

Influence of Physical, Chemical, Enzymatic, and Fermentation Modifications on the Physicochemical Characteristics and Microstructure of Jack Bean (*Canavalia ensiformis*) Flour

R. Haryo Bimo Setiarto^{1,2,*}, Andre Wijaya³, Tjahja Muhandri^{3,4}, Uswatun Hasanah^{3,4},
Lutfi Anshory¹

¹ Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), KST Soekarno, Cibinong, Bogor, 16911, Indonesia; rhar002@brin.go.id (R.H.B.S.); lutf012@brin.go.id (L.A.);

² Research Collaboration Center for Traditional Fermentation, National Research and Innovation Agency (BRIN), KST Soekarno, Jl. Raya Jakarta-Bogor KM. 46, Cibinong, Bogor, 16911, Indonesia; rhar002@brin.go.id (R.H.B.S.);

³ Department of Food Science and Technology, IPB University, Bogor, 16680, Indonesia; andrewijaya@apps.ipb.ac.id (A.W.); tjahjamuhandri@apps.ipb.ac.id (T.M.); uswatun.hasanah@apps.ipb.ac.id (U.H.);

⁴ Southeast Asia Food and Agricultural Science and Technology (SEAFast) Center, IPB University, Bogor, 16680, Indonesia; tjahjamuhandri@apps.ipb.ac.id (T.M.); uswatun.hasanah@apps.ipb.ac.id (U.H.);

* Correspondence: rhar002@brin.go.id;

Received: 25.08.2025; Accepted: 27.10.2025; Published: 15.02.2026

Abstract: Jack bean (*Canavalia ensiformis*) is a protein- and carbohydrate-rich legume with limited food application due to poor physicochemical properties and beany flavour. This study examined the effects of physical, chemical, enzymatic, and fermentation modifications on the functional and structural characteristics of jack bean flour. Treatments included autoclaving–cooling (one and two cycles), annealing, microwave–cooling, heat moisture treatment, acid hydrolysis, pullulanase debranching, and combined fermentation–autoclaving. Modified and native flours were evaluated for colour, pasting properties, starch digestibility, and microstructure using scanning electron microscopy (SEM). The modifications significantly influenced flour characteristics, with pullulanase debranching (DP) showing the most desirable attributes: higher peak viscosity (11.50 cP), lower setback viscosity (3.50 cP), and reduced starch digestibility (33.02%). SEM images revealed disrupted and porous granule structures, especially in enzymatically and fermentatively modified samples. Overall, the modification treatments enhanced thermal stability, reduced retrogradation, and improved the functional potential of jack bean flour. These findings highlight its suitability for use in functional foods, bakery formulations, and plant-based ingredients, supporting industrial applications in starch-based product innovation.

Keywords: enzymatic; fermentation; jack bean flour; microstructure; physicochemical properties.

© 2026 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

1. Introduction

Canavalia ensiformis, commonly known as jack bean, is a legume from the pea family that is widely available in Indonesia but remains underutilised by local communities [1]. Jack bean flour has a promising nutritional composition, containing high levels of carbohydrates (58.4%) and proteins (25.2%) with a low fat content (5.21%) [2], making it a potential raw material for food formulation. However, native jack bean flour exhibits several limitations, including low thermal stability, poor resistance to shear forces, and a high tendency to retrograde [3–5]. These drawbacks hinder its application in food systems that undergo intense

processing conditions, including high temperatures, low pH, and repeated freezing–thawing cycles [6]. Therefore, modifying jack bean flour is essential to enhance its functionality and expand its industrial applications.

Flour or starch modification involves structural alterations aimed at improving physicochemical and functional properties, such as viscosity, solubility, and granule morphology. Various modification methods, such as physical, chemical, enzymatic, and fermentation, have been employed to achieve these improvements. For instance, physical modifications, such as heat–moisture treatment, can increase the pasting temperature while reducing setback and breakdown viscosity in corn starch [6]. Chemical modification using acid hydrolysis has been shown to enhance starch lightness and stability [7], while enzymatic modification through pullulanase debranching can alter granule crystallinity and improve digestibility [8]. Similarly, lactic acid bacteria (LAB)-fermented porang flour has been reported to decrease total starch content and enhance starch digestibility [9]. These findings suggest that different modification techniques can distinctly influence the physicochemical and morphological characteristics of flour or starch.

Physical modification generally relies on thermal processes that promote starch chain hydrolysis or reduce the degree of polymerisation (DP), forming shorter amylose chains capable of realignment into double-helix structures during retrogradation [10,11]. Common thermal-based methods include autoclaving–cooling, annealing, microwave–cooling, and heat–moisture treatment (HMT) [12]. Each method differs in its operating conditions, such as temperature, pressure, and moisture content, but all are considered safe and effective techniques for improving flour functionality in industrial applications [13–17].

Chemical modification, typically performed using strong acids such as hydrochloric acid, involves the random hydrolysis of glycosidic bonds in amylose and amylopectin [18]. This treatment produces starches with stable viscosity, enhanced thermal and acid resistance, and desirable thickening properties suitable for industrial use [7]. In contrast, enzymatic modification utilises specific enzymes such as pullulanase to selectively hydrolyse α -1,6 linkages in the amylopectin fraction, thereby influencing starch structure, crystallinity, and in vitro digestibility [19,20].

Fermentation modification, on the other hand, offers a biological approach that combines enzymatic action and microbial metabolism. In this study, LAB fermentation was performed in combination with a single autoclaving–cooling cycle. LAB cultures, such as *Lactobacillus plantarum* D-240 and *Leuconostoc mesenteroides* SU-LS67, produce hydrolytic enzymes including amylase (2.53 U/mL) and pullulanase (2.81 U/mL), which can degrade α -1,4 and α -1,6 glycosidic bonds in starch chains [11,12,21]. Accordingly, this study aims to evaluate the effects of physical, chemical, enzymatic, and fermentation modifications on the physicochemical properties and granule morphology of jack bean flour. The findings are expected to identify the most suitable modification technique for improving the functional characteristics of this underutilised legume flour and to support its potential application in food processing industries.

2. Materials and Methods

2.1. Materials and reagents.

The flour used in this study was derived from jack bean (*Canavalia ensiformis*), locally known as *kacang koro pedang*, obtained from PT Yasa Jamur Sriwijaya, West Java, Indonesia.

The reagents used in this study included sodium hydrogen carbonate (NaHCO₃, Supelco), hydrochloric acid (HCl, Merck), sodium hydroxide (NaOH, Merck), microbial pullulanase (Sigma), *Lactobacillus plantarum* IIA-1A5 culture from the Laboratory of Animal Product Technology (THT), IPB University, phenol (Merck), sulfuric acid (H₂SO₄, Supelco), glucose (Merck), maltose (Merck), 3,5-dinitrosalicylic acid (DNS, Sigma), and α -amylase from *Aspergillus oryzae* (Sigma).

2.2. Jack bean flour preparation.

The procedure of Putro *et al.* [22] was adopted with minor adjustments. Jack bean seeds were sorted to remove damaged seeds and foreign materials. The seeds were weighed and soaked in a 1% sodium bicarbonate (NaHCO₃) solution at a seed-to-solution ratio of 1:3 (w/v) for 24 h at room temperature. After soaking, the seed coats were removed, and the seeds were drained and chopped using a chopper. The chopped seeds were dried in a hot-air oven at 40°C, ground using a milling machine, and sieved through an 80-mesh sieve. Jack bean flour was then placed in impermeable plastic bags and stored at 25°C for later analysis.

2.3. Sample preparation.

2.3.1. Physical modifications.

Physical modification of jack bean flour included autoclaving–cooling (AC), annealing (ANN), microwave–cooling (MC), and heat–moisture treatment (HMT). The AC modification followed the method of Rahmawati and Wirawan [23], which involved heating a 20% (w/v) flour suspension in distilled water using an autoclave at 121°C for 15 min. The ANN modification referred to Ariyantoro *et al.* [24], in which a 30% (w/v) flour suspension in distilled water was heated in a water bath at 50°C for 24 h. The MC modification followed Wang *et al.* [25], involving heating a 30% (w/v) flour suspension in distilled water using a microwave at 800 W for 10 min. The HMT modification followed Ariyantoro *et al.* [24], in which the flour moisture content was adjusted to 20% before heating in an oven at 120°C for 3 h. After each hydrothermal treatment, the flour suspensions were cooled at 4°C, then dried in an oven at 60°C for 24 hours until flour flakes were formed. The dried flakes were then milled using a milling machine and sieved through an 80-mesh screen.

2.3.2. Chemical modification.

The chemical modification of jack bean flour followed the method of Sudheesh *et al.* [26], with a slight modification: 2.2 N HCl was added at a flour-to-acid ratio of 1:2 (w/v) for 2 h at 35°C in a water bath. Acid neutralisation was carried out by adding 1 M NaOH solution until the pH reached 6, followed by washing with distilled water. The resulting flour paste was cooled to 4°C for 24 hours, then dried in an oven at 60°C for 24 hours until flour flakes formed. The dried flakes were then milled using a milling machine and sieved through an 80-mesh screen.

2.3.3. Enzymatic modification.

The enzymatic modification of jack bean flour was prepared according to the method reported by Setiarto *et al.* [8] with a slight modification. First, jack bean flour was suspended in distilled water at 20% (w/v). The suspension was initially heated in a water bath at 70°C for

5 min, followed by autoclaving at 121°C for 15 min. The resulting flour paste was left at room temperature for 1 h, then cooled to 4°C for 24 h. The paste was reheated in a water bath to 50°C, after which 100 mL of 0.1 M acetate buffer (pH 5.2) and 2.5 mL of pullulanase enzyme (10.4 U/g flour) were added. The mixture was incubated in a shaker incubator at 50°C and 110 rpm for 6 h. Enzyme inactivation was carried out using one cycle of autoclaving–cooling (AC 1 S), after which the flour was milled and sieved through an 80-mesh screen.

2.3.4. Fermentation modification.

The fermentation modification method for jack bean flour was carried out as described by Setiarto *et al.* [9] with minor modifications. Prior to fermentation, the flour was sterilised. The sterilised flour was suspended in sterile distilled water at a 1:3 (w/v) ratio. A 5% inoculum of *Lactobacillus plantarum* IIA-1A5, previously revitalised in MRS broth (MRSB), was added to the suspension and homogenised. The mixture was then incubated at 37°C for 18 h. Upon completion of fermentation, one cycle of autoclaving–cooling (AC 1 S) was applied, after which the flour was milled and sieved through an 80-mesh screen.

2.4. Physicochemical characteristics and microstructure analysis.

2.4.1. Colour characteristics analysis.

The colour scale for L* (lightness), a* (redness), and b* (yellowness) of native and modified flour was measured using a Chromameter CR-310 (Konica Minolta Co., Osaka, Japan). The colour measurement includes L* (lightness, 0 = black /100 = white), a* (+a* = redness/-a* = greenness), and b* (+b* = yellowness/-a* = blueness). Whiteness index (WI) was calculated using the equation [6]:

$$WI(\%) = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

2.4.2. Pasting properties analysis.

Both the native and the modified flours were analysed to determine their gelatinisation profiles and pasting properties, using the technique described by Faridah *et al.* [27] with a modification of the Rapid Visco Analyser (RVA, Perten Instrument, Sweden). About 3 g of flour sample (on a 10% moisture basis) was added to 25 g of distilled water and mixed to form a starch suspension. The suspension was then poured into an RVA canister. The sample was maintained at 50°C for 1 min, heated at 12.0°C/min to 95°C, and then held at 95°C for 2.5 min. Afterwards, it was cooled to 50°C for 2.5 minutes and then kept at 50°C for 2 minutes. All viscosity measurements were reported in cP units.

2.4.3. Total starch content analysis.

Total starch content was determined according to the method of Setiarto *et al.* [8]. An aliquot (0.5 mL) of the diluted sample was mixed with 0.5 mL of 5% phenol, followed by the rapid addition of 2.5 mL of concentrated H₂SO₄. The mixture was incubated at room temperature for 10 minutes, vortexed, and then incubated for an additional 20 minutes at room temperature. Absorbance was measured at 490 nm using a UV–Vis spectrophotometer (Shimadzu, Japan). Glucose concentration (mg/mL) was determined from a standard curve. The total sugar content (% dry basis) was obtained from the standard curve, and the total starch

content (% dry basis) of the jack bean flour was calculated by multiplying the total sugar content by 0.9, where is used to represent experimental factors for the conversion of monosaccharides to polysaccharides. In this study, the calculation of the total starch content (% db) was shown in the following formula:

$$\text{Total sugar content (\% db)} = \frac{\text{Glucose level(mg/mL)}}{\text{Sample weight (mg)}} \times \text{vol. tot. reaction (mL)} \times \text{DF} \times 100 \quad (2)$$

$$\text{Total starch content (\% db)} = \text{Total sugar content (\% db)} \times 0.9 \quad (3)$$

2.4.4. Starch digestibility analysis.

In vitro starch digestibility of modified jack bean flour was determined based on maltose production from enzymatic hydrolysis using α -amylase from *Aspergillus oryzae*, following Otemuyiwa and Aina [28]. Absorbance was measured at 520 nm using a UV–Vis spectrophotometer (Shimadzu, Japan). Negative controls (blank jack bean flour and blank starch) were prepared without enzyme treatment. Digestibility was expressed as the ratio of maltose content in the sample to that in the pure starch control. In this study, the calculation of the starch digestibility (%) was shown in the following formula:

$$\text{Starch digestibility (\%)} = \frac{\text{Maltose of sample} - \text{Maltose of blank sample}}{\text{Maltose of pure starch} - \text{Maltose of blank pure starch}} \times 100 \quad (4)$$

2.4.5. Microstructure analysis.

Granule morphology was characterised directly from the flour (not isolated starch) by scanning electron microscopy (SEM). In the SEM analysis, a sputter coater (Hitachi E102 Ion Sputter, Tokyo, Japan) was used to coat the jack bean flour samples. The samples were examined at an accelerating voltage of 20.0 kV using a Hitachi S-2400 scanning electron microscope (Hitachi, Tokyo, Japan). Representative digital images of starch granules were obtained at 1000 \times magnification.

2.5. Statistical analysis.

Statistical analysis was performed using IBM SPSS Statistics 27.0. Determinations of colour characteristics, total starch content, and starch digestibility were performed in triplicate (n=3), whereas pasting properties were determined in duplicate (n=2). Data were expressed as mean \pm standard deviation (SD) of experiments and were compared through one-way ANOVA (analysis of variance) using Duncan's Multiple Range test. Statistical significance was set at a confidence level of 0.95 ($P < 0.05$). Values followed by the same letter in the comparison were not significantly different ($P > 0.05$).

3. Results and Discussion

3.1. Colour characteristics.

Colour is an important quality attribute of jack bean flour that reflects the extent of starch modification, protein interactions, and non-enzymatic browning reactions during processing. The effects of various modification methods on colour can be evaluated in terms of lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) [24].

Modification treatments significantly affected the colour parameters (L^* , a^* , b^* values, and WI). The colour characteristics of native and modified flours are presented in Table 1. The Maillard reaction during the modification process contributed to lower lightness (L^*) and whiteness index (WI) values than those of native flour. The thermal process of flour modification significantly induces Maillard reactions [29] and gelatinisation [30], resulting in flour that tends to be darker in colour. A different result was observed in the HMT modification, where the lightness value was not significantly different from that of native flour or the control (K) because the low water content limits the mobility of reactants in the Maillard reaction [31]. Meanwhile, the WI value in the HA modification was not significantly different from that of native flour because, during modification, reactive chloride ions are formed, which act as bleaches in starch modified with HCl [7]. The decrease in L^* and WI values in this study is in line with the research on ANN-modified jack bean starch [24] and modified mung bean starch with a combination of AC 2S and HA [32].

Table 1. Colour characteristics of native and modified jack bean flours.

Samples	Parameter			
	L^* (lightness)	a^* (redness)	b^* (yellowness)	Whiteness index
K	90.80±1.76 ^f	-1.44±0.05 ^a	14.74±0.55 ^a	82.53±1.39 ^f
AC 1 S	81.86±2.05 ^c	3.03±0.15 ^c	22.48±1.24 ^{fg}	70.95±2.23 ^c
AC 2 S	82.00±1.31 ^c	4.01±0.26 ^g	21.05±0.66 ^d	72.01±1.35 ^{cd}
ANN	87.98±1.07 ^e	2.50±0.21 ^d	17.23±0.48 ^c	78.83±0.96 ^e
MC	84.32±1.27 ^d	3.57±0.08 ^f	21.86±0.58 ^{ef}	72.85±1.21 ^d
HMT	91.09±1.23 ^f	-0.04±0.03 ^b	16.46±0.36 ^b	81.27±0.90 ^f
HA	89.16±1.14 ^e	0.41±0.07 ^c	14.26±0.33 ^a	82.07±0.92 ^f
DP	79.45±1.48 ^b	5.77±0.19 ⁱ	22.59±0.75 ^g	68.92±1.56 ^b
FAC	75.28±1.90 ^a	4.55±0.40 ^h	21.45±0.84 ^{de}	66.95±1.99 ^a

K (control); AC 1 S (autoclaving-cooling 1 cycle); AC 2 S (autoclaving-cooling two cycles); ANN (annealing); MC (microwave cooling); HMT (heat moisture treatment); HA (acid hydrolysis); DP (debranching pullulanase); FAC (fermentation followed by AC 1 S); nd (not detected). Means within columns with different superscripts are significantly different ($P < 0.05$).

Colour profile analysis plays a crucial role in determining product quality and consumer preference. According to Lisa *et al.* [33] and Marta *et al.* [34], high lightness and WI values in flour or starch are preferred by consumers. HA and HMT-modified jack bean flour have the potential to achieve higher consumer acceptance through colour profile attributes. Thermal processing during autoclaving can promote Maillard reactions between reducing sugars and proteins, as well as caramelisation of sugars. After one cycle, a moderate reduction in L^* (lightness) is often observed, accompanied by slight increases in a^* (toward redness) and b^* (toward yellowness). With two cycles, these effects are more pronounced due to repeated autoclaving-cooling stresses, resulting in darker flour with lower L^* and higher chroma [9,35].

Annealing is a low-temperature, moisture-based treatment that does not trigger Maillard browning. As a result, L^* values remain relatively high, and there is little change in a^* and b^* . Colour stability is maintained, making annealed flour closer to native flour in appearance [8,9,36]. Microwave treatment generates rapid heating, which can create localised “hot spots” leading to slight browning. Consequently, flour shows decreased L^* and slight increases in a^* and b^* . However, because the treatment time is shorter than autoclaving, colour changes are less intense [9,37]. HMT applies high temperature in low moisture conditions, limiting extensive Maillard reactions. Colour changes are therefore mild, with small reductions in L^* and minimal increases in a^* or b^* . The flour generally remains light, but with a slightly duller tone compared to native or annealed samples [9,38]. The results of this study are consistent with the findings of Ariyantoro *et al.* [24], who reported that ANN modification

(50°C, for 24 hours, air ratio 1:2) had a significant effect on reducing the L* (81.40) and WI (70.30) values in jack bean starch.

Acid hydrolysis primarily affects starch at the molecular level without inducing thermal browning. Thus, flour colour tends to remain stable, with only slight increases in L* (due to the breakdown of amorphous regions that may scatter light differently). No significant changes in a* and b* are usually observed, making this treatment favourable when maintaining natural colour is desirable [9,39]. Enzymatic debranching does not involve high heat; therefore, colour alterations are minimal. Jack bean flour treated with pullulanase retains high L* values and stable a* and b* values, similar to those of acid hydrolysis. Any minor shifts may arise from protein–enzyme interactions, but remain negligible [8,9,40]. Fermentation introduces acids and microbial enzymes that can slightly lighten flour (higher L* values) by breaking down proteins and removing pigments. However, when followed by autoclaving–cooling, Maillard reactions intensify, leading to significant darkening (lower L*, higher a* and b*). The net effect is a more pronounced colour change compared to AC alone, with flour appearing darker and more yellowish–brown [9,11]. This finding is in line with Gonçalves *et al.* [41], who reported a significant decrease in brightness value for HA-modified damar seed starch incubated with 2% HCl at 22°C compared to the control.

The greatest colour changes (darkening) were observed in AC with two cycles and FAC, attributed to repeated heating and the Maillard reaction. Moderate colour changes occurred during microwave cooling and HMT, reflecting mild browning. In contrast, minimal alterations were found in annealing, acid hydrolysis, and pullulanase debranching, where the absence of intense heat or extensive Maillard reactions preserved colour stability. From an application perspective, annealing, acid hydrolysis, and pullulanase treatments are most suitable when colour uniformity and lightness are required (e.g., baked goods, functional powders). Conversely, autoclaving–cooling and fermentation–autoclaving–cooling are advantageous when a darker colour is acceptable or desirable, such as in high-fibre or resistant starch-enriched products, where distinct colour shifts complement functionality.

3.2. Pasting properties.

Pasting properties describe the response of starch to heating, swelling, shearing, and cooling in aqueous systems, often measured by Rapid Visco Analyser (RVA). These properties are strongly affected by starch granule integrity, amylose–amylopectin ratio, and retrogradation tendency, all of which are altered by modification treatments [42].

The pasting properties of native and modified jack bean flour are shown in Table 2. The results showed that the modification process of jack bean flour can increase the pasting temperature (PT) and breakdown viscosity (BV), and reduce the setback viscosity (SV). The increase in PT results from enhanced molecular interactions during modification, which imply increased intragranular bonded forces [43], allowing the starch to absorb more heat before its structure disintegrates. The lowest BV and SV values were observed in the DP modification, indicating greater resistance to heating [44] and a tendency toward retrogradation [45]. This is because the increase in crystallinity during the modification process increases granule resistance to swelling [42], due to increased intragranular bonded forces that can minimise intensive amylose leaching. According to Zhang *et al.* [46], the lower the amylose leaching level, the lower the BV value. In addition, molecular interactions that occur during modification also increase resistance to destabilisation of the amorphous region [42]. This resistance reduces the opportunity for starch re-crystallisation after gelatinisation and cooling,

which is associated with low SV values. The results of this study are in line with those of Demirkesen-Bicak *et al.* [47] and Halim *et al.* [48], who reported a decrease in BV and SV values in DP-modified black chickpea starch and HA-modified faba bean starch. A similar finding was also found in ANN-modified mung bean starch, which reported an increase in PT [49].

Table 2. Pasting properties of native and modified jack bean flours.

Samples	Parameter				
	PT (°C)	PV (cP)	BV (cP)	SV (cP)	FV (cP)
K	88.70±0.57	788.00±46.67	106.00±8.49	370.50±68.59	1052.50±106.77
AC 1 S	95.03±0.04	306.00±11.31	192.00±7.07	8.50±2.12	122.50±16.26
AC 2 S	95.00±0.07	385.00±55.15	288.00±55.15	-18.00±1.41	79.00±1.41
ANN	90.33±0.53	645.00±59.40	163.50±10.61	138.50±71.42	620.00±1.41
MC	94.93±0.11	270.00±52.33	108.00±36.77	-26.00±12.73	136.00±2.83
HMT	89.53±0.04	797.50±00.71	403.00±0.71	-33.50±3.54	360.50±2.12
HA	90.70±0.07	734.00±42.43	77.50±3.54	196.00±33.94	852.50±12.02
DP	Nd	60.00±5.66	11.50±2.12	3.50±4.95	52.00±2.83
FAC	95.03±0.04	314.50±38.89	161.50±3.54	-35.00±26.87	118.00±15.56

PT (pasting temperature); PV (peak viscosity); BV (breakdown viscosity); SV (setback viscosity); FV (final viscosity); K (control); AC 1 S (autoclaving-cooling 1 cycle); AC 2 S (autoclaving-cooling two cycles); ANN (annealing); MC (microwave cooling); HMT (heat moisture treatment); HA (acid hydrolysis); DP (debranching pullulanase); FAC (fermentation followed by AC 1 S); Nd (not detected).

Based on pasting properties, DP and HA-modified jack bean flour was identified as having ideal characteristics for application in the food industry. This is due to its low BV and SV values, which indicate thermal stability and a low tendency for retrogradation. This suggests that DP and HA-modified flour can be used as ingredients in food products requiring high-temperature cooking, such as bakery products and canned foods.

Autoclaving and cooling disrupt the native granule structure through gelatinisation and promote amylose retrogradation during cooling. After one cycle, pasting viscosity parameters are reduced: peak viscosity decreases due to weakened swelling capacity, while breakdown values also decline, reflecting greater granule stability. With two cycles, these effects intensify, and setback and final viscosity increase significantly, indicating enhanced retrogradation and resistant starch formation. Thus, AC treatments shift pasting behaviour toward lower peak/thinness but higher retrograded stability [8,9,35]. Similar results were also observed in the study by Faridah *et al.* [50] on 2-cycle AC-modified corn flour, which showed a lower SV value than the control.

Annealing improves crystalline alignment without causing gelatinisation. This treatment usually lowers peak viscosity and breakdown, as granules swell less extensively and resist shear disruption. Setback and final viscosity may increase slightly due to stronger gel formation during cooling. ANN-treated flour, therefore, shows more stable pasting behaviour, with reduced viscosity fluctuations [9,36]. Similar results were also reported in a study of ANN-modified jack bean starch by Ariyantoro *et al.* [24], which reported an increase in BV compared to the control.

Microwave treatment causes localised overheating, resulting in heterogeneous gelatinisation. Peak viscosity may initially increase due to rapid starch swelling, but the breakdown is high because swollen granules are easily disrupted. After cooling, retrogradation is partial, resulting in moderate setback viscosity. Overall, microwave-treated flour displays unstable pasting properties compared with AC or ANN [9,37].

HMT increases crystalline order and restricts granule swelling. As a result, peak viscosity and breakdown are significantly reduced, while pasting temperature increases.

Setback and final viscosity are higher, indicating strong reassociation of amylose and amylopectin chains during cooling. HMT therefore produces flour with low swelling capacity but stable paste characteristics, suitable for products requiring thermal stability [9,38]. Similar results were also reported in a study of HMT-modified barley flour by Lv *et al.* [51], which found an increase in BV compared to the control.

Acid hydrolysis selectively degrades amorphous regions, yielding smaller starch fragments with limited swelling capacity. Consequently, peak viscosity is markedly reduced, and pasting profiles show flattened curves with low breakdown. Setback viscosity may increase slightly due to aggregation of degraded chains, but overall, pasting ability is impaired. This treatment is better suited for applications requiring low-viscosity starches [9,39]. A decrease in BV value was reported by Polnaya *et al.* [7] in HA-modified sago starch (HCl 2.2 N).

Pullulanase treatment generates linear amylose chains that retrograde readily. Peak viscosity decreases due to reduced amylopectin branching (which normally supports swelling), but setback and final viscosity increase strongly because of amylose reassociation during cooling. Thus, debranched flour exhibits low initial swelling but high gel stability [9,40]. Similar results were also reported in research on sweet potato starch enzymatically modified with pullulanase (Babu and Parimalavalli [52]), which showed a lower SV value than the control.

Fermentation produces enzymatic hydrolysis and porous structures, while subsequent AC induces retrogradation. The pasting profile reflects both effects: peak viscosity is low due to weakened granule integrity, while setback and final viscosity are markedly higher, indicating strong retrogradation of the starch network. Compared to AC alone, FAC results in even more stable final pastes, but with reduced swelling power [8,9,11]. Similar results were also reported in the study by Yuliana *et al.* [53] on modified composite sweet potato flour fermented by LAB, which showed a lower SV value than the control.

The lowest peak viscosity was observed in acid hydrolysis, debranching, and FAC treatments, primarily due to structural weakening and reduced swelling capacity. The most stable pastes, characterised by high setback and final viscosity, were produced by AC with two cycles, HMT, pullulanase debranching, and FAC. In contrast, microwave-cooled pastes were the least stable, with higher breakdown values due to heterogeneous heating. Meanwhile, annealing had the mildest impact on the pasting profile, resulting in only a slight reduction in peak viscosity while maintaining paste stability. The findings demonstrate that the pasting properties of jack bean flour can be tailored through different modification methods. AC, two cycles, pullulanase, HMT, and FAC produce high gel stability and resistant starch. Annealing provides smooth viscosity with low breakdown. Acid hydrolysis yields low-viscosity starch suitable for specialised uses, while microwave cooling results in high swelling and viscosity, but with unstable pastes.

3.3. Total starch.

Total starch content in jack bean flour can be influenced by thermal, enzymatic, chemical, or fermentation-based modifications. While starch is the dominant carbohydrate fraction, its measurable content may decrease following treatments that promote hydrolysis, leaching, retrogradation, or microbial utilisation, and remain more stable in treatments that reorganise starch without extensive degradation [11]. The change in total starch in jack bean flour following modification treatments is presented in Figure 1. Changes in total starch that occur in modified jack bean flour are caused by breaking the glycosidic bonds of the starch

fraction, either in the linear α -1,4 glycosidic bonds or the branched α -1,6 glycosidic bonds. Changes in total starch that occur in modified flour are caused by the breaking of glycosidic bonds in the fraction due to thermal processing [9]. The more AC cycles applied, the greater the amount of starch degradation, resulting in a significant decrease in total starch content. A significant decrease in total starch content was also found in DP modification, which involves hydrothermal and enzymatic processes that break glycosidic bonds in the starch fraction. Enzymatic modification with pullulanase significantly hydrolyses the amylopectin chain to produce short-chain amylose [54]. Modified AC 2S and DP flours had significantly lower total starch content than native flour. This is because the intensive thermal process degrades the starch structure into simpler ones. Setiarto *et al.* [11] reported that increased starch damage indicates a lower total starch content compared to unmodified flour. Test results showed that the total starch content of modified jack bean flour ranged from 54.91 to 62.92% db. The total starch content of modified jack bean flour with AC 2S was 55.31% db, lower than that of modified jack bean starch with AC 3S (68.42% db) [55] and that of modified black chickpea starch with AC 1S (84.8% db) [47].

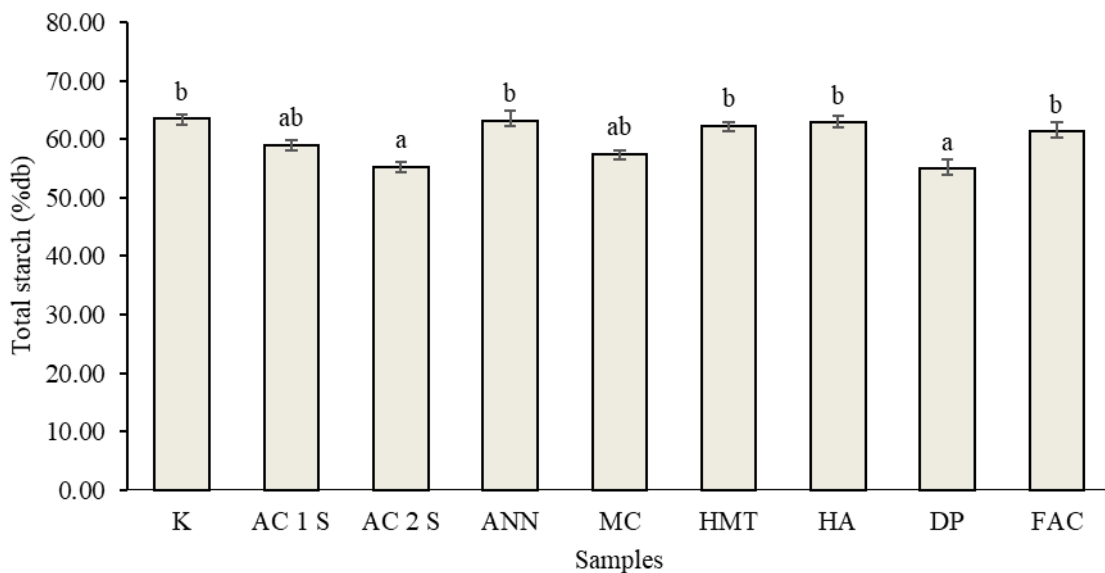


Figure 1. Total starch of native and modified jack bean flours. K (control), AC 1 S (autoclaving-cooling 1 cycle), AC 2 S (autoclaving-cooling two cycles), ANN (annealing), MC (microwave cooling), HMT (heat-moisture treatment), HA (acid hydrolysis), DP (debranching pullulanase), and FAC (fermentation followed by AC 1 S). Means within bar charts with different superscripts are significantly different ($P < 0.05$).

Autoclaving causes gelatinisation, during which soluble starch components may leach into the surrounding medium, slightly reducing measurable total starch. After cooling, retrogradation generates resistant starch fractions; however, these are still classified as starch, so the overall loss is moderate. With two cycles, further leaching and chain breakdown may occur, leading to a greater reduction compared with one cycle [9,35]. The results of this study are consistent with those of Setiarto *et al.* [56], who reported a decrease in total starch content in campolay flour modified with AC 1 S and AC 2 S compared to unmodified flour.

Annealing operates under sub-gelatinisation conditions and primarily reorganises crystalline regions without solubilising starch. Thus, total starch content remains largely unchanged, with only minor variations due to subtle molecular rearrangements [8,9,36].

Microwave treatment causes rapid localised gelatinisation and may enhance starch solubilisation, leading to slight reductions in total starch as soluble fractions become more extractable or degraded. However, the short processing time limits the extent of starch loss

compared with AC [9,37]. The decrease in total starch content observed in this study is consistent with the findings of Marta *et al.* [6] on MC-modified corn starch.

HMT involves high temperature but limited moisture, restricting gelatinisation and starch leaching. The total starch is therefore mostly preserved, with minimal losses. Any reductions are primarily associated with minor molecular fragmentation rather than solubilisation [8,9,38].

Acid hydrolysis preferentially cleaves glycosidic bonds in amorphous regions, producing low-molecular-weight sugars such as glucose and maltose. This leads to a significant reduction in measurable total starch content, as a portion is converted into soluble sugars not quantified as starch in standard assays [8,9,39]. The decrease in total starch during HA modification is consistent with the findings of Kusnandar *et al.* [57] on HA-modified sago starch.

Pullulanase hydrolyses α -(1 \rightarrow 6) linkages, converting amylopectin into linear chains. While much of this material still qualifies as starch, prolonged hydrolysis may release shorter chains, or dextrans, resulting in a moderate decrease in total starch values. Nonetheless, the loss is generally less pronounced than with strong acid hydrolysis [8,9,40]. The decrease in total starch during DP modification is consistent with the findings of Setiarto *et al.* [9] on DP-modified porang flour.

During fermentation, microorganisms metabolise part of the starch, directly reducing its total content through enzymatic hydrolysis into organic acids and other metabolites. Subsequent autoclaving–cooling can further promote leaching and retrogradation, compounding the effect. Thus, FAC results in one of the largest reductions in total starch compared with other treatments, as it combines biological consumption with thermal restructuring [8,9,11]. The decrease in total starch content in FAC is consistent with the findings of Setiarto *et al.* [11] and Faozi *et al.* [58] on FAC-modified taro flour.

The largest reductions in total starch were observed with acid hydrolysis and FAC, as these processes convert starch into sugars or microbial metabolites. Moderate decreases occurred with pullulanase debranching, autoclaving–cooling (particularly two cycles), and microwave cooling, where partial hydrolysis and leaching contribute to starch loss. In contrast, annealing and HMT caused only minimal changes, since starch integrity is largely preserved under these conditions. These outcomes suggest that when maintaining high starch content is desired, annealing and HMT are preferred. Conversely, for applications aiming at starch hydrolysis, functional oligosaccharide release, or reduced starch availability, acid hydrolysis and FAC are most effective.

3.4. Starch digestibility.

Figure 2 shows that the digestibility of modified jack bean flour decreased or increased compared to native flour. Modified jack bean flours with AC, MC, DP, and FAC significantly reduced starch digestibility compared with native jack bean flour. Gelatinisation and retrogradation during modification contribute to the rearrangement of starch molecules, both within amylose and between amylose and amylopectin, forming double helix structures that result in stronger starch bonds and increased difficulty in digestion [50].

Furthermore, decreased starch digestibility is related to α -amylase being unable to recognise the double helix bonds in starch formed by gelatinisation and retrogradation [59]. Low starch digestibility indicates high levels of resistant starch and a low glycemic index in a product [8].

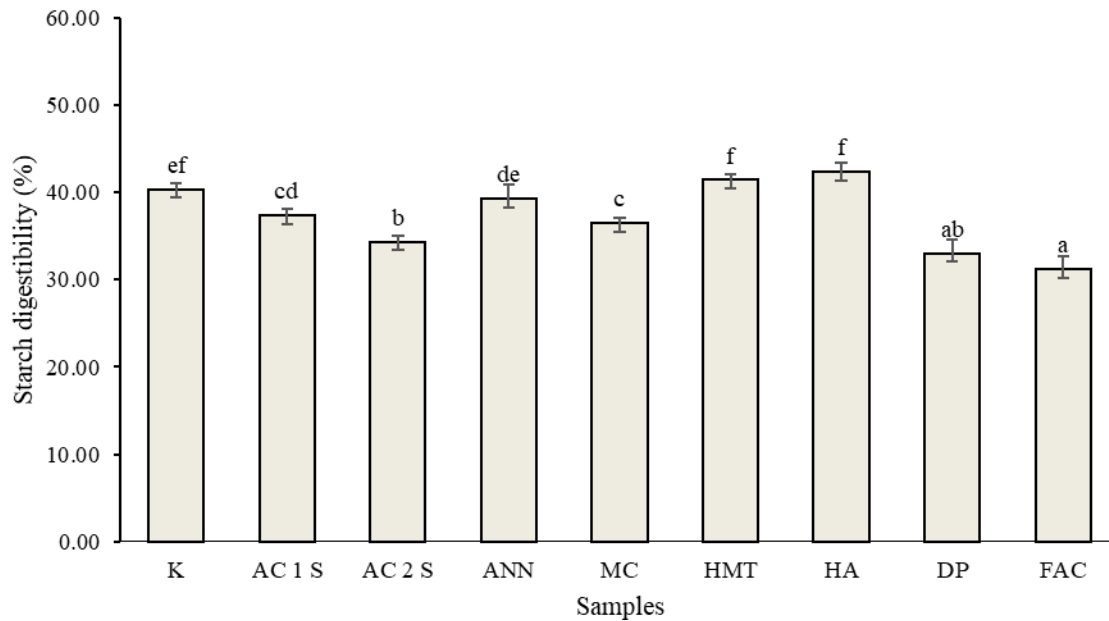


Figure 2. Starch digestibility of native and modified jack bean flours. K (control), AC 1 S (autoclaving-cooling 1 cycle), AC 2 S (autoclaving-cooling two cycles), ANN (annealing), MC (microwave cooling), HMT (heat-moisture treatment), HA (acid hydrolysis), DP (debranching pullulanase), and FAC (fermentation followed by AC 1 S). Means within bar charts with different superscripts are significantly different ($P < 0.05$).

The results of the starch digestibility test of modified jack bean flour ranged from 39.26-31.23%, lower than that of modified black chickpea starch AC 1S at 62.2% [47], modified field bean starch HMT at 45% [60], and modified pea starch DP at 36.1% [61]. This shows that jack bean flour samples treated with AC, MC, DP, and FAC have the potential to serve as raw materials for functional foods.

Starch digestibility of jack bean flour is closely linked to the extent of structural disruption, retrogradation, and crystalline reorganisation induced by different modification methods. Each approach alters the accessibility of starch granules to digestive enzymes, resulting in varying proportions of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [8,9].

Autoclaving and cooling cause gelatinisation during heating and retrogradation during cooling. After one cycle, partial gelatinisation increases enzyme accessibility, raising RDS levels. However, retrogradation of amylose produces RS fractions. With two cycles, retrogradation is intensified, leading to denser, more ordered aggregates. Thus, digestibility shifts toward higher RS content and reduced RDS. This indicates that repeated AC cycles enhance starch resistance to enzymatic hydrolysis, which is beneficial for lowering glycemic response [9,35]. The results of this study are consistent with those of Faridah et al. [50], who reported that AC-modified corn flour and corn starch showed a significant decrease in starch digestibility compared to the control.

Annealing, performed at sub-gelatinisation temperatures with excess moisture, does not destroy granule integrity but reorganises crystalline lamellae. This increases the stability of the double helix and reduces enzyme accessibility. Digestibility is therefore reduced, with higher SDS content and moderate RS formation. Compared to AC, ANN favours slow digestibility rather than extensive resistant starch production [8,9,36]. This is in line with the findings of Setiarto *et al.* [9], who reported that the digestibility of ANN-modified porang flour starch was not significantly different from that of the control.

Microwave treatment rapidly disrupts starch granules through localised heating. Initial gelatinisation enhances RDS, but subsequent cooling promotes limited retrogradation. The heterogeneous microstructure yields both easily digestible and partially resistant fractions. Overall, microwave cooling increases RDS relative to AC or ANN but produces less RS [9,37]. A similar study by Deng *et al.* [62] reported a significant decrease in starch digestibility in MC-modified potato starch compared to the control.

HMT strengthens crystalline domains without gelatinisation, resulting in more ordered, compact granules. This reduces enzyme penetration and lowers RDS, while enhancing SDS. RS levels may also increase slightly, but the dominant effect is slower digestibility rather than complete resistance. Thus, HMT-modified jack bean flour exhibits delayed glucose release profiles [8,9,38]. The increase in starch digestibility in HMT compared to the control is consistent with Zhao *et al.* [63], who reported higher starch digestibility in mung bean starch modified by repeated HMT (RHMT).

Acid hydrolysis preferentially removes amorphous regions, enriching crystalline fractions. This increases relative crystallinity and generates smaller, more compact starch fragments. The enhanced crystallinity reduces enzymatic accessibility, leading to lower RDS and higher RS content. However, excessive hydrolysis may increase surface porosity, slightly improving the digestibility of fragmented starch particles [8,9,39]. The increased starch digestibility is consistent with the findings of Setiarto *et al.* [8] on HA-modified corn flour.

Pullulanase cleaves α -(1 \rightarrow 6) linkages in amylopectin, producing linear amylose chains. These chains retrograde readily, forming tightly packed resistant structures. As a result, digestibility shifts toward increased RS content and reduced RDS. Debranching is one of the most effective enzymatic strategies for producing resistant starch in jack bean flour [8,9,40]. This finding aligns with the research of Liu *et al.* [64] and Bodjrenou *et al.* [20], which reported a significant decrease in the digestibility of waxy corn starch and purple sweet potato starch enzymatically modified with pullulanase.

The combination of fermentation and AC produces a hybrid effect. Fermentation generates porous structures and increases linear chains through microbial enzymatic action. Subsequent autoclaving–cooling promotes retrogradation of these chains, yielding a dense RS-rich matrix. Digestibility is markedly reduced, with substantial RS and SDS formation. Compared to AC alone, FAC produces more porous yet retrograded structures, balancing initial enzyme penetration with strong resistance during digestion [8,9,11]. The decreased starch digestibility is consistent with the research of Setiarto *et al.* [11] on FAC-modified taro flour.

These results indicate that the starch digestibility of jack bean flour can be strategically tailored through specific modification methods. Debranching, AC with two cycles, and FAC are most effective for developing functional foods with a low glycemic index. At the same time, HMT and annealing are better suited for products designed for controlled, gradual glucose release. In contrast, microwave cooling yields the highest RDS fraction, making it appropriate for energy-dense or easily digestible food formulations.

3.5. Microstructure of jack bean flour.

Physical modification methods profoundly influence the starch–protein matrix of jack bean flour, resulting in distinct microstructural changes that correlate with functional and physicochemical properties. Autoclaving followed by cooling promotes partial gelatinisation and subsequent retrogradation. After one cycle, starch granules typically exhibit surface disruption, swelling, and fissures, indicating the breakdown of crystalline regions. A second

autoclaving–cooling cycle intensifies these effects, leading to more compact retrograded structures and a denser matrix due to repeated gelatinisation retrogradation processes. This results in reduced granule integrity and the formation of resistant starch aggregates, as retrograded amylose chains become more crystalline and less susceptible to enzymatic digestion [8,9,35].

Annealing involves exposure to excess moisture at sub-gelatinisation temperatures, which does not destroy granule integrity but alters internal organisation. Jack bean starch granules under ANN usually retain their smooth surfaces, yet internal rearrangements occur, enhancing crystallinity and alignment of double helices. This leads to more compact, thermally stable granules without significant fissures or pores, distinguishing ANN from more disruptive treatments such as autoclaving [8,9,36].

Microwave energy induces rapid molecular vibration, promoting localised gelatinisation and disruption of starch granule morphology. Unlike conventional heating, microwave treatment produces heterogeneous microstructural effects, with some granules swollen, ruptured, or partially collapsed. Cooling afterwards facilitates partial retrogradation, yielding irregular aggregates and porous structures. This microstructural heterogeneity may enhance water absorption and solubility but reduce structural uniformity compared to ANN or HMT [9,37].

HMT applies high temperature with limited moisture, leading to structural reorganisation without complete gelatinisation. Under HMT, jack bean starch granules generally maintain their integrity and birefringence, but internal reorientation increases crystallinity and molecular order. Surface morphology remains relatively intact, though slight roughening and a slight increase in compactness are observed. The result is a more rigid and less porous granule structure, enhancing thermal stability and resistance to enzymatic hydrolysis [8,9,38].

Autoclaving–cooling (particularly two cycles) produces the greatest structural disruption, as evidenced by fissures and retrograded aggregates. Annealing and HMT are stabilising treatments that maintain granule integrity while enhancing internal crystalline organisation. Microwave cooling yields the most heterogeneous and porous structures, reflecting its rapid and uneven heating mechanism. These modifications demonstrate how processing strategies differentially alter the microstructure of jack bean flour. Treatments such as ANN and HMT are suited for improving stability and functionality without extensive granule damage, while autoclaving, cooling, and microwave approaches induce more pronounced retrogradation and aggregation, potentially useful for generating resistant starch fractions or modifying textural attributes in food applications [8,9,35].

Enzymatic modification using pullulanase, a debranching enzyme that hydrolyses α -(1→6) glycosidic linkages, induces substantial changes in the starch–protein matrix of jack bean flour. This treatment selectively removes branch points in amylopectin, producing shorter linear amylose chains and altering the overall supramolecular organisation of starch granules. The microstructural alterations of modified jack bean flour observed under SEM are summarised in Table 3.

Unlike physical treatments such as autoclaving or HMT, pullulanase modification does not immediately destroy the external morphology of starch granules. Under scanning electron microscopy (SEM), granules typically maintain their original oval or polygonal shapes, although minor surface roughening and small pits may appear (Figure 3). These features reflect localised enzymatic attack at vulnerable amorphous regions near the granule surface.

Table 3. Comparative summary of microstructural characteristics of modified jack bean flour observed under SEM.

Modification method	Granule morphology	Structural observation (SEM analysis)
Native (Control)	Intact, polygonal granules with smooth surfaces	Granules are discrete with well-defined edges and no visible surface damage; typical legume starch morphology.
Autoclaving–Cooling (AC)	Partially disrupted and aggregated	Granule swelling and partial gelatinisation were observed, with aggregation due to retrogradation during cooling cycles.
Annealing (ANN)	Smooth and slightly swollen	The surface remains mostly intact with minor expansion, indicating structural rearrangement without full gelatinisation.
Microwave (MW)	Fragmented and irregular	Localised melting and surface erosion; presence of fissures and collapsed granules due to rapid heating.
Heat–Moisture Treatment (HMT)	Densely compacted and rough	Partial granule deformation, accompanied by surface roughness, suggests increased crystalline alignment and reduced swelling capacity.
Acid Hydrolysis (HA)	Eroded and porous	Pronounced surface corrosion and pitting due to acid penetration; evidence of molecular degradation.
Debranching (DP)	Distorted and slightly compacted	Partially disrupted granules with smoother internal surfaces; enhanced formation of linear chains and resistant starch structure.
Fermentation–Autoclaving–Cooling (FAC)	Deformed and aggregated	Complete gelatinisation and retrograded matrix formation; microbial activity promotes starch breakdown before re-crystallisation.

The primary impact of debranching is internal. Hydrolysis of α -(1→6) linkages reduces branching density in amylopectin, resulting in the generation of linear chains capable of forming more stable double helices. During subsequent cooling or storage, these linear chains retrograde more readily, promoting the formation of tightly packed, crystalline domains. Microstructurally, this leads to denser aggregates and the appearance of compact retrograded clusters rather than loose granule arrangements. Enzymatic debranching often increases porosity within the flour matrix. Hydrolytic activity creates microchannels, which appear as cavities or erosion marks in SEM images (Figure 3). These modifications enhance water diffusion pathways, facilitating hydration and swelling. As amylose retrogrades, granules may aggregate into block-like or irregular structures, reducing overall granule individuality and producing a more continuous gel-like matrix [8,9,40].

Compared to physical modification techniques (autoclaving, microwave, or HMT), pullulanase treatment introduces more subtle surface changes but pronounced internal restructuring. While treatments like autoclaving-cooling fracture and fissure the granule surface, pullulanase primarily alters the molecular architecture, leading to enhanced crystallinity and the formation of resistant starch. This structural reorganisation makes debranched jack bean flour particularly suitable for applications requiring slow digestibility and improved textural stability. The microstructural effects of pullulanase debranching highlight its potential for tailoring the functional properties of jack bean flour. The denser, retrograded microstructure may contribute to reduced glycemic response, improved thermal stability, and desirable textural properties in food systems [8,9,40].

The sequential application of fermentation followed by autoclaving–cooling (AC) induces more profound microstructural alterations in jack bean flour compared to either method alone. This combined approach integrates biological enzymatic degradation with thermal retrogradation restructuring, generating unique starch–protein morphologies. During fermentation, microbial enzymes (amylases, proteases) and organic acids partially hydrolyse starch granules and disrupt the protein matrix surrounding them. SEM images typically show surface erosion, shallow pits, and localised fragmentation (Figure 3). The flour becomes more porous, with weakened crystalline–amorphous boundaries. This pre-conditioning reduces

granule rigidity, creating vulnerable sites for subsequent physical disruption during autoclaving [8,9,11].

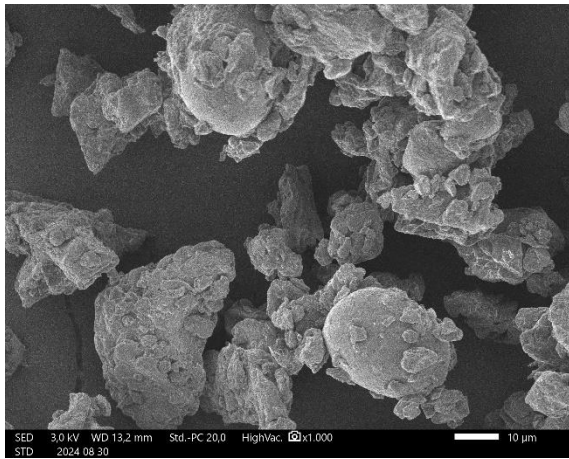
When fermented flour is subjected to autoclaving–cooling cycles, starch granules undergo more extensive gelatinisation due to their pre-weakened structure. Upon cooling, retrogradation occurs more efficiently, especially with the higher proportion of linear amylose chains produced during fermentation. The result is a dense network of retrograded starch aggregates, with compact block-like or gel-like clusters visible under SEM (Figure 3). Compared to non-fermented flour subjected to AC, the retrograded structures here are more continuous and interconnected, reflecting synergistic restructuring of starch chains. The combined treatment produces a hybrid microstructure with both porous surface features (from fermentation) and densely retrograded crystalline aggregates (from AC). Small cavities and fissures may remain embedded within larger retrograded clusters, illustrating the dual action of microbial degradation and thermal retrogradation. The flour matrix appears more compact overall, with reduced individual granule visibility and greater aggregation [8,9,11].

The porous–retrograded hybrid structure may balance digestibility and resistance: initial porosity enhances water penetration, but the dense retrograded clusters slow enzymatic hydrolysis (Figure 3). This could yield flour with moderate digestibility, improved textural stability, and enhanced resistant starch content, making it suitable for functional foods aimed at glycemic control. In comparison, fermentation alone favours digestibility, while autoclaving–cooling alone favours resistant starch; their combination creates an intermediate but tailored functionality. The combination of fermentation and autoclaving–cooling produces a synergistically modified microstructure in jack bean flour, integrating microbial erosion with thermally induced retrogradation. This results in a porous yet compact matrix, with potential advantages for functional food applications requiring both nutrient bioavailability and slow starch digestibility [8,9,11].

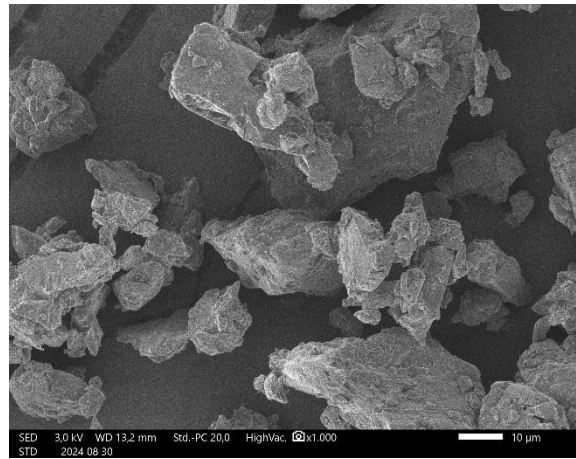
Acid hydrolysis is a common chemical modification technique that selectively cleaves the glycosidic bonds in starch, particularly targeting the amorphous regions while leaving the crystalline domains relatively intact. When applied to jack bean flour, this treatment induces distinct microstructural transformations that influence both granular morphology and molecular arrangement [8,9,39].

Scanning electron microscopy (SEM) observations typically reveal that acid hydrolysis does not destroy the native shape of starch granules (Figure 3). Granules often retain their polygonal or oval outlines; however, surface roughness, shallow pits, and localised erosions are also present. These changes reflect preferential hydrolysis of amorphous zones at or near the granule surface, resulting in etched or pitted granules with irregular textures. At the molecular level, acid hydrolysis selectively degrades amorphous lamellae, exposing and sharpening the crystalline regions. This leads to the formation of smaller, more compact crystalline fragments within the flour matrix. The relative crystallinity of the starch may increase, since hydrolysis removes less-ordered components and enriches the proportion of crystalline domains. Consequently, the microstructure becomes more rigid and less porous compared to enzymatic modifications, such as fermentation [8,9].

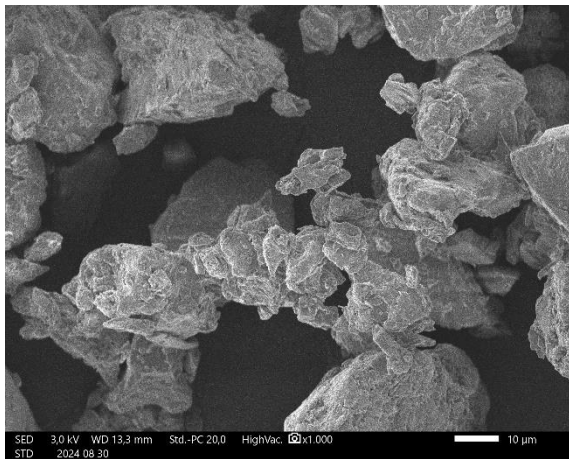
Prolonged hydrolysis can intensify surface erosion, leading to the formation of porous structures, cracks, and, in some cases, partial granule fragmentation. Aggregates of degraded starch fragments may form, resulting in a less uniform microstructure in the flour. Such porosity enhances the accessibility of hydrolytic enzymes in subsequent processing, but extensive hydrolysis can also reduce granule integrity and yield fine starch debris [8,9].



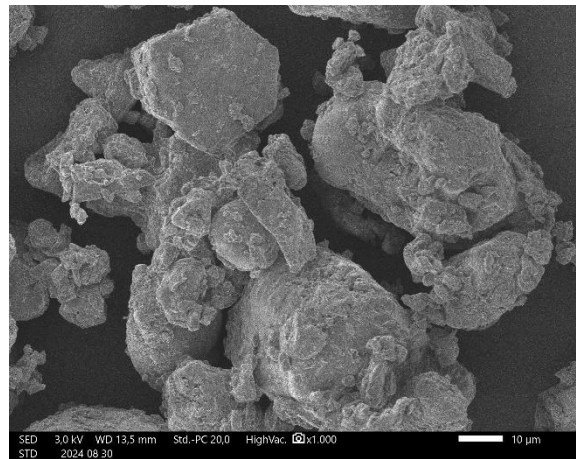
(a)



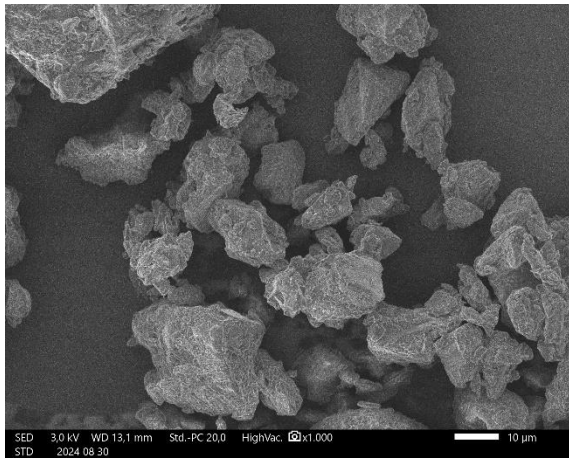
(b)



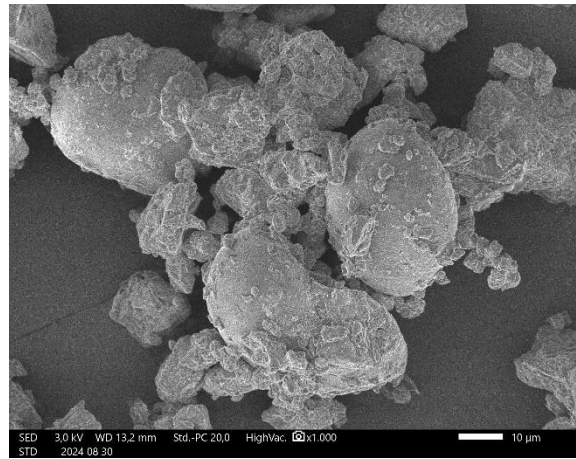
(c)



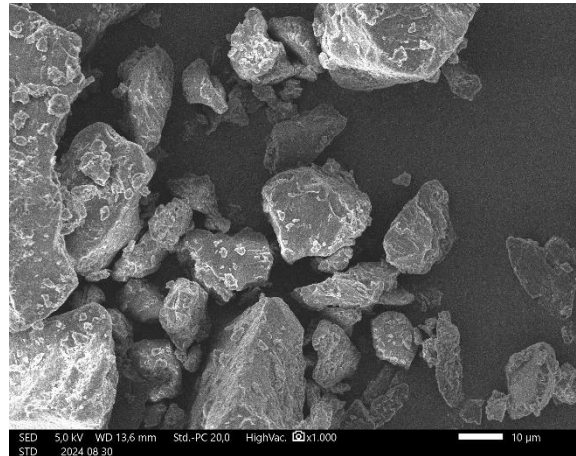
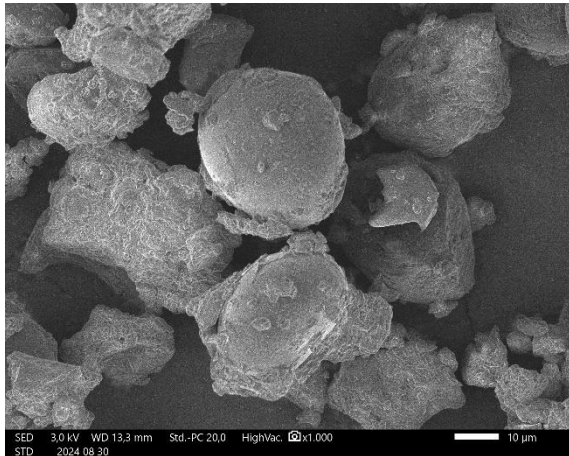
(d)



(e)



(f)



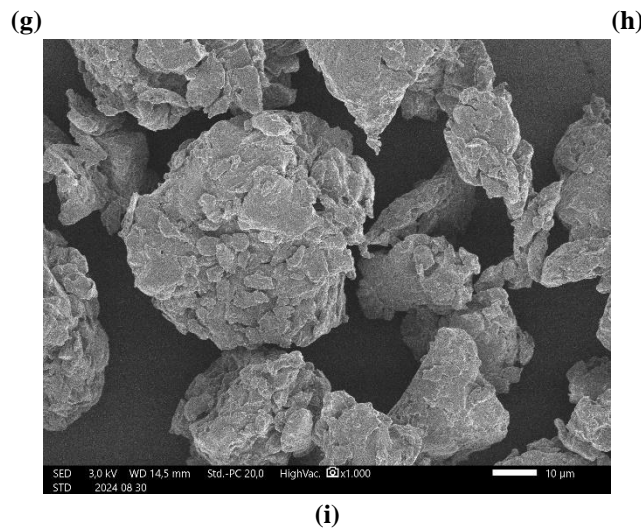


Figure 3. Scanning electron micrographs of native and modified jack bean flours with 1000x magnification, (a) control; (b) autoclaving-cooling 1 cycle; (c) autoclaving-cooling two cycles; (d) annealing; (e) microwave cooling; (f) heat-moisture treatment; (g) acid hydrolysis; (h) debranching pullulanase; (i) fermentation followed by autoclaving-cooling 1 cycle.

Compared to physical modifications (e.g., autoclaving–cooling), acid hydrolysis produces less gelatinisation and retrogradation, but more targeted erosion of amorphous regions. In contrast to enzymatic debranching (pullulanase), which increases linear chains and retrogradation, acid hydrolysis reduces granule uniformity without promoting ordered chain reassembly. The result is a microstructure characterised by intact outlines, roughened surfaces, and porous interiors rather than collapsed or fully aggregated clusters [8,9]. The microstructural effects of acid hydrolysis can be exploited in food applications that require higher digestibility, as erosion of amorphous regions enhances enzymatic susceptibility. Additionally, the increase in relative crystallinity may improve flour stability in certain formulations. However, excessive hydrolysis may compromise functionality by over-fragmenting the starch matrix [8,9].

Observation of starch granule morphology provides an initial indication of increased crystallinity, with implications for digestive enzyme resistance through the reduction of the amorphous region. Morphological changes of starch granules were observed using an SEM and are presented in Figure 3. The SEM analysis was conducted using 1,000x magnification. The results of the study identified the morphology of jack bean starch flour as spherical or ellipsoidal with sizes ranging from 22.54 to 30.11 micrometres, which is in line with [65]. Jack bean flour modified with AC, DP, MC, and FAC exhibited a rough, lump-like structure due to damage to the starch granule structure, with implications for increased crystallinity. Setiarto *et al.* [9] reported that an increase in the crystalline region has implications for an increase in the density of the starch granule structure, making it more resistant to digestive enzymes such as alpha amylase.

Starch granules in flour, when fully gelatinised (AC, MC, DP, and FAC), become shapeless or irregularly shaped. This irregular crystalline shape was formed because the granules were damaged and swollen during the gelatinisation and retrogradation process. These results are similar to those of Faridah *et al.* [50] and Setiarto *et al.* [8], who analysed AC- and MC-modified corn flour and starch and observed irregularly shaped starch granules. Similar results were also identified in DP-modified black chickpea starch [47] and FAC-modified porang flour [9], which reported granule morphology deformation compared to native flour or starch.

4. Conclusions

This study demonstrates that the physicochemical, structural, and functional properties of jack bean flour can be effectively modified through various physical, chemical, enzymatic, and fermentation-based approaches. Each treatment uniquely influences starch organisation, pasting behaviour, and digestibility. Among the tested methods, pullulanase debranching (DP) produced the most favourable characteristics, enhanced thermal stability, low retrogradation tendency, and improved starch digestibility, indicating its suitability for functional food development. The findings suggest that modified jack bean flour, particularly the DP variant, has promising potential for food industry applications, ranging from low-glycaemic, resistant-starch-enriched products to energy-dense, easily digestible formulations. Future work should include rheological and sensory evaluations to validate functional performance and consumer acceptance, thereby strengthening its industrial applicability. However, this study is limited by the absence of rheological and sensory evaluations, which are essential to confirm processing performance and consumer acceptability. Future research should therefore incorporate these assessments to comprehensively validate the functional potential of modified jack bean flour for industrial utilisation.

Author Contributions

Conceptualization, R.H.B.S. and A.W.; methodology, R.H.B.S., A.W., T.M., U.H., and L.A.; software, A.W. and L.A.; validation, R.H.B.S., T.M., U.H., and L.A.; formal analysis, A.W. and L.A.; investigation, R.H.B.S., A.W., T.M., U.H., and L.A.; resources, R.H.B.S.; data curation, A.W. and L.A.; writing—original draft preparation, A.W., R.H.B.S., T.M., and U.H.; writing—review and editing, A.W., R.H.B.S., T.M., and U.H.; visualization, A.W.; supervision, R.H.B.S., T.M., U.H., and L.A.; project administration, R.H.B.S., T.M., and U.H. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding.

Acknowledgments

The author would like to thank all parties who have helped complete this research. All authors made equal contributions as the main contributors to this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Virly, S.A.M.; Trisnawati, C.Y.; Kaharso, V.C.; Ivana, F.; Asali, K. Physicochemical characteristics of jack bean (*Canavalia ensiformis* (L.) DC.) milk, a non-dairy milk alternative developed using various pretreatment methods. *Food Res.* **2024**, *8*, 170–178, [https://doi.org/10.26656/fr.2017.8\(4\).382](https://doi.org/10.26656/fr.2017.8(4).382).
2. Okomoda, V.T.; Tiamiyu, L.O.; Uma, S.G. Effects of hydrothermal processing on nutritional value of *Canavalia ensiformis* and its utilization by *Clarias gariepinus* (Burchell, 1822) fingerlings. *Aquac. Rep.* **2016**, *3*, 214–219, <https://doi.org/10.1016/j.aqrep.2016.04.003>.
3. Niu, H.; Han, Q.; Cao, C.; Liu, Q.; Kong, B. Short-term retrogradation behaviour of corn starch is inhibited by the addition of porcine plasma protein hydrolysates. *Int. J. Biol. Macromol.* **2018**, *115*, 393–400, <https://doi.org/10.1016/j.ijbiomac.2018.04.047>.
4. Cahyana, Y.; Wijaya, E.; Halimah, T.S.; Marta, H.; Suryadi, E.; Kurniati, D. The effect of different thermal modifications on slowly digestible starch and physicochemical properties of green banana flour (*Musa acuminata colla*). *Food Chem.* **2019**, *274*, 274–280, <https://doi.org/10.1016/j.foodchem.2018.09.004>.
5. Pasca, B.D.; Muhandri, T.; Hunaefi, D.; Nurtama, B. Physicochemical characteristics of cassava flour with several modification methods. *J. Mutu Pangan Indones. J. Food Qual.* **2022**, *8*, 97–104, <https://doi.org/10.29244/jmpi.2021.8.2.97>.
6. Marta, H.; Cahyana, Y.; Bintang, S.; Soeherman, G.P.; Djali, M. Physicochemical and pasting properties of corn starch as affected by hydrothermal modification by various methods. *Int. J. Food Prop.* **2022**, *25*, 792–812, <https://doi.org/10.1080/10942912.2022.2064490>.
7. Polnaya, F.J.; Huwae, A.A.; Tetelepta, G. Physico-chemical and functional properties of ihur sago (*Metroxylon sylvestre*) starch by modified acid hydrolysis. *Agritech* **2018**, *38*, 7–15, <https://doi.org/10.22146/agritech.16611>.
8. Setiarto, R.H.B.; Isra, M.; Andrianto, D.; Widhyastuti, N.; Masrukhin. Improvement of prebiotic properties and resistant starch content of corn flour (*Zea mays* L.) momala gorontalo using physical, chemical and enzymatic modification. *Trop. Life Sci. Res.* **2023**, *34*, 255–278, <https://doi.org/10.21315/tlsr2023.34.2.13>.
9. Setiarto, R.H.B.; Adyeni, W.D.; Puspawati, N.N.; Wardana, A.A.; Anshory, L.; Khusniati, T. Physicochemical, enzymatic and fermentation modifications improve resistant starch levels and prebiotic properties of porang (*Amorphophallus oncophyllus*) flour. *Int. J. Food Sci. Technol.* **2024**, *59*, 9353–9367, <https://doi.org/10.1111/ijfs.17580>.
10. Setiarto, R.H.B.; Jenie, B.S.L.; Faridah, D.N.; Saskiawan, I. Study of development resistant starch contained in food ingredients as prebiotic source. *J. Ilmu Pertan. Indones.* **2015**, *20*, 191–200, <https://doi.org/10.18343/jipi.20.3.191>.
11. Setiarto, R.H.B.; Jenie, B.S.L.; Faridah, D.N.; Saskiawan, I.; Sulistiani. Effect of lactic acid bacteria fermentation and autoclaving-cooling for resistant starch and prebiotic properties of modified taro flour. *Int. Food Res. J.* **2018**, *25*, 1691–1697.
12. Wijaya, A.; Muhandri, T.; Hasanah, U.; Setiarto, R.H.B. Physical, chemical, fermentation and enzymatic modification technology to increase resistant starch and improve prebiotic properties of high carbohydrate foods. *Rev. Agric. Sci.* **2024**, *12*, 377–400, https://doi.org/10.7831/ras.12.0_377.
13. Setiarto, R.H.B.; Widhyastuti, N.; Setiadi, D. Improvement resistant starch from modified sorghum flour by using fermentation and autoclaving-cooling cycling. *J. Ilmu Pertan. Indones.* **2018**, *23*, 10–20, <https://doi.org/10.18343/jipi.23.1.10>.
14. Iuga, M.; Mironeasa, S. A review of the hydrothermal treatments impact on starch based systems properties. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 3890–3915, <https://doi.org/10.1080/10408398.2019.1664978>.
15. Karsodimejo, S.M.; Kusnandar, F.; Lioe, H.N.; Jayanegara, A. Resistant starch content modulation of carbohydrates sources through microwave heating: meta-analysis. *J. Teknol. dan Ind. Pangan* **2023**, *34*, 210–223, <https://doi.org/10.6066/jtip.2023.34.2.210>.
16. Faridah, D.N.; Damaiyanti, S.; Indrasti, D.; Jayanegara, A.; Afandi, F.A. Effect of heat moisture treatment on resistant starch content among carbohydrate sources: a meta-analysis. *Int. J. Food Sci. Technol.* **2022**, *57*, 1965–1974, <https://doi.org/10.1111/ijfs.15276>.

17. Molavi, H.; Razavi, S.M.A.; Farhoosh, R. Impact of hydrothermal modifications on the physicochemical, morphology, crystallinity, pasting and thermal properties of acorn starch. *Food Chem.* **2018**, *245*, 385–393, <https://doi.org/10.1016/j.foodchem.2017.10.117>.
18. Syafii, F.; Fajriana, H.; Ma'rifatullah, F.R. Effect of modification of kepok banana flour to resistant starch content and physicochemical properties. *J. Agritech Sci.* **2023**, *7*, 86–102, <https://doi.org/10.30869/jasc.v7i01.1153>.
19. Rashwan, A.K.; Younis, H.A.; Abdelshafy, A.M.; Osman, A.I.; Eletmany, M.R.; Hafouda, M.A.; Chen, W. Plant starch extraction, modification, and green applications: a review. *Environ. Chem. Lett.* **2024**, *22*, 2483–2530, <https://doi.org/10.1007/s10311-024-01753-z>.
20. Bodjrenou, D.M.; Li, X.; Chen, W.; Zhang, Y.; Zheng, B.; Zeng, H. Effect of pullulanase debranching time combined with autoclaving on the structural, physicochemical properties, and in vitro digestibility of purple sweet potato starch. *Foods* **2022**, *11*, 3779, <https://doi.org/10.3390/foods11233779>.
21. Shao, D.; Zhang, J.; Shao, T.; Li, Y.; He, H.; Wang, Y.; Ma, J.; Cao, R.; Li, A.; Du, X. Modification of structure, pasting, and in vitro digestion properties of glutinous rice starch by different lactic acid bacteria fermentation. *Foods* **2025**, *14*, 367, <https://doi.org/10.3390/foods14030367>.
22. Putro, N.T.L.; Arisasmita, J.K.; Srianta, I. Study of HCN content and starch characteristics in jack bean flour (*Canavalia ensiformis*) based on seed soaking time in hot water and sodium bicarbonate solution (NaHCO₃). *J. Teknol. Pangan dan Gizi* **2015**, *14*, 72–82.
23. Rahmawati, A.; Wirawan. Formulation of food bars made from white jack bean (*Canavalia ensiformis*) autoclaving–cooling. *Teknol. Pangan Media Inf. dan Komun. Ilm. Teknol. Pertan.* **2021**, *12*, 154–165, <https://doi.org/10.35891/tp.v12i2.2342>.
24. Ariyantoro, A.R.; Fitriyani, A.; Affandi, D.R.; Muhammad, D.R.A.; Yulviatun, A.; Nishizu, T. The effect of dual modification with annealing and Heat Moisture Treatment (HMT) on physicochemical properties of jack bean starch (*Canavalia ensiformis*). *Food Res.* **2022**, *6*, 189–198, [https://doi.org/10.26656/fr.2017.6\(4\).497](https://doi.org/10.26656/fr.2017.6(4).497).
25. Wang, M.; Sun, M.; Zhang, Y.; Chen, Y.; Wu, Y.; Ouyang, J. Effect of microwave irradiation-retrogradation treatment on the digestive and physicochemical properties of starches with different crystallinity. *Food Chem.* **2019**, *298*, 125015, <https://doi.org/10.1016/j.foodchem.2019.125015>.
26. Sudheesh, C.; Sunooj, K.V.; Bhavani, B.; Aaliya, B.; Navaf, M.; Akhila, P.P.; Sabu, S.; Sasidharan, A.; Sinha, S.K.; Kumar, S.; Sajeevkumar, V.A.; George, J. Energetic neutral atoms assisted development of kithul (*Caryota urens*) starch–lauric acid complexes: a characterisation study. *Carbohydr. Polym.* **2020**, *250*, 116991, <https://doi.org/10.1016/j.carbpol.2020.116991>.
27. Faridah, D.N.; Fardiaz, D.; Andarwulan, N.; Sunarti, T.C. Physicochemical characterisation of arrowroot starch (*Maranta arundinaceae*). *Agritech* **2014**, *34*, 14–21.
28. Otemuyiwa, I.O.; Aina, A.F. Physicochemical properties and in vitro digestibility studies of microwave-assisted chemically modified breadfruit (*Artocarpus altilis*) starch. *Int. J. Food Prop.* **2021**, *24*, 140–151, <https://doi.org/10.1080/10942912.2020.1861007>.
29. Ridhani, M.A.; Vidyaningrum, I.P.; Akmalia, N.N.; Fatihatunisa, R.; Azzahro, S.; Aini, N. Potential of adding various types of sugar on the sensory and physicochemical properties of sweet bread: a review. *Pas. Food Technol. J.* **2021**, *8*, 61–68, <https://doi.org/10.23969/pftj.v8i3.4106>.
30. Syafutri, M.I.; Pratama, F.; Malahayati, N.; Hamzah, B. Profile of modified sago starch by heat moisture treatment and autoclaving. *Int. J. Sci. Res.* **2017**, *6*, 2111–2114.
31. Huang, C.; Cui, H.; Hayat, K.; Zhang, X.; Ho, C.T. Variation of moisture state and taste characteristics during vacuum drying of maillard reaction intermediates of hydrolyzed soybean protein and characterization of browning precursors via fluorescence spectroscopy. *Food Res. Int.* **2022**, *162*, 112086, <https://doi.org/10.1016/j.foodres.2022.112086>.
32. Triwitono, P.; Marsono, Y.; Murdiati, A.; Marseno, D.W. The effect of two cycles autoclaving and citric acid hydrolysis combination to chemical and physical characteristic of mung beans (*Vigna radiata* L.) starch RS-3. *Agritech* **2018**, *37*, 312–318, <https://doi.org/10.22146/agritech.11620>.
33. Lisa, M.; Lutfi, M.; Susilo, B. Effect of temperature variation and long drying of the quality flour white oyster mushroom (*Plaeotus ostreatus*). *J. Keteknikan Pertan. Trop. dan Biosist.* **2015**, *3*, 270–279.
34. Marta, H.; Rismawati, A.; Soeherman, G.P.; Cahyana, Y.; Djali, M.; Yuliana, T.; Sondari, D. The effect of dual-modification by heat-moisture treatment and octenylsuccinylation on physicochemical and pasting properties of arrowroot starch. *Polymers* **2023**, *15*, 3215, <https://doi.org/10.3390/polym15153215>.

35. Isra, M.; Andrianto, D.; Setiarto, R.H.B. Effect of autoclaving-cooling on resistant starch content and prebiotic properties of high carbohydrate foods: meta-analysis study. *J. Teknol.* **2023**, *85*, 81–90, <https://doi.org/10.11113/jurnalteknologi.v85.18515>.
36. Isra, M.; Andrianto, D.; Setiarto, R.H.B. Effect of annealing treatment on resistant starch content and prebiotic properties of high-carbohydrate foods: meta-analysis study. *Biointerface Res. Appl. Chem.* **2023**, *13*, 540, <https://doi.org/10.33263/BRIAC136.540>.
37. Isra, M.; Andrianto, D.; Setiarto, R.H.B. Effects of Microwave Heat Treatment for Resistant Starch Levels and Prebiotic Properties of High Carbohydrate Foods: Meta-Analysis Study. *CMU J. Nat. Sci* **2022**, *21*, e2022032, <https://doi.org/10.12982/CMUJNS.2022.032>.
38. Isra, M.; Andrianto, D.; Setiarto, R.H.B. Effect heat moisture treatment for resistant starch levels and prebiotic properties of high carbohydrate food: meta-analysis study. *Food Res.* **2023**, *7*, 144–150.
39. Isra, M.; Andrianto, D.; Setiarto, R.H.B. Effect of lintnerization (acid hydrolysis) on resistant starch levels and prebiotic properties of high carbohydrate foods: a meta-analysis study. *Songklanakarinn J. Sci. Technol.* **2022**, *44*, 1331–1338, <https://doi.org/10.14456/sjst-psu.2022.173>.
40. Isra, M.; Andrianto, D.; Setiarto, R.H.B. Effect of debranching pullulanase for resistant starch levels and prebiotic properties of high carbohydrate foods: meta-analysis study. *Philipp. J. Sci.* **2023**, *152*, 173–183.
41. Gonçalves, P.M.; Noreña, C.P.Z.; da Silveira, N.P.; Brandelli, A. Characterization of starch nanoparticles obtained from *Araucaria angustifolia* seeds by acid hydrolysis and ultrasound. *LWT* **2014**, *58*, 21–27, <https://doi.org/10.1016/j.lwt.2014.03.015>.
42. Subroto, E.; Indriarto, R.; Marta, H.; Shalihah, S. Effect of heat-moisture treatment on functional and pasting properties of potato (*Solanum tuberosum* L. var. Granola) starch. *Food Res.* **2019**, *3*, 469–476, [https://doi.org/10.26656/fr.2017.3\(5\).110](https://doi.org/10.26656/fr.2017.3(5).110).
43. Ashwar, B.A.; Gani, A.; Shah, A.; Wani, I.A.; Masoodi, F.A. Preparation, health benefits and applications of resistant starch—a review. *Starch - Stärke* **2016**, *68*, 287–301, <https://doi.org/10.1002/star.201500064>.
44. Arifa, A.H.; Syamsir, E.; Budijanto, S. Physicochemical properties of black rice (*Oryza sativa* L.) from West Jawa, Indonesia. *AgriTech* **2021**, *41*, 15–24, <https://doi.org/10.22146/agritech.53307>.
45. Setiarto, R.H.B.; Kusumaningrum, H.D.; Jenie, B.S.L.; Khusniati, T.; Widhyastuti, N.; Ramadhani, I. Microstructure and physicochemical characteristics of modified taro starch after annealing, autoclaving-cooling and heat moisture treatment. *Food Res.* **2020**, *4*, 1226–1233, [https://doi.org/10.26656/fr.2017.4\(4\).079](https://doi.org/10.26656/fr.2017.4(4).079).
46. Zhang, B.; Bai, B.; Pan, Y.; Li, X.M.; Cheng, J.S.; Chen, H.Q. Effects of pectin with different molecular weight on gelatinization behavior, textural properties, retrogradation and in vitro digestibility of corn starch. *Food Chem.* **2018**, *264*, 58–63, <https://doi.org/10.1016/j.foodchem.2018.05.011>.
47. Demirkesen-Bicak, H.; Tacer-Caba, Z.; Nilufer-Erdil, D. Pullulanase treatments to increase resistant starch content of black chickpea (*Cicer arietinum* L.) starch and the effects on starch properties. *Int. J. Biol. Macromol.* **2018**, *111*, 505–513, <https://doi.org/10.1016/j.ijbiomac.2018.01.026>.
48. Halim, A.; Torley, P.J.; Farahnaky, A.; Majzoobi, M. Investigating the effects of acid hydrolysis on physicochemical properties of quinoa and faba bean starches as compared to cassava starch. *Foods* **2024**, *13*, 3885, <https://doi.org/10.3390/foods13233885>.
49. Sitanggang, A.; Sani, P.; Mastuti, T. Modification of mung bean starch by annealing treatment and acetylation. Proceedings of the 2nd SEAFast International Seminar—2nd SIS, 4-5 September **2019**, Bogor, Indonesia; SciTePress: Setubal, Portugal, **2020**; Volume 1, pp. 10-19, <http://dx.doi.org/10.5220/0009977100100019>.
50. Faridah, D.N.; Silitonga, R.F.; Indrasti, D.; Afandi, F.A.; Jayanegara, A.; Anugerah, M.P. Verification of autoclaving-cooling treatment to increase the resistant starch contents in food starches based on meta-analysis result. *Front. Nutr.* **2022**, *9*, 904700, <https://doi.org/10.3389/fnut.2022.904700>.
51. Lv, Y.; Ma, S.; Yan, J.; Sun, B.; Wang, X. Effect of heat-moisture treatment on the physicochemical properties, structure, morphology, and starch digestibility of highland barley (*Hordeum vulgare* L. var. *nudum* Hook. f) flour. *Foods* **2022**, *11*, 3511, <https://doi.org/10.3390/foods11213511>.
52. Babu, A.S.; Parimalavalli, R. Effect of pullulanase debranching and storage temperatures on structural characteristics and digestibility of sweet potato starch. *J. Saudi Soc. Agric. Sci.* **2018**, *17*, 208–216, <https://doi.org/10.1016/j.jssas.2016.04.005>.
53. Yuliana, N.; Nurdjanah, S.; Setyani, S.; Novianti, D. The benefits of fermentation in improving the pasting properties of composite sweet potato flour and its application in composite white salted noodles. *Food Res.* **2023**, *7*, 120–127, [https://doi.org/10.26656/fr.2017.7\(1\).712](https://doi.org/10.26656/fr.2017.7(1).712).

54. Shi, J.; Sweedman, M.C.; Shi, Y.C. Structural changes and digestibility of waxy maize starch debranched by different levels of pullulanase. *Carbohydr. Polym.* **2018**, *194*, 350–356, <https://doi.org/10.1016/j.carbpol.2018.04.053>.
55. Rahmawati, A.; Murdiati, A.; Marsono, Y.; Anggrahini, S. Effects of complex carbohydrate from white jack bean (*Canavalia ensiformis* L. DC.) flour after autoclaving-cooling cycles on short-chain fatty acids, digesta cholesterol content and bile acid binding in hypercholesterolemic rats. *Pakistan J. Nutr.* **2018**, *17*, 586–595, <https://doi.org/10.3923/pjn.2018.586.595>.
56. Setiarto, R.H.B.; Amalia, L.; Febriani, Y.; Fitriana, T.; Widhyastuti, N. The effect of autoclaving-cooling cycle on chemical composition and biological quality of campolay flour (*Pouteria campheciana*). *J. Ris. Teknol. Ind.* **2019**, *13*, 54–69.
57. Kusnandar, F.; Hastuti, H.P.; Syamsir, E. Resistant starch of sago from acid hydrolysis and autoclaving-cooling processes. *J. Teknol. dan Ind. Pangan* **2015**, *26*, 52–62, <https://doi.org/10.6066/jtip.2015.26.1.52>.
58. Faozi, I.; Karseno; Handayani, I. Combination of lactic acid bacteria fermentation and autoclaving-cooling in the formation of resistant starch talas beneng flour (*Xanthosoma undipes* K. Koch). *J. Ilmu Pertanian Indones.* **2023**, *28*, 255–264, <https://doi.org/10.18343/jipi.28.2.255>.
59. Zailani, M.A.; Kamilah, H.; Husaini, A.; Seruji, A.Z.R.A.; Sarbini, S.R. Functional and digestibility properties of sago (*Metroxylon sagu*) starch modified by microwave heat treatment. *Food Hydrocoll.* **2022**, *122*, 107042, <https://doi.org/10.1016/j.foodhyd.2021.107042>.
60. Piecyk, M.; Domian, K. Effects of heat-moisture treatment conditions on the physicochemical properties and digestibility of field bean starch (*Vicia faba* var. minor). *Int. J. Biol. Macromol.* **2021**, *182*, 425–433, <https://doi.org/10.1016/j.ijbiomac.2021.04.015>.
61. Lu, Z.H.; Belanger, N.; Donner, E.; Liu, Q. Debranching of pea starch using pullulanase and ultrasonication synergistically to enhance slowly digestible and resistant starch. *Food Chem.* **2018**, *268*, 533–541, <https://doi.org/10.1016/j.foodchem.2018.06.115>.
62. Deng, X.; Huang, H.; Huang, S.; Yang, M.; Wu, J.; Ci, Z.; He, Y.; Wu, Z.; Han, L.; Zhang, D. Insight into the incredible effects of microwave heating: Driving changes in the structure, properties and functions of macromolecular nutrients in novel food. *Front. Nutr.* **2022**, *9*, 941527, <https://doi.org/10.3389/fnut.2022.941527>.
63. Zhao, K.; Zhang, B.; Su, C.; Gong, B.; Zheng, J.; Jiang, H.; Zhang, G.; Li, W. Repeated heat-moisture treatment: A more effective way for structural and physicochemical modification of mung bean starch compared with continuous way. *Food Bioprocess Technol.* **2020**, *13*, 452–61, <https://doi.org/10.1007/s11947-020-02405-0>.
64. Liu, W.; Hong, Y.; Gu, Z.; Cheng, L.; Li, Z.; Li, C. In structure and in-vitro digestibility of waxy corn starch debranched by pullulanase. *Food Hydrocoll.* **2017**, *67*, 104–110, <https://doi.org/10.1016/j.foodhyd.2016.12.036>.
65. Anuntagool, J.; Soonthonsun, S. Effect of particle size classification on properties of flour from jack bean: an under-utilized high protein legumes. *LWT* **2023**, *189*, 115418, <https://doi.org/10.1016/j.lwt.2023.115418>.

Publisher's Note & Disclaimer

The statements, opinions, and data presented in this publication are solely those of the individual author(s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.