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Structural Reorganisation and Reduced In Vitro Digestibility of Jackfruit Seed Starch Induced by Sequential Hydrogen Peroxide Oxidation and Heat–Moisture Treatment

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ABSTRACT

Hydrogen peroxide oxidation and heat–moisture treatment (HMT) modify starch through distinct mechanisms, yet little is known about how their sequential application affects starch structure and digestibility. This study evaluated the structural reorganisation and in vitro digestibility of jackfruit seed starch subjected to hydrogen peroxide oxidation followed by HMT. Oxidation resulted in a degree of oxidation of 1.26% and slightly disrupted the ordered structure, reducing relative crystallinity from 18.4% to 16.8% and the A_{1047}/A_{1022} ratio from 0.684 to 0.666. In contrast, subsequent HMT promoted structural reorganisation and granule aggregation, increasing relative crystallinity to 23.4% and the A_{1047}/A_{1022} ratio to 0.709. These structural changes were accompanied by a marked shift in digestibility: rapidly digestible starch decreased from 68.5% in native starch to 18.8% after sequential treatment, while resistant starch increased from 15.7% to 55.6%. Correspondingly, the hydrolysis index decreased from 70.76 to 17.81, with the predicted glycaemic index decreasing from 78.56 to 49.49. Together, these findings suggest that HMT-induced structural reorganisation following oxidation is closely associated with reduced enzymatic susceptibility in jackfruit seed starch, providing a useful dual-modification approach for tailoring starch structure and digestibility.

KEYWORDS: jackfruit seed starch, hydrogen peroxide oxidation, heat–moisture treatment, resistant starch, starch digestibility, structural reorganisation.

1. INTRODUCTION

Starch is a major dietary carbohydrate and an important determinant of the postprandial glycaemic response. Native starches, however, are generally susceptible to enzymatic hydrolysis, resulting in rapid glucose release following ingestion [1, 2]. Consequently, structural modification of starch has emerged as an important strategy for modulating its enzymatic susceptibility. Resistant starch (RS), which escapes digestion in the small intestine, has attracted considerable interest because of its slower contribution to glucose availability and associated physiological benefits [1, 3]. Importantly, starch digestibility is strongly influenced by structural characteristics at multiple length scales, including granule morphology, crystalline organisation and short-range molecular order. Changes in these structural features can alter enzyme accessibility and consequently shift the relative proportions of rapidly digestible, slowly digestible and resistant starch fractions [4].

Jackfruit (*Artocarpus heterophyllus* Lam.) seeds are an abundant by-product of jackfruit processing in tropical regions, particularly in South and Southeast Asia. Despite their high starch content, the seeds remain underutilised as a starch source [5, 6]. Jackfruit seed starch has attracted interest as an alternative starch source for food applications [5, 7]. Recent studies have also explored ways to reduce its enzymatic digestibility. Heat–moisture treatment (HMT), for example, can increase resistant starch content while altering granule aggregation and crystalline organisation [8, 9]. Chemical modification similarly affects the molecular architecture and enzymatic digestibility of jackfruit seed starch [10, 11]. Together, these findings indicate that its enzymatic susceptibility can be tailored through structural modification [12].

Hydrogen peroxide oxidation is a relatively mild chemical method for starch modification, in which hydroxyl groups are converted into carbonyl and carboxyl groups. The introduction of these functional groups can alter molecular interactions within starch granules, leading to changes in structural organisation and physicochemical properties [13, 14]. In jackfruit seed starch, hydrogen peroxide oxidation has been shown to produce measurable

carbonyl and carboxyl groups together with changes in granule morphology and molecular organisation [14]. HMT, in contrast, is a physical modification carried out at elevated temperature under limited-moisture conditions. It promotes molecular rearrangement and chain reassociation within starch granules, which can alter crystalline organisation, short-range molecular order and enzyme accessibility [15, 16]. The extent of these changes depends on the starch source and treatment conditions, but increases in slowly digestible and resistant starch have been reported following HMT [16].

The different effects of oxidation and HMT on starch organisation provide a rationale for combining the two treatments [17]. Oxidation may partially disrupt the native molecular organisation of starch, whereas subsequent HMT can promote chain rearrangement and reassociation under limited-moisture conditions. Applying these treatments sequentially may therefore produce structural characteristics that differ from those resulting from either treatment alone. Although previous studies have examined hydrogen peroxide oxidation and HMT of jackfruit seed starch separately [8, 9, 14], little is known about their sequential application. In particular, it remains unclear how the structural changes introduced by oxidation influence subsequent starch reorganisation during HMT, and how these changes are associated with enzymatic digestibility.

We hypothesised that hydrogen peroxide oxidation would partially disrupt the ordered structure of jackfruit seed starch, whereas subsequent HMT would promote molecular reassociation and structural reorganisation, thereby reducing susceptibility to enzymatic hydrolysis. To test this hypothesis, we investigated the effects of sequential hydrogen peroxide oxidation and HMT on the structural characteristics and in vitro digestibility of jackfruit seed starch. Changes in granule morphology, crystalline structure and short-range molecular order were examined alongside starch digestibility fractions, hydrolysis index (HI) and predicted glycaemic index (pGI) to examine the relationship between structural reorganisation and enzymatic susceptibility.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Mature jackfruit (*Artocarpus heterophyllus* Lam.) seeds were obtained from a local market in Dong Nai Province, Vietnam. Native jackfruit seed starch (JS) was isolated according to the method described by Tung et al. [14]. Hydrogen peroxide (H_2O_2, 30%, w/w), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), hydrochloric acid (HCl), potassium chloride (KCl), sodium hydroxide (NaOH), and other analytical-grade reagents were purchased from Xilong Scientific Co., Ltd. (Guangdong, China). Pepsin, pancreatic α-amylase, amyloglucosidase, and 3,5-dinitrosalicylic acid (DNS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Digestible and Resistant Starch Assay Kit (K-DSTRS, Megazyme, Bray, Ireland) was used for the determination of starch digestibility fractions.

2.2. Hydrogen peroxide oxidation followed by heat–moisture treatment of jackfruit seed starch

Hydrogen peroxide oxidation was carried out according to Tung et al., with minor modifications [14]. Native jackfruit seed starch (50 g) was dispersed in distilled water at a starch-to-water ratio of 1:4 (w/v). The suspension was adjusted to pH 9.0 using 1 M NaOH, after which $CuSO_4 \cdot 5H_2O$ was added at 0.1% (w/w, dry starch basis) as a catalyst. Hydrogen peroxide (30%, w/w) was then added to achieve a final concentration of 3% (w/v) in the reaction mixture. Oxidation was performed at 50 °C for 90 min under continuous stirring at 700 rpm, with the pH maintained at 9.0 throughout the reaction using 0.1 M NaOH or 0.1 M H_2SO_4 as required.

Following oxidation, the suspension was neutralised to pH 7.0, vacuum-filtered and washed thoroughly with distilled water. A portion of the resulting oxidised jackfruit seed starch was retained for analysis and designated OJS. The remaining oxidised starch was used for subsequent HMT. The moisture content of the oxidised starch was determined, and distilled water was added to adjust the moisture content to 30% (wet basis). Heat–moisture treatment (HMT) was then performed according to Sun et al., with minor modifications [16]. The moisture-adjusted starch was heated at 110 °C for 8 h in a hot-air oven. After treatment, the sample was stored at 4 °C for 24 h, dried at 45 °C, ground, passed through a 100-mesh sieve and stored in sealed polyethylene bags until further analysis.

2.3. Analytical methods

2.3.1. Determination of carbonyl and carboxyl contents and degree of oxidation

Carbonyl and carboxyl contents were determined according to Naknaen et al., with minor modifications [18]. For carboxyl determination, starch (2 g) was suspended in 25 mL of 0.1 N HCl and stirred for 30 min. The suspension was filtered, washed thoroughly with distilled water and diluted to 300 mL. After heating in a boiling-water bath for 15 min, the mixture was titrated with 0.01 N NaOH to pH 8.3, monitored using a pH meter. Native starch was analysed under the same conditions and used as a blank.

For carbonyl determination, starch (4 g) was dispersed in 100 mL of distilled water at 40 °C and adjusted to pH 3.2 with 0.1 N HCl. Hydroxylamine hydrochloride reagent (15 mL) was added, and the mixture was incubated at 40 °C for 4 h with gentle stirring before titration with 0.1 N HCl to pH 3.2, monitored using a pH meter. A reagent blank was analysed under the same conditions. Carbonyl and carboxyl contents were expressed as percentages on a dry starch basis, and their sum was reported as the degree of oxidation (DO).

2.3.2. Determination of starch digestibility fractions

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were determined using a Digestible and Resistant Starch Assay Kit (K-DSTRS; Megazyme, Bray, Ireland), following the manufacturer's protocol, consistent with AOAC Official Method 2017.16. Starch samples were digested with pancreatic α-amylase and amyloglucosidase in maleate buffer (pH 6.0) at 37 °C under continuous stirring. Aliquots were collected after 20, 120 and 240 min, and the released glucose was quantified using glucose oxidase–peroxidase (GOPOD) reagent. RDS represented starch digested within the first 20 min, whereas SDS corresponded to starch digested between 20 and 120 min. Resistant starch was determined from the starch fraction remaining after 240 min of enzymatic digestion, following the K-DSTRS assay protocol.

2.3.3. In vitro starch hydrolysis, hydrolysis index, and predicted glycaemic index

In vitro starch hydrolysis was determined according to Pham et al., with minor modifications [10]. Starch (1.5 g) was suspended in 10 mL of HCl–KCl buffer (pH 1.5) containing pepsin (1 mg) and incubated at 40 °C for 60 min. The suspension was then adjusted to pH 6.9 with phosphate buffer and hydrolysed with pancreatic α-amylase at 37 °C. Aliquots were collected at 30, 60, 90, 120, 150 and 180 min and heated at 100 °C for 15 min to stop the reaction. Amyloglucosidase was then added, and the samples were incubated at 60 °C for 45 min. Reducing sugars were measured as glucose equivalents using the 3,5-dinitrosalicylic acid (DNS) assay at 540 nm.

Starch hydrolysis was calculated using a glucose-to-starch conversion factor of 0.9:

$$\text{Starch hydrolysis (\%)} = \frac{\text{glucose equivalents} \times 0.9}{\text{initial starch mass}} \times 100 \quad \text{(Eq.1)}$$

The area under the hydrolysis curve (AUC) over 180 min was calculated using the trapezoidal rule. White bread was analysed under the same conditions and used as the reference for calculating the hydrolysis index (HI):

$$\text{HI} = \frac{\text{AUC}_{\text{sample}}}{\text{AUC}_{\text{white bread}}} \times 100 \quad \text{(Eq.2)}$$

The predicted glycaemic index (pGI) was calculated according to Goñi et al. [19]:

$$\text{pGI} = 39.71 + 0.549 \times \text{HI} \quad \text{(Eq.3)}$$

2.3.4. Structural characterisation of starch

X-ray diffraction (XRD): The crystalline structure of the starch samples was analysed using a D2 PHASER X-ray diffractometer (Bruker, Germany) with CuKα radiation (λ = 1.5406 Å). Diffraction patterns were recorded over a 2θ range of 5–80°. Relative crystallinity (RC) was determined from the obtained diffraction patterns.

Fourier-transform infrared spectroscopy (FTIR): FTIR spectra of the starch samples were recorded using a JASCO Fourier-transform infrared spectrometer (JASCO, Japan) over the range of 4000–400 cm^{-1}. The absorbance ratio at 1047 and 1022 cm^{-1} (A_{1047}/A_{1022}) was used as an indicator of short-range molecular order.

Scanning electron microscopy (SEM): The morphology of the starch granules was examined using a field-emission scanning electron microscope (FE-SEM; JEOL, Japan). Dried starch samples were mounted on aluminium stubs using conductive carbon tape prior to observation.

2.4. Statistical analysis

Experimental measurements were performed in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analyses were performed using Minitab Statistical Software (Version 20, Minitab LLC, State College, PA, USA). Differences among means were assessed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p < 0.05$. Structural parameters derived from the XRD patterns and FTIR spectra are reported as single values.

3. RESULTS AND DISCUSSION

3.1. Effects of hydrogen peroxide oxidation and heat–moisture treatment on oxidation characteristics and starch digestibility

Hydrogen peroxide oxidation resulted in the formation of carbonyl and carboxyl groups in jackfruit seed starch (Table 1). OJS contained 0.98% carbonyl and 0.28% carboxyl groups, corresponding to a DO of 1.26%. Similar values were observed in OHJS after HMT, indicating that the subsequent hydrothermal treatment had little effect on the oxidised functional groups formed during the initial oxidation step. This is consistent with HMT acting primarily through physical rearrangement of starch chains rather than further chemical oxidation [13, 14].

Oxidation was accompanied by a marked shift in starch digestibility. RDS decreased from 68.5% in JS to 42.6% in OJS, whereas SDS increased from 15.8% to 23.0% and RS from 15.7% to 34.4%. These changes suggest that oxidation reduced the proportion of starch readily accessible to digestive enzymes. The introduction of carbonyl

and carboxyl groups, together with structural changes associated with oxidation, may alter molecular interactions within the starch matrix and thereby influence enzyme accessibility [14, 20, 21].

HMT produced a further change in the digestibility profile, reducing RDS to 18.8% and increasing RS to 55.6%. Notably, this increase in enzyme resistance occurred without a corresponding increase in DO, suggesting that the additional effect of HMT was likely associated with physical reorganisation of the oxidised starch rather than further chemical modification. Molecular rearrangement and chain reassociation during HMT can alter starch organisation in ways that restrict enzyme accessibility [16, 22–24].

Table 1 Carbonyl and carboxyl contents, degree of oxidation, and starch digestibility fractions of JS, OJS and OHJS

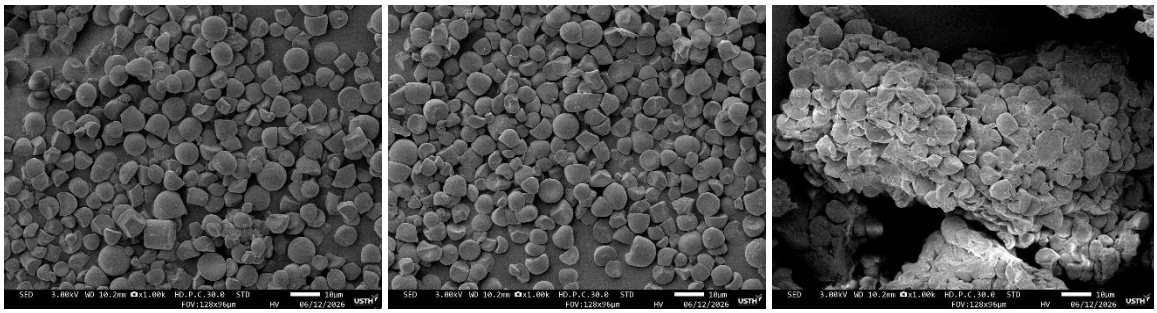
Sample	Carbonyl (%)	Carboxyl (%)	DO (%)	RDS (%)	SDS (%)	RS (%)
JS	ND	ND	ND	68.5 ± 1.2^{a}	15.8 ± 0.5^{c}	15.7 ± 0.4^{c}
OJS	0.98 ± 0.03^{a}	0.28 ± 0.01^{a}	1.26 ± 0.04^{a}	42.6 ± 1.0^{b}	23.0 ± 0.6^{b}	34.4 ± 0.6^{b}
OHJS	0.97 ± 0.01^{a}	0.26 ± 0.02^{a}	1.23 ± 0.02^{a}	18.8 ± 0.8^{c}	25.6 ± 0.7^{a}	55.6 ± 0.7^{a}

Different superscript letters within the same column indicate significant differences ($p < 0.05$). ND, not detected.

3.2. Structural characteristics of modified starch

3.2.1. Surface morphology

SEM images revealed clear differences in granule morphology among JS, OJS and OHJS (Fig. 1). Native JS granules were predominantly oval to spherical, with relatively smooth surfaces and well-defined boundaries. After hydrogen peroxide oxidation, OJS retained the overall granule shape, although slight surface roughening and localised erosion were observed. These changes suggest that oxidation mainly affected the granule surface without causing extensive disruption of granule integrity. Similar surface changes have been reported for oxidised starches and are generally associated with oxidative attack on the starch granules [14, 20].



a. JS *b. OJS* *c. OHJS*

Fig. 1 Scanning electron micrographs of native (JS), oxidised (OJS), and oxidised starch after heat–moisture treatment (OHJS)

More pronounced morphological changes were observed after HMT. OHJS showed greater granule aggregation and a less uniform surface, although individual granule outlines remained visible. This aggregation may arise from increased interactions among starch chains during heating under limited-moisture conditions, favouring closer association between adjacent granules [16, 22, 25, 26]. Nevertheless, the persistence of identifiable granule

boundaries indicates that the sequential treatment altered granule organisation without completely disrupting granular integrity.

3.2.2. Crystalline structure

The XRD patterns of JS, OJS and OHJS showed the characteristic A-type crystalline structure, with major diffraction peaks at approximately 15°, 17°, 18° and 23° (2θ) (Fig. 2). The overall diffraction pattern was retained after both oxidation and HMT, indicating that neither treatment altered the crystalline polymorph of jackfruit seed starch. However, relative crystallinity (RC) changed with modification. RC decreased from 18.4% in JS to 16.8% in OJS, suggesting partial disruption of the ordered crystalline regions during hydrogen peroxide oxidation. This decrease is consistent with previous reports that oxidative modification can disturb starch molecular organisation and reduce crystalline order without necessarily altering the crystalline type [14, 21, 27]. In contrast, RC increased to 23.4% after HMT, exceeding that of both JS and OJS. This increase suggests that heating under limited-moisture conditions promoted rearrangement and reassociation of starch chains within the oxidised matrix. Similar structural reorganisation following HMT has been reported for other starch systems [16, 28, 29].

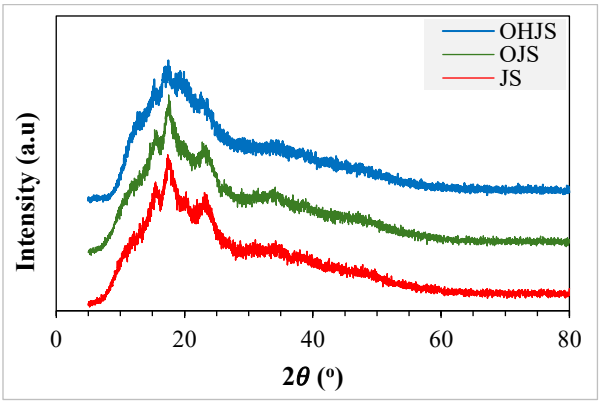


Fig. 2 X-ray diffraction patterns of JS, OJS and OHJS

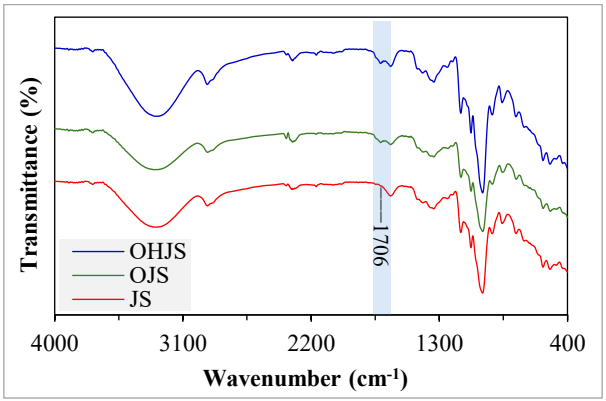


Fig. 3 Fourier-transform infrared spectra of JS, OJS and OHJS

3.2.3. Short-range molecular order

The FTIR spectra of JS, OJS and OHJS showed similar characteristic absorption bands, indicating that the main molecular features of starch were retained after modification (Fig. 3). A band at approximately 1706 cm^{-1} was observed in OJS and OHJS, which can be assigned to C=O stretching and is consistent with the formation of carbonyl groups during oxidation [13, 14]. Particular attention was given to the bands at approximately 1047 and 1022 cm^{-1}, which are associated with ordered and amorphous regions of starch, respectively. The A_{1047}/A_{1022} ratio, commonly used as an indicator of short-range molecular order, decreased from 0.684 in JS to 0.666 in OJS (Table 2), indicating a slight loss of local molecular order following hydrogen peroxide oxidation [14, 30]. Following HMT, the A_{1047}/A_{1022} ratio increased to 0.709, exceeding the values of both JS and OJS. This increase suggests greater short-range ordering of starch chains after treatment under limited-moisture conditions. HMT can promote molecular rearrangement and chain reassociation, favouring the development of more ordered local structures [15, 16, 28]. The FTIR results complement the XRD observations, indicating that the increased crystalline order after HMT was accompanied by greater short-range molecular organisation.

Table 2 Relative crystallinity and FTIR absorbance ratio of JS, OJS and OHJS

Sample	Relative crystallinity, RC (%)	A_{1047}/A_{1022}
JS	18.4	0.684
OJS	16.8	0.666
OHJS	23.4	0.709

Table 3 Hydrolysis index and predicted glycaemic index of JS, OJS and OHJS

Sample	HI	pGI
JS	70.76 ± 1.32^{a}	78.56 ± 1.98^{a}
OJS	27.48 ± 0.57^{b}	54.80 ± 1.03^{b}
OHJS	17.81 ± 0.12^{c}	49.49 ± 0.48^{c}

Different superscript letters within the same column indicate significant differences ($p < 0.05$).

3.3. In vitro starch hydrolysis, hydrolysis index and predicted glycaemic index

The *in vitro* hydrolysis profiles of JS, OJS and OHJS differed markedly throughout the 180 min digestion period (Fig. 4). Native JS showed the greatest extent of hydrolysis, whereas OJS was hydrolysed more slowly and OHJS consistently exhibited the lowest hydrolysis. This pattern agrees with the digestibility fractions in Table 1, where oxidation reduced RDS and increased RS, with subsequent HMT producing a further shift towards the resistant fraction. The lower hydrolysis of the modified starches therefore reflects a progressive reduction in their susceptibility to enzymatic digestion.

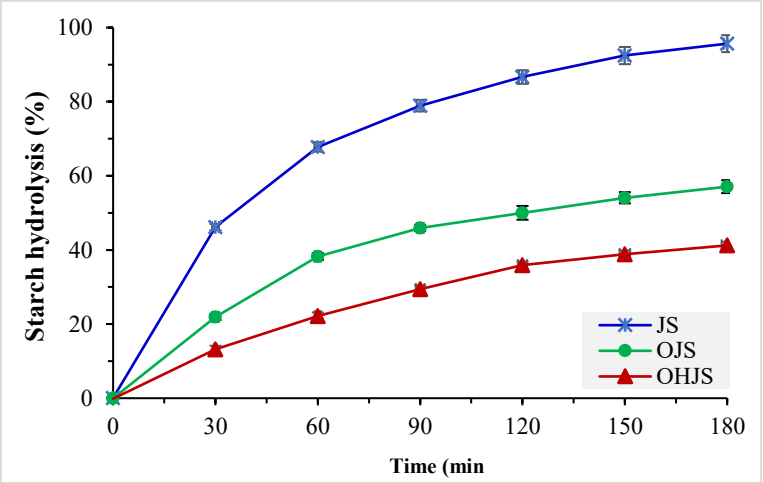


Fig. 4 In vitro starch hydrolysis curves of JS, OJS and OHJS during digestion (*Values are expressed as mean ± SD (n = 3)*)

The lower hydrolysis observed in OHJS was consistent with the structural changes induced by HMT. Its higher relative crystallinity and A_{1047}/A_{1022} ratio indicate greater long- and short-range molecular order, while SEM revealed more pronounced granule aggregation. Together, these structural changes may restrict enzyme accessibility through a more ordered and aggregated starch organisation, thereby slowing hydrolysis [16, 28, 31–33]. These observations provide a plausible structural explanation for the greater resistance of OHJS to enzymatic digestion.

Differences in hydrolysis behaviour were further reflected in the HI and pGI values (Table 3). Both HI and pGI decreased significantly following modification. HI decreased from 70.76 in JS to 27.48 in OJS and 17.81 in OHJS, while the corresponding pGI values decreased from 78.56 to 54.80 and 49.49, respectively. These results indicate that hydrogen peroxide oxidation markedly reduced starch hydrolysis, with a further reduction following HMT. The lower pGI of OHJS suggests a reduced predicted glycaemic response relative to native jackfruit seed starch.

CONCLUSIONS

Sequential hydrogen peroxide oxidation and heat–moisture treatment markedly altered the structural organisation and in vitro digestibility of jackfruit seed starch. Oxidation introduced carbonyl and carboxyl groups and partially disrupted starch order, whereas subsequent HMT promoted structural reorganisation without further increasing the degree of oxidation. These effects were reflected in greater granule aggregation, an increase in relative crystallinity from 16.8% in OJS to 23.4% in OHJS, and an increase in the A_{1047}/A_{1022} ratio from 0.666 to 0.709. These structural changes were accompanied by a substantial reduction in enzymatic susceptibility, with RDS decreasing to 18.8% and RS increasing to 55.6% in OHJS. Consistent with these changes, HI and pGI decreased to 17.81 and 49.49, respectively. The findings suggest that the enhanced digestive resistance following HMT was associated primarily with physical reorganisation of the oxidised starch rather than further chemical oxidation. These findings demonstrate the potential of sequential oxidation–HMT as an approach for tailoring the structure and digestibility of jackfruit seed starch.

Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Le Thi Hong Thuy: Conceptualisation, methodology, formal analysis, writing – original draft, writing – review and editing, project administration, funding acquisition.

Pham Ngoc Bao Tram: Investigation, data curation, formal analysis, visualisation.

Tran Manh Hai: Investigation, methodology, validation, resources.

All authors read and approved the final manuscript.

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