of native starch characteristics.

Various processing methods significantly impact RS formation. Safe and efficient modified starch production in the food industry often involves physical and enzyme treatments. Pullulanase, a specific debranching enzyme, targets α -(1,6) glycosidic bonds in granular and gelatinised starch and is commonly used to enhance RS by increasing apparent amylose levels through debranching amylopectin. Subsequent retrogradation aligns amylose within RS3's crystalline structure, enhancing resistance to enzymatic hydrolysis (Ma et al. 2020).

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Research extensively explores RS formation in high-amylose corn starch (Mutlu et al. 2018), potatoes (Babu and Parimalavalli 2018), and peas (Lu et al. 2018). However, tropical crops like breadfruit lack sufficient study. Microwave treatment (MT) offers advantages like rapid heating, energy efficiency, and effectiveness in altering starch structures. Microwaves have successfully modified starch in rice, potato, and lotus seeds, impacting their physicochemical properties and digestion characteristics (Villanueva et al. 2018).

While previous research has explored microwave-starch interactions and their effects (Li et al. 2018), the impact of MT on breadfruit starch is largely unexplored due to limited studies. This investigation addresses the underutilisation of breadfruit and its starch modifications, assessing how debranching enzymatic treatment and MT enrich RS content and affect functional properties.

MATERIAL AND METHODS

Material

Mature seedless 'white flesh' breadfruits were sourced from a local farm in Southern Vietnam. Chemicals obtained from Duksan, Scharlau, and Sigma-Merck (USA) were of analytical grade, including sodium hydroxide pellets, ethyl alcohol 99.9%, acetic acid glacial, calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), maleic acid, sulfuric acid 95%, methyl red, methyl blue, boric acid, copper (II) sulfate pentahydrate, potassium sulphate, petroleum ether, and hydrochloric acid. The Resistant Starch Assay Kit (Rapid) (K-RAPRS 11/19) and pullulanase enzyme M1 (from *Klebsiella planticola*) were purchased from Megazyme (Ireland).

Isolation of breadfruit starch

To obtain breadfruit starch (BS), the fruit was cleaned, latex-drained, peeled, and cored. Mixing the pulp with water (1:5, *w/w*), grinding, and cheesecloth filtration followed. After overnight settling (15 °C), starch collected at the bottom was purified through washing cycles. The starch was suspended in water, settled, and washed with 50% ethanol. Dry-

ing at 50 °C to < 13% moisture, grinding, and passage through a 250-mesh sieve occurred.

Preparation of resistant starch from breadfruit starch

Debranching by enzyme pullulanase. BS (5 g, 20% *w/v*) was mixed with 25 mL of 0.1 M acetate buffer (pH 5), treated by boiling (10 min), gelatinisation (130 °C for 1 h), and pullulanase enzymatic treatment [(0.5, 1.0, 1.5, and 2.0 $\text{U} \cdot \text{g}^{-1}$ dry basis (d.b.) at 40 °C for 4–20 h]. After enzyme deactivation (85 °C for 20 min), the starch was dried (moisture < 13%) at 50 °C, ground through a 250 μm mesh sieve, and stored in a desiccator for analysis, following a modified Ozturk et al. (2009) method.

Microwave heating of breadfruit starch. BS was placed in oven-safe plastic containers with lids and adjusted to moisture levels of 10, 15, 20, and 25% by adding distilled water. After 24 h at 4 °C for 24 h, samples underwent MT using different durations (60–240 s) and intensity levels (20–50 $\text{W} \cdot \text{g}^{-1}$). After MT, samples were dried and stored in a desiccator for analysis.

Analytical methods

Proximate analysis of isolated starch. Fresh breadfruit and breadfruit starch underwent triplicate proximate composition, determining moisture (925.10), ash (923.03), total crude fat (920.85), and the total crude protein (920.87) via standard Association of Official Analytical Chemists (AOAC) methods (1990). The amylose content of fresh breadfruit was determined using Megazyme's K-AMYL 02/20 method.

Determination of resistant starch. RS content in fresh breadfruit, native breadfruit starch, and modified starch was assessed with the Megazyme Resistant Starch Assay Kit, adhering to AOAC Method 2002.02 and AACC Method 32–40.

Swelling power and solubility

Starches (1% *w/v*, d.b.) were dispersed in water and heated (70, 80, 90 °C, 30 min) with stirring. After cooling and centrifugation ($3\,000 \times g$, 15 min), the liquid was analysed for solubility after drying at 105 °C. Swelling power ($\text{g} \cdot \text{g}^{-1}$) and solubility (%) were calculated (see Equations 1 and 2).

$$\text{solubility} = \frac{\text{residue weight (g)} \times \text{water weight (g)}}{\text{aliquot volume (mL)} \times \text{sample weight (g)}} \times 100 \quad (1)$$

$$\text{swelling power} = \frac{\text{weight of sedimental starch (g)}}{\text{weight of sample (g)}} \quad (2)$$

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Water absorption capacity and oil binding capacity

BS was mixed with distilled water (1:15, *w/w*) or rapeseed oil (1:10, *w/v*), vortexed for 2 min, and centrifuged at $1\,250 \times g$ for 20 min. Water absorption capacity (WAC) and oil binding capacity (OBC) were calculated by measuring g of water or oil bound per g of the dried sample after removing the supernatant and drops.

Syneresis

Starch samples (2%, *w/v*) were suspended in distilled water, heated at 85 °C for 30 min, and rapidly cooled in an ice-water bath to room temperature, and stored at 4 °C for 3, 5, and 7 days. After storage, gels were centrifuged at $3\,000 \times g$ for 15 min. Syneresis, the released water from the gels, was measured as a percentage of total water content (Yousif et al. 2012).

Scanning electron microscopy (SEM)

BS granule surfaces were studied using the Hitachi S-4800 SEM (Japan). Samples were coated with thin platinum film, covered with conductive tape, and imaged at 10 kV and 8.0–8.1 mm working distance for detailed visualisation.

Fourier transform infrared (FT-IR) measurement

For FT-IR analysis, 2 mg starch samples were mixed with 180 mg KBr to form a 1 cm diameter sheet. The Frontier FT-IR/NIR Spectrum (Perkin Elmer Spectrum, USA) recorded spectra in the $4\,000\text{--}400\text{ cm}^{-1}$ range, averaging 20 scans per spectrum for comprehensive infrared absorption insights.

Statistical analysis

Data were analysed with SPSS software (version 20.0) for Windows. Experiments were con-

ducted in triplicate, and results were presented as mean \pm standard deviation (SD). Treatment mean differences were determined using Duncan's 5% significance level test.

RESULTS AND DISCUSSION

Composition analysis of fresh breadfruit and breadfruit starch. Mature seedless breadfruits with oval shapes and distinct features were used. The fruit had a greenish-yellow with hexagonal markings and a bumpy texture. The firm, creamy white pulps had a pale yellow edge. The chemical composition of the fresh fruit, isolated breadfruit starch, and starch yield are shown in Table 1.

Starch yield percentages and the chemical composition of BS show notable variations due to factors like variety, maturity, and conditions. As per Table 1, the BS is classified as low amylose. The total starch content (99.3%) aligns with prior research on Chinese breadfruit varieties (99.13% and 98.90%) (Li et al. 2022). Furthermore, protein and lipid contents in the Vietnamese variety are slightly lower than those in the Indonesia cultivar (2.12% and 0.86%) (Yulistyani 2013). Thus, the isolation method used here has potential as an alternative ingredient, enabling more efficient extraction and purer isolation from other sources.

Effect of enzymatic concentration and hydrolysis time on the yield of RS. In Figure 1, enzyme concentration and hydrolysis time impact BS debranching. Peak RS content (16.69%) occurred after 12 h of $1\text{ U}\cdot\text{g}^{-1}$ d.b. enzyme concentration hydrolysis. Further hydrolysis yielded stable levels ($P < 0.05$), but prolonged hydrolysis decreased RS, indicating excessive hydrolysis hindrance.

Table 1. Chemical composition of fresh breadfruit and native breadfruit starch

Breadfruit sample (%)	Fresh pulp	Isolated starch
Starch yield	NM	12.90 ± 0.30
Moisture	78.88 ± 0.60	10.44 ± 0.20
Total carbohydrate	18.50 ± 0.50	88.88 ± 0.20
Protein	1.28 ± 0.01	0.50 ± 0.02
Lipid	0.33 ± 0.03	ND
Ash	1.15 ± 0.01	0.20 ± 0.01
Total starch (d.b.)	NM	99.3
Amylose content (d.b.)	NM	10.4

ND – not detected; d.b. – dry basis; NM – not measurable

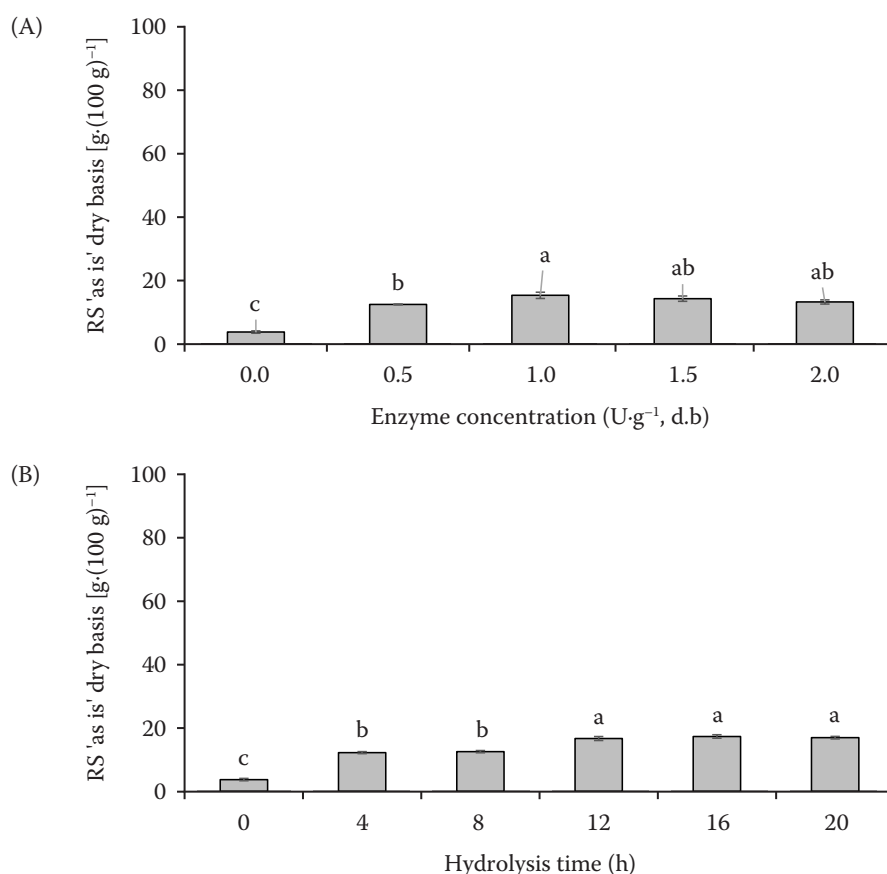


Figure 1. The effect of (A) enzyme concentration and (B) hydrolysis duration on resistant starch (RS) content of bread-fruit starch at 20% (w/v) solid-to-liquid ratio, 40 °C

a–c – distinct letters indicate significant differences at $P < 0.05$

Higher enzyme concentration promotes linear structure via gelatinisation and retrogradation. Gelatinisation releases amylose, coiling upon pullulanase exposure. Cooling leads to crystalline retrograded starch. Excessive enzymes and prolonged hydrolysis don't boost RS due to hindered crystallite formation from short chains. Ideal starch recrystallisation requires a specific chain length. Pullulanase mainly converts branched polysaccharides into small fermentable sugars, raising digestible starch. RS formation depends on amylose quantity and length, favouring more and longer chains.

Effect of microwave radiation treatment on the yield of RS. MT starch reached a peak RS content of 74.8% under the most suitable conditions at 15% starch moisture, 30 W·g⁻¹ intensity level for 3 min (Figure 2). This content significantly surpassed native starch (9.8%) and enzyme hydrolysis samples (17.3%). The increase in RS content is attributed to microwave electromagnetic radiation. Microwaves prompt water molecules' rapid movement within the starch

matrix, leading to granule collisions, accelerated motion, and temperature rise. Gelatinisation involves water absorption by starch particles, causing swelling and loss of ordered structures like double helices with rising temperature. Hydrated starch granules absorb microwave energy, generating more kinetic energy and transforming ordered structures into disordered ones, augmenting RS content. Moisture's role is evident, aligning with prior research (Li et al. 2019). Higher moisture enhances starch gelatinisation and energy absorption, but continuous granule movement may hinder ordered structure transformation, potentially lowering RS formation during prolonged or varying treatments.

Xu et al. (2019) showed that MT reduced double helices and branching in maize and potato starches. Similarly, MT improved digestion in rice starch, enhancing slowly digestible starch (Li et al. 2020). This study observed increased RS with MT starch, but other studies, like microwaved sago starch, reported reduced RS content (Zailani et al. 2022). These find-

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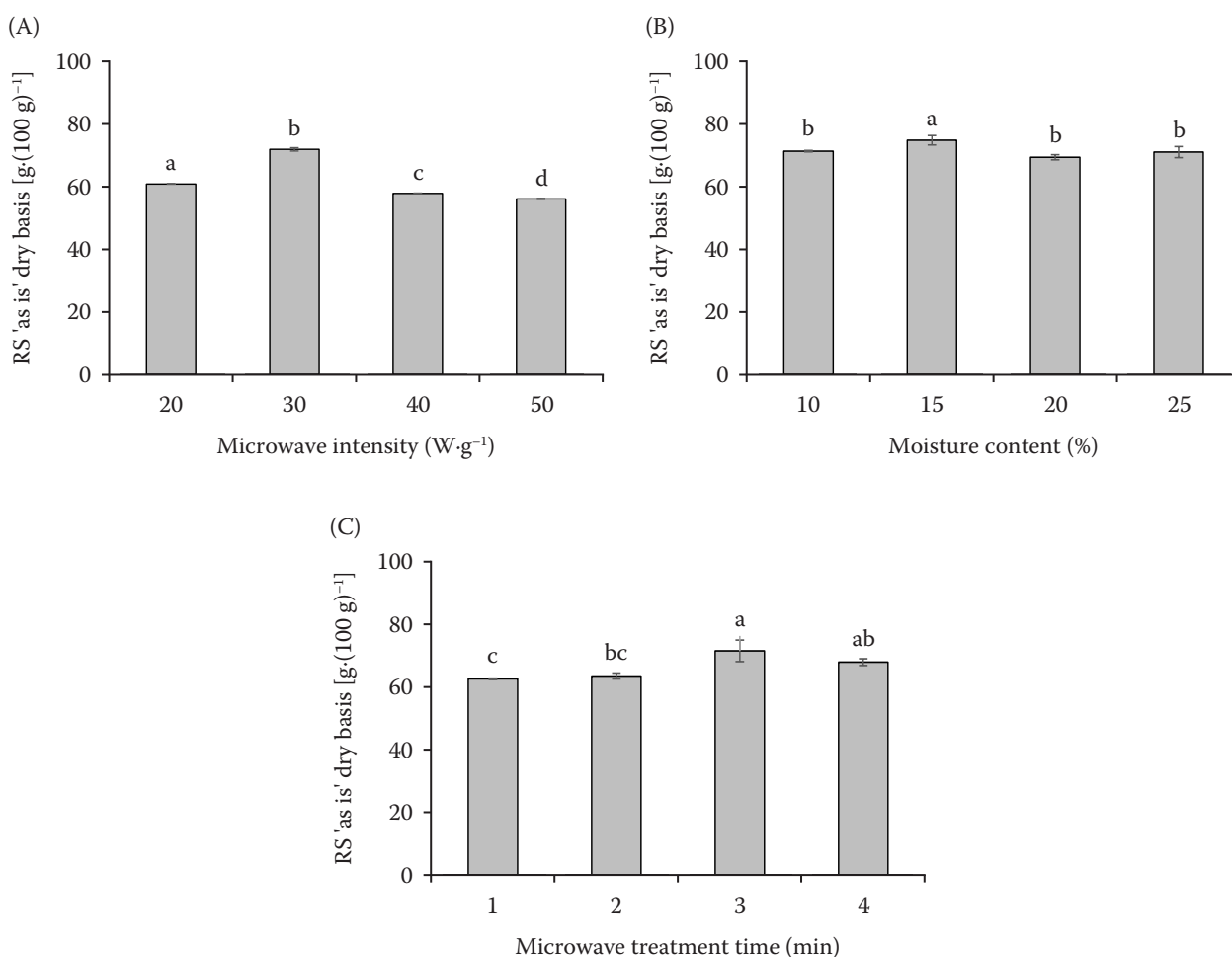


Figure 2. The effect of (A) microwave intensity, (B) starch moisture content, and (C) treatment time on breadfruit resistant starch (RS) content

a–d – distinct letters indicate significant differences at $P < 0.05$

ings emphasise starch's complex behaviour under different conditions.

This study combined debranching enzyme and MT to modify native BS. Surprisingly, the combined method reduced RS compared to individual treatments (data not shown), suggesting MT alone was more effective in inducing changes. High RS content in modified BS has health and food application potential. Further research is needed to understand RS formation's molecular mechanisms and assess different processing methods with breadfruit varieties. This study provides insights into BS utilisation, emphasising MT's role in enhancing RS and altering functional properties for potential food applications.

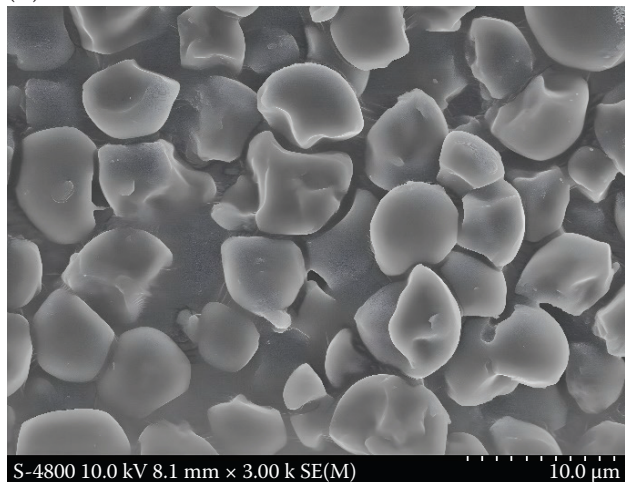
Morphology. Figure 3 shows SEM images of breadfruit starches at different magnifications, revealing small, irregular granules with block-like shapes. Particle size analysis confirms compact granules,

often $< 8 \mu\text{m}$ for native starch, consistent with prior studies (Marta et al. 2019; Li et al. 2022). MT led to visible granule deformation without significant size change. The average MT starch particle size was slightly larger ($< 11 \mu\text{m}$), showing irregular shapes, defects, and cracks. MT's temperature rises, and structural changes can damage starch.

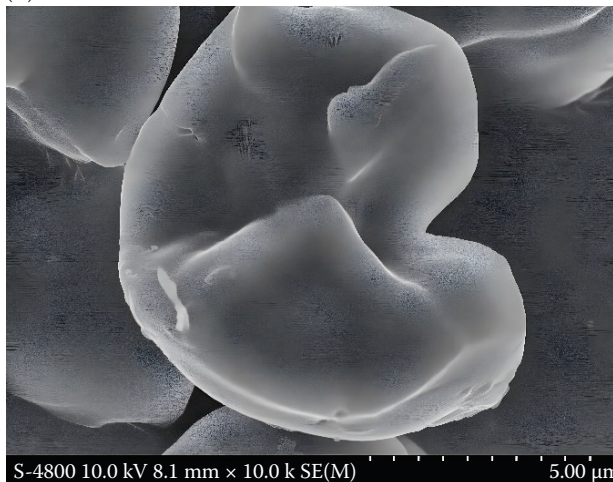
Under irradiation and low water content, BS granules may become sticky or enlarge. MT disrupts granules, reducing integrity. New hydrogen bonds between starch and water create clustering. The SEM image highlights BS's unique polyhedral morphology, resembling rice starch. These findings showcase BS as an alternative in the starch industry due to its distinctive granule structure.

FT-IR analysis. FT-IR analysis (Figure 4) of BS revealed distinct absorption peaks corresponding to functional groups and molecular vibrations. Peaks

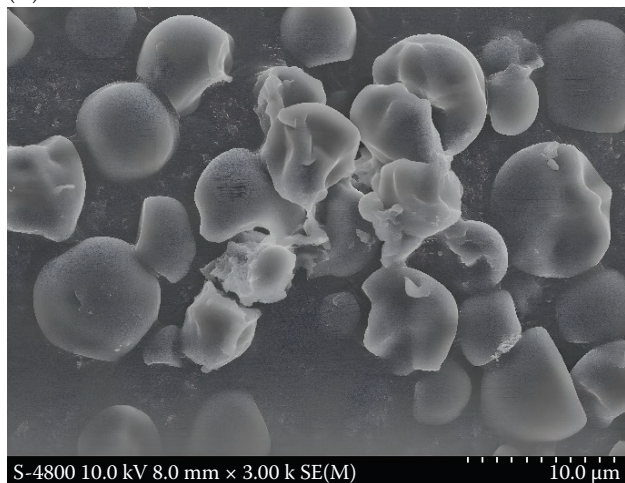
(A) Native starch 3 000×



(B) Native starch 10 000×



(C) MT starch 3 000×



(D) MT starch 10 000×

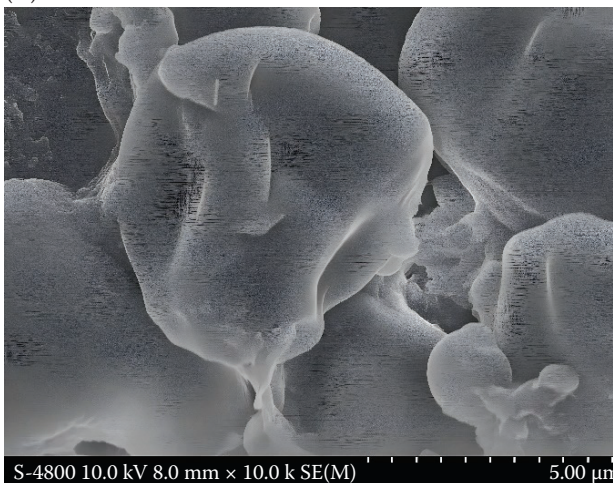


Figure 3. Scanning electron microscopy images of (A) native starch at 3 000×, (B) native starch at 10 000×, (C) microwave treated breadfruit starch at 3 000×, and (D) microwave treated breadfruit starch at 10 000×

MT – microwave treatment

in the range of 3 382–3 386, 2 931, 2 106, 1 643–1 641, 1 415–1 413, 1 203, 1 154, 1 021, 930, 855, and 764–763 cm^{-1} appeared in both native and MT samples, with reduced intensity after MT.

In the 4 000–2 500 cm^{-1} range, with a sharp peak at 3 382–3 386 cm^{-1} signifies O-H stretch (hydroxyl groups), while C-H stretching (methylene linkages) absorption occurs at 2 931.43–2 931.98 cm^{-1} . Peaks at 1 641 and 2 930 cm^{-1} are related to the crystalline structure, as in other studies (Parikh et al. 2023). Peaks at 1 200–800 cm^{-1} correspond to C-O bending (OH group) and C-O stretching. The ~2 100 cm^{-1} peak links to free water content. A shift to ~2 087 cm^{-1} in MT starch suggests starch dehydration. Water molecules binding to starch via

OH bonds may serve a structural role (Dankar et al. 2018). A 1 653 cm^{-1} peak appeared solely in MT starch, indicating water bending in starch's amorphous regions.

The fingerprint region (1 500–400 cm^{-1}) yielded starch's polymeric structure and conformation changes. Bands at ~1 415, ~1 203, ~1 154, and 930–763 cm^{-1} indicate CH_2 symmetric scissoring, C-O stretching, C-O-C asymmetric stretching, and C-O-C ring vibration of carbohydrate. The 1 022 cm^{-1} spectra point to amorphous regions in starch, most intense in BS. The native starch's 551 cm^{-1} peak disappears after MT, suggesting water's role in preserving starch structure. FT-IR analysis unveils BS's molecular structure changes due to MT.

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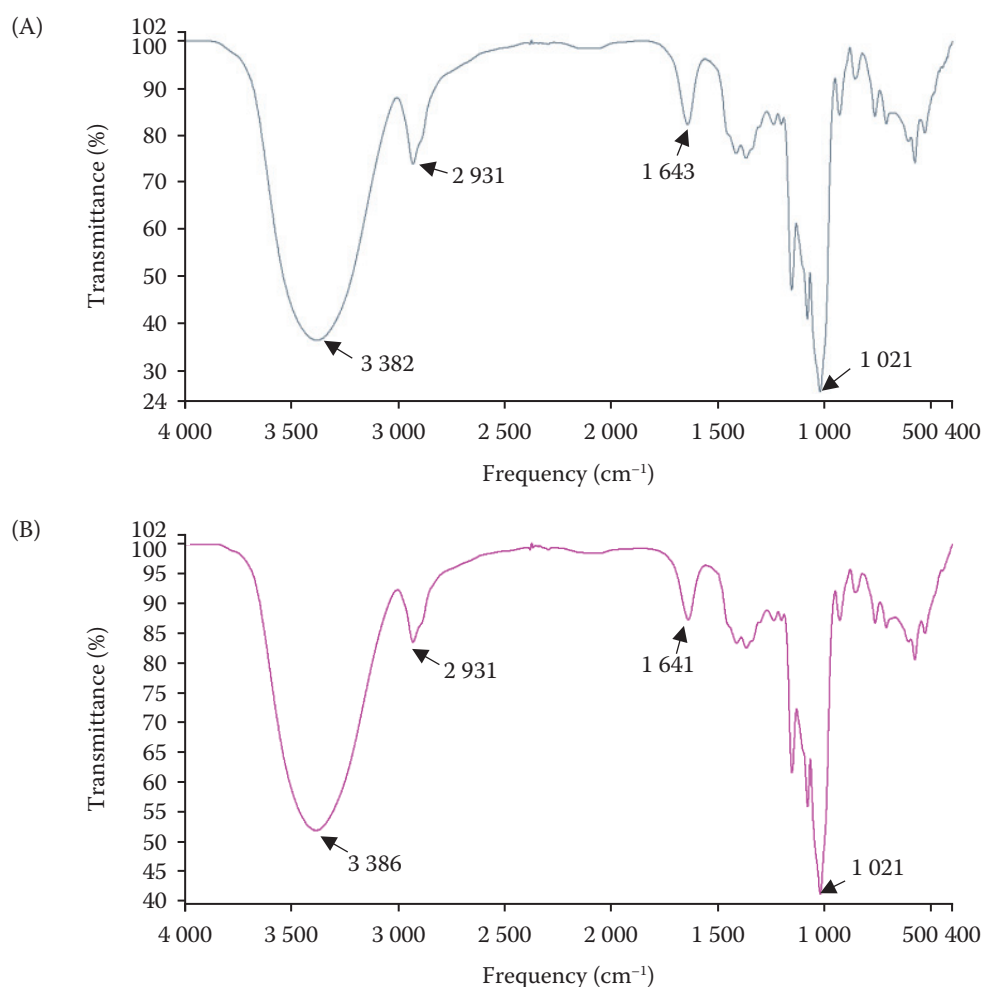


Figure 4. Fourier transform infrared spectrum of (A) native and (B) microwave heat treatment breadfruit starch

Solubility and swelling power. Table 2 displays temperature-driven solubility and swelling power improvements due to MT-induced starch swelling. At 90 °C, native starch had 22.05 g·g⁻¹ swelling power, while MT starch showed 18.88 g·g⁻¹. Solubility followed a similar trend, rising at higher temperatures due to enhanced starch mobility. Modified starches generally had higher solubility, but native starch exhibited significantly superior swelling power ($P < 0.05$).

MT starches' reduced swelling power is due to MT disrupting the crystalline structure, promoting hydrogen bonding with water. This increased solubility forms shorter-chain soluble dextrins. Sensitive polar groups in starch chains, influenced by microwaves, decrease water binding to hydroxyl groups, reducing swelling power. This aligns with a previous study on MT lotus seed starch (Zeng et al. 2016).

Water absorption capacity and oil binding capacity. MT altered BS's properties (Table 2). The modified starch had slightly higher WAC and OBC than the native starch.

Statistical analysis revealed significant water and oil binding differences ($P < 0.05$). WAC rose due to granular heat damage, enhancing gelatinisation and solubility. However, starch retrogradation reduced water-binding sites via hydrogen bonding, resulting in a noticeable difference in modified starch's WAC compared to native starch.

The SEM image (Figure 3) displays gelatinised starch granules, increasing water-binding surface area, aligning with higher WAC in modified BS (Marta et al. 2019) and potato starch (Kumar et al. 2020), enhancing food texture. OBC of modified BS follows this trend. SEM images reveal starch granule structural integrity loss, causing deformed granules to readily absorb oil into amorphous regions linked to the amylose-lipid complex. MT and retrogradation may create a double helix structure from shorter amylose chains, possibly trapping oil.

Syneresis. In Table 2, both native and MT breadfruit starch gels displayed significantly higher syneresis values, indicating reduced storage stability in modified starch gels. Over 7-day storage at 4 °C, syneresis ranged

Table 2. Functional properties of native and microwave treatment (MT) breadfruit starches

Properties	Native breadfruit starch	MT breadfruit starch
Solubility (%)		
70	3.058 ± 1.073 ^{aB}	6.568 ± 0.870 ^{bB}
80	6.011 ± 0.230 ^{aA}	10.240 ± 0.931 ^{bA}
90	7.707 ± 0.196 ^{aA}	10.713 ± 0.032 ^{bA}
Swelling power (g·g⁻¹)		
70	10.797 ± 0.205 ^{cC}	9.453 ± 0.154 ^{cC}
80	16.163 ± 0.001 ^{aB}	13.794 ± 1.120 ^{bB}
90	22.072 ± 0.030 ^{aA}	18.880 ± 0.243 ^{bA}
Water absorption capacity (g·g⁻¹)		
	2.300 ± 0.130 ^a	2.270 ± 0.070 ^a
Oil binding capacity (g·g⁻¹)		
	2.140 ± 0.020 ^a	2.830 ± 0.070 ^b
Syneresis (%)		
Day 1	66.040 ± 0.010 ^{aC}	77.620 ± 0.700 ^{bB}
Day 3	75.750 ± 2.790 ^{aAB}	74.480 ± 0.560 ^{aAB}
Day 5	72.500 ± 2.740 ^{aBC}	78.790 ± 0.710 ^{bAB}
Day 7	78.500 ± 2.280 ^{aA}	80.520 ± 0.710 ^{aA}

a–c, A–D Distinct lowercase and uppercase letters within rows and columns signify statistically significant differences ($P < 0.05$), respectively

from 78.50% to 80.52% for native and modified starches. Both exhibited decreased syneresis on the second day, with modified starch having lower values. Yet, subsequent days showed syneresis rise due to retrogradation in both starches. Gel concentration notably impacts syneresis, with higher concentrations generally reducing it. The amylopectin-to-amylose ratio influences water release from the gel, which is determined by the starch structure's chemical composition and interactions.

MT disrupts the starch granule structure, forming an amorphous region. Granule swelling upon water absorption creates a more stable crystalline structure. However, this change reduces polymer swelling pressure, leading to liquid phase separation and syneresis. Furthermore, centrifugation speed and mechanical disturbance influence syneresis. MT's impact on syneresis and starch stability is evident, similar to MT sago starch (Zailani et al. 2022).

CONCLUSION

MT increased breadfruit's RS content compared to pullulanase enzymatic debranching, altering its functional properties and morphology. Changes encompassed enhanced WAC and solubility, reduced swelling power, and granule shape/size modifications. MT prompted intragranular rearrangement and crys-

tallisation. The RS-enriched modified breadfruit starch holds potential for health-focused food applications, necessitating further research on underlying molecular mechanisms and suitability across breadfruit varieties. This study provides valuable insights into the utilisation of breadfruit starch and underscores the significance of MT in enhancing RS content and modifying functional properties for potential food applications.

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