JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ACETYLATION OF OZONATED SAGO (*Metroxylon sagu Rottb.*) STARCH: IMPLICATIONS FOR ITS PROPERTIES ON STARCH NOODLES

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https://doi.org/10.55251/jmbfs.12490

ARTICLE INFO

Received 6. 3. 2025
Revised 26. 4. 2026
Accepted 30. 4. 2026
Published 1. 6. 2026

Regular article

OPEN ACCESS

ABSTRACT

This study aimed to enhance the physicochemical and functional properties of sago starch for noodle production using sequential ozonation and acetylation. Native sago starch was first ozonated to disrupt its crystalline structure, followed by acetylation with varying acetic anhydride ratios (4–8 g per 100 g starch) and reaction times (5–25 min). The acetyl content (%) and degree of substitution (DS) increased with higher acetylation agent ratios and longer reaction times, peaking at 6.644% acetyl content and 0.268 DS for the highest ratio (A-OS 8) at 25 min. Functional properties were significantly altered, with acetylated-ozonated starches showing increased moisture content (up to 15.168%), a higher water solubility index (WSI: 16.835%), and a reduced water absorption index (WAI: 30.419–33.692%) compared to native starch. Rheological analysis revealed that ozonation reduced the peak viscosity of native starch (5344 cP) to 4474 cP, while acetylation partially restored the viscosity (up to 5214 cP for A-OS 4). Structural characterization via SEM confirmed surface roughness and fissures in modified starches, whereas XRD and FTIR demonstrated retained A-type crystallinity with reduced order and successful acetyl group incorporation (evidenced by C=O peaks at 1740 cm^{-1}). In noodle applications, dual-modified starch improved texture, with reduced hardness (302.37 g for A-OS 8 vs. 593.46 g for native starch) and lower cooking loss (12.85% vs. 19.39%), alongside enhanced brightness (L* = 73.3) and shorter cooking times (5 min for A-OS 8). These results demonstrate that ozonation-acetylation synergistically modifies sago starch, optimizing its properties for noodle production by balancing its solubility, viscosity, and structural stability.

Keywords: sago starch, starch modification, ozonation, acetylation, functional properties, noodle texture

INTRODUCTION

Ozonated starch in noodle production has both benefits and drawbacks owing to its modified properties. Ozonated starch, which is produced by introducing carboxyl and carbonyl groups through controlled chemical reactions, has a lower viscosity, swelling power, and retrogradation tendency than native starches **(Dimri *et al.*, 2023; Vanier *et al.*, 2017)**. These changes enhance clarity, binding strength, and storage stability, and affect the physicochemical properties of starch, such as viscosity, gelatinization properties, and texture **(Satmalawati *et al.*, 2020)**. These alterations in noodle-making can influence cooking quality, for example, cooking yield, cooking loss, and hardness **(Bao *et al.*, 2021; R. Shi *et al.*, 2023)**. These challenges entail maintaining the desired texture and cooking functionality because ozonated starch can extend cooking time, alter adhesiveness, and affect color values **(Konya & Aktaş, 2024; M. Shi *et al.*, 2025)**.

The simultaneous modification of starch by oxidation and acetylation is attracting interest because of its potential to improve the functional characteristics of starch for noodle-making. Oxidation, a chemical reaction that entails the introduction of carboxyl and carbonyl groups into the structure of starch, increases its prospects for acetylation, thereby promoting the effective absorption of acetyl groups (Pietrzyk *et al.*, 2014). Sequential modification is a process that improves the texture and stability of noodles by reducing retrogradation and increasing elasticity, resulting in high-quality products of higher quality **(Halal *et al.*, 2015; Sindhu & Khatkar, 2023)**.

The combination of acetylation and oxidation significantly changes the gelatinization and viscosity characteristics of starch, leading to reduced gelatinization temperatures and peak viscosities, which are advantageous for achieving improved cooking qualities and smoother textures for noodle production **(Ma *et al.*, 2021; Pietrzyk *et al.*, 2014)**. Oxidation-induced structural changes, including partial depolymerization and higher porosity, significantly increase the efficacy of acetylation, thereby leading to enhanced water uptake and swelling ability, which are essential for noodle texture and hydration **(Halal *et al.*, 2015; R. Shi *et al.*, 2023)**. These modifications cumulatively produce innovative starches with functional properties tuned for noodle applications, which surpasses the drawbacks of native starch. This approach focuses on the synergy between oxidation and acetylation, with potential advances in the production of high-quality noodle products **(Subroto *et al.*, 2023)**.

The texture of noodles is considerably affected by the characteristics of ozonated and acetylated starches **(S. Huang *et al.*, 2025)**. Ozonated starches are observed to exhibit decreased viscosity with high gelatinization temperature and enthalpy owing to the induction of carboxyl groups, which may be able to restrict starch retrogradation and help produce noodles with quality **(Hou *et al.*, 2024; R. Shi *et al.*, 2023)**. Lowered peak viscosity through elevated carboxyl content affects the noodle texture by upgrading the shear resistance. In contrast, acetylated starches have higher swelling power, water-holding capacity, and elasticity, resulting in more elastic and cohesive noodles. In addition, acetylation modifies the surface of starch granules to become rough and more porous, enhancing their mechanical properties and cooking quality **(Sindhu *et al.*, 2021)**.

Nonetheless, most of the studies reported thus far have involved starches that have undergone high levels of oxidation and acetylation, with the possibility of mild oxidation prior to acetylation, which remains widely unreported **(Cahyono *et al.*, 2023; R. Shi *et al.*, 2023)**. The present work aims to address this shortfall by investigating the physicochemical and structural changes in sago starch that undergoes mild oxidation before acetylation, as a novel approach to enhance its functionality for noodle production. This study indicates that slight oxidation can enhance the functional qualities of sago starch under acetylation, improving important parameters, such as water absorption, oil absorption, and texture properties. This study investigated the sequential ozonation-acetylation of sago starch as a novel approach to improve its functionality for noodle production. These changes will be analyzed, and their effects on the quality of noodles will be determined using advanced analytical methods such as rapid visco analysis (RVA), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Instrumental analysis is expected to gain new knowledge on the dual modification of sago starch for high-moisture food systems, with special reference to improving the texture and stability of wet noodles. These results are intended to fill knowledge gaps and facilitate the development of sustainable and novel starch-based food products.

MATERIAL AND METHODS

Materials

The materials used in this study included ozonated sago starch (OS), which was obtained through ozonation in a previous study **(Cahyono *et al.*, 2024)** and native sago starch (100% purity), which served as the raw material for producing ozonated starch, commercially manufactured by PT Kekal Jaya Sentosa in Palembang, Indonesia. The other chemicals used were glacial acetic acid (Merck,

Germany), sodium hydroxide (NaOH) (Merck, Germany), and hydrochloric acid (HCl) (Merck, Germany). Distilled water was used for the solution preparation and washing steps. All reagents were of analytical grade and were used without further purification.

Acetylation of Ozonated Sago Starch

The acetylation process was adapted from Kumoro and Amalia (2015) with modifications. Briefly, 280 g of oxidized sago starch was suspended in water (35 wt. %), and the pH was adjusted to 8.5 using 0.1 M NaOH under constant stirring (300 rpm, 10 min). Varying concentrations of glacial acetic acid were added to prepare the acetylated A-OS (Acetylated-Ozonation Starch) samples: A-OS 4 (4 g/100 g starch), A-OS 5 (5 g/100 g), A-OS 6 (6 g/100 g), A-OS 7 (7 g/100 g), and A-OS 8 (8 g/100 g). The mixture was stirred for 1 hour pH adjusted to 5.5 with 1 M HCl. The resulting product was filtered, washed thoroughly, and dried. (Kumoro & Amalia, 2015).
Acetyl content and DS were quantified via alkaline hydrolysis (Kumoro & Amalia, 2015). Briefly, 0.1–0.2 g of acetylated starch was hydrolyzed in a mixture of glacial acetic acid and concentrated sulfuric acid by heating. The liberated acetic acid was titrated with standardized NaOH to neutrality. Acetyl content (%) and DS were calculated as follows **(Kumoro & Amalia, 2015)**:

$$\% \text{ Acetyl content } = \frac{V\text{NaOH (mL)x } M\text{NaOH x 0.043 x 100)}}{\text{W sample (g)}} \quad (1)$$

$$\text{DS } = \frac{162 \text{ x \% Acetyl}}{4300-(42 \text{ x \%Acetyl})} \quad (2)$$

Functional Properties

Water content was analyzed according to the standard method outlined in AOAC 925.10-1995 **(Horwitz, 2010)**. The Water Absorption Index (WAI) and Water Solubility Index (WSI) were assessed using the procedure described by Chan *et al.* (2009). A one-gram sample was combined with 10 mL of distilled water and heated in a water bath at 60°C for 30 min. After heating, the mixture was centrifuged at 3000 rpm for 20 min to separate the supernatant from sago starch paste. The obtained supernatant (10 mL) was then transferred to a drying cup and dried in an oven at 150°C **(Chan *et al.*, 2009)**.

$$\text{WAI (\%)} = \frac{\text{paste weight}}{\text{weight of dry sample}} \times 100\% \quad (3)$$

$$\text{WSI (\%)} = \frac{\text{weight of dry residue}}{\text{volume supernatant}} \times 100\% \quad (4)$$

Oil Absorption Capacity (OAC) was determined using a modified centrifugation method **(Beuchat, 1977)**. Briefly, 1.0 g of the starch sample (mi) was mixed with 10 mL of refined vegetable oil and vortexed for 2 min. After incubating for 30 min at room temperature (25°C), the mixture was centrifuged at 3,000 rpm for 20 min. The supernatant was carefully decanted and the pellet weight (ma) was measured after drying for 10 min. The OAC was calculated using the following equation:

$$\text{OAC (g/g)} = \frac{\text{weight of dry residue} - \text{Initial weight of dry starch (g)}}{\text{Initial weight of dry starch (g)}} \quad (5)$$

Pasting Properties

Gelatinization behavior was analyzed using a Rapid Visco Analyzer (RVA 4500, Perten Instruments). Around 3.0 g of each sample (dry weight) was combined with 25 g distilled water in an RVA container. The mixture was heated from 60°C to 95°C at 6°C/min and held at 95°C for 4 min, followed by a cooling phase with continuous stirring at 160 rpm (Seet Chi *et al.*, 2021).

Morphology, Functional Groups, and Crystallinity

The morphology of the native, ozonated starch, and acetylated ozonated sago starch granules was examined using SEM-EDX (JEOL JSM-6510LA) at 2000× magnification, where a focused electron beam (2–30 kV) scanned the surface to generate detailed images. The functional groups of the starch were identified by FTIR spectroscopy (Shimadzu IR Prestige 21). The crystallinity of starch samples was evaluated via X-Ray Diffraction (XRD) (SHIMADZU XRD-7000) by recording diffraction patterns across a 2θ range of 5°–40° at 2° per minute **(Sumardiono, Jos, Pudjihastuti, Sari, *et al.*, 2021; Sumardiono, Jos, Pudjihastuti, Yafiz, *et al.*, 2021)**. A-OS 6 was a representative acetylated ozonated sago starch sample for all three analyses.

Formulation and Processing of Sago Starch-Based Noodles

A 5% portion of modified starch was initially pregelatinized in distilled water at a ratio of 1:9 (w/v). This pregelatinized starch was then combined with the remaining 95% modified starch (based on 500 g total). The mixture was kneaded with water to achieve dough consistency suitable for bain-marie processing at 40°C. The dough, with a moisture content of 55%, was directly extruded into hot water (95–98°C) and maintained at this temperature for 50–70 s before being transferred into cold water **(Chen *et al.*, 2002)**.

Texture Properties

Texture analysis was performed using a TA-XT Plus texture analyzer (Stable Micro Systems, UK). Three strands of the cooked noodles were arranged in parallel on a flat metal plate for testing. Several textural attributes were evaluated, including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. The instrument was configured with the following settings: pre-test speed, 1 mm/s; test speed, 1 mm/s; post-test speed, 1 mm/s; compression level, 60%; and a 1-second interval between consecutive compression tests **(Li *et al.*, 2020)**.

Cooking Quality

The noodle cooking performance was evaluated by measuring cooking time and cooking loss. Cooking time was determined by monitoring the disappearance of opacity at the noodle core **(Yang *et al.*, 2021)**. Cooking loss and water absorption were calculated by determining the weight gain ratio relative to dry noodle solids **(K. Zhang *et al.*, 2021)**. Noodle color was analyzed using the color measurement system established by the International Commission on Illumination (CIE) in 1976, considering L*, a*, and b* parameters **(Goñi & Salvadori, 2017)**.

Statistical Analysis

Data on acetyl and degree acetylation, functional properties, viscosity properties, noodle texture, and cooking performance were analyzed using SPSS 26. First, normality tests were performed. For normally distributed data, one-way ANOVA followed by Tukey HSD was applied. Samples with the same letter notation indicated no significant difference, while different notations signified significant differences **(Sumardiono, Budiyono, *et al.*, 2021)**.

RESULTS AND DISCUSSION

Acetyl and Degree Acetylation

The data presented in Figure 1 show the acetyl content (%) of acetylated ozonated sago starches measured at various reaction times (5, 10, 15, 20, and 25 min) and acetylation agent ratios (6, 7, 8, 9, and 10). At 5 min, the acetylation content was relatively low at the lower acetylation agent ratios (6–8), ranging from 0.246±0.001% to 0.369±0.174%, while ratio 10 began to show significantly higher values (2.461±0.001%). At 20 and 25 min, the acetyl content reached its peak values, particularly at a ratio of 10, stabilizing at 6.644±1.044% and 6.644±0.696%, respectively. This plateau suggests that the reaction reached a saturation point, likely due to the exhaustion of the reactive hydroxyl groups.

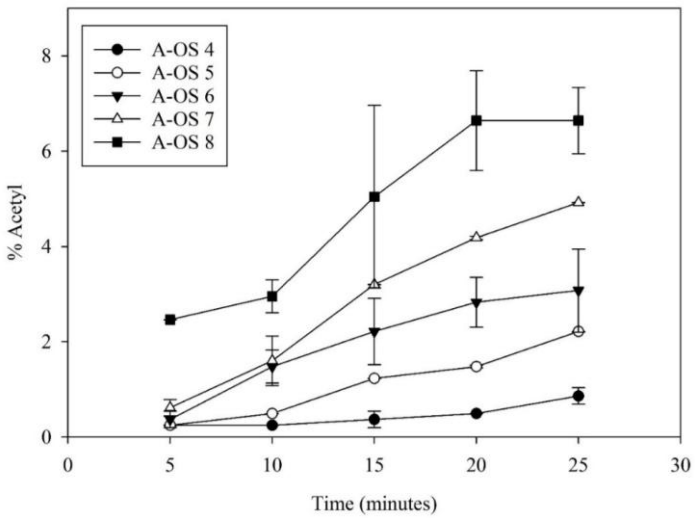


Figure 1 Acetyl content (%) of Acetylated-Ozonated Sago Starches with Various Ratios of Acetylation Agents

The increasing acetyl content can be attributed to the progressive substitution of hydroxyl groups (-OH) in the starch molecule with acetyl groups (-$COCH_3$) as the reaction proceeds. Higher concentrations of acetylation agents (e.g., A-OS 8) provide excess acetyl donors, accelerating the esterification process. Pretreatment with ozonation enhances this process by disrupting the crystalline structure of starch granules, thereby increasing the accessibility of hydroxyl groups for acetylation. Consistent with the theoretical basis of starch modification, where acetylation occurs primarily in the amorphous regions of starch granules, as

suggested by **(Pietrzyk, Fortuna, Łabanowska, *et al.*, 2018; Sumardiono *et al.*, 2024)**.

The ozonolysis process begins with ozone (O_3) decomposition into reactive species, such as hydroxyl radicals (•OH), which oxidize the hydroxyl groups (-OH) of starch into carbonyl (C=O) functionalities. The Criegee mechanism subsequently cleaves glycosidic bonds (C-O-C), generating aldehydes that are further oxidized into carboxyl groups (-COOH). This oxidative depolymerization produces low-molecular-weight starch fragments with enhanced reactivity. These oxidized fragments then serve as ideal substrates for acetylation, where the remaining hydroxyl groups (at the C2, C3, and C6 positions) react with acetic anhydride through liquid-phase diffusion to starch particles and surface reactions, replacing -OH with acetyl groups ($-COCH_3$) (Rahim *et al.*, 2022; von Gunten, 2007).

Table 1 Degree of substitution of Acetylated-Ozonated Sago Starches (A-OS) at Various Reaction Times

Time (min)	A-OS 4	A-OS 5	A-OS 6	A-OS 7	A-OS 8
5	0,009 ± 0,021^{a}	0,0093 ± 0,022^{a}	0,014 ± 0,007^{a}	0,023 ± 0,007^{ab}	0,095 ± 0,001^{c}
10	0,009 ± 0,007^{a}	0,0186 ± 0,051^{a}	0,056 ± 0,013^{bc}	0,061 ± 0,020^{bc}	0,115 ± 0,014^{d}
15	0,014 ± 0,004^{a}	0,047 ± 0,001^{bc}	0,085 ± 0,027^{c}	0,124 ± 0,012^{d}	0,200 ± 0,076^{f}
20	0,019 ± 0,001^{a}	0,056 ± 0,005^{bc}	0,110 ± 0,020^{cd}	0,164 ± 0,001^{e}	0,268 ± 0,042^{g}
25	0,033 ± 0,007^{b}	0,085 ± 0,011^{c}	0,120 ± 0,034^{d}	0,195 ± 0,004^{f}	0,268 ± 0,028^{g}

Note: Values are presented as means ± standard deviations. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

The results of this study were consistent with those of previous studies on starch acetylation. **Subroto *et al.* (2023)** and **Pietrzyk *et al.* (2018)** reported that higher acetylation agent ratios significantly increased acetyl content, particularly when pretreatment methods, such as ozonation or heat exposure, were applied to disrupt starch granules. The current study corroborates this finding by demonstrating an enhanced acetylation efficiency in ozonated starches **(Pietrzyk, Fortuna, Juszczak *et al.*, 2018; Subroto *et al.*, 2023)**.

As shown in Table 1, the degree of substitution (DS) increased progressively with longer reaction times across all samples. At 5 min, the DA values were relatively low and statistically similar for A-OS 4 to A-OS 6, ranging from 0.009 to 0.014, with a significantly higher DA observed in A-OS 8 (0.095±0.001). As the reaction time increased to 10 and 15 min, A-OS 5 to A-OS 8 exhibited noticeable increments in the DA values, with A-OS 8 achieving 0.115±0.014 and 0.200±0.076, respectively. At 20 and 25 min, A-OS 8 reached the highest degree of substitution at 0.268±0.042 and 0.268±0.028, respectively, significantly exceeding those of the other samples.

These findings highlight a strong relationship between reaction time and the extent of acetylation, with higher acetylation agent ratios (A-OS 8) facilitating a more pronounced increase in DS values over time. Statistical analysis further confirmed that the DA values among the samples differed significantly ($P < 0.05$), particularly at extended reaction times. The observed increase in DS values with longer reaction times and higher acetylation agent concentrations can be attributed to the increased availability of acetyl groups and enhanced reactivity of starch molecules following ozonation. Ozonation disrupts the crystalline regions of starch granules, increasing their accessibility to chemical modifications such as acetylation. This phenomenon is consistent with the findings of **Thakur *et al.* (2023)**, who reported that ozonation enhanced starch susceptibility to chemical derivatization by creating amorphous regions that facilitated the introduction of functional groups **(Thakur *et al.*, 2023)**.

At the molecular level, the acetylation process involves substituting hydroxyl groups in the starch molecule with acetyl groups ($-COCH_3$), reducing intermolecular hydrogen bonding, and altering the physicochemical properties of the starch. Samples with higher acetylation agent ratios (e.g., A-OS 8) exhibited the most significant DS values because of the increased availability of the acetyl donors. The results of this study are consistent with those of previous studies on starch acetylation. Similarly, **Maqbool *et al.* (2024)** and **Huang *et al.* (2024)** found that ozonated starch exhibited enhanced reactivity during acetylation compared to native starch owing to structural disruptions caused by oxidative treatment **(X. Huang *et al.*, 2024; Maqbool *et al.*, 2024)**.

Functional properties

The functional properties of sago starch were significantly altered by ozonation and acetylation, as demonstrated by the data in Table 2. The moisture content of native sago starch (S) was 12.796%, which increased slightly to 12.857% after ozonation (OS). Acetylated ozonated sago starches (A-OS) exhibited further increases in moisture content, with A-OS 8 reaching the highest value of 15.168%. These changes suggest a shift in the hydrophilic characteristics of starch, a common outcome of acetylation, which introduces ester groups that tend to attract water molecules, making starch more absorbent **(Rahim *et al.*, 2022)**.

Table 2 Functional Properties of Native, Ozonated, and Acetylated-Ozonated Sago Starches with Various Ratios of Acetylation Agents

Sago Starch	Sample code	water content (%)	WSI (%)	WAI (%)	OAC (g/g)
Native Sago Starch	S	12,796 ± 0,041^{a}	12,711 ± 0,162^{a}	36,605 ± 0,069^{d}	0,319 ± 0,001^{a}
Ozonated Sago Starch	OS	12,857 ± 0,042^{a}	14,0597 ± 0,106^{b}	30,586 ± 0,172^{a}	0,3796 ± 0,008^{b}
Acetylated-Ozonated Sago Starch	A-OS 4	13,743 ± 0,020^{b}	13,966 ± 0,159^{a}	30,585 ± 0,057^{a}	0,318 ± 0,009^{a}
	A-OS 5	13,922 ± 0,146^{bc}	14,710 ± 0,105^{c}	30,419 ± 0,179^{a}	0,314 ± 0,005^{a}
	A-OS 6	13,687 ± 0,272^{b}	15,689 ± 0,155^{d}	33,059 ± 0,061^{c}	0,314 ± 0,011^{a}
	A-OS 7	14,839 ± 0,193^{c}	15,679 ± 0,100^{d}	33,692 ± 0,196^{c}	0,310 ± 0,010^{a}
	A-OS 8	15,168 ± 0,228^{d}	16,835 ± 0,259^{e}	32,179 ± 0,130^{b}	0,308 ± 0,004^{a}

Note: Values are presented as means ± standard deviations. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

The WSI profile followed a similar trend, with the native sago starch displaying a WSI of 12.711%, which increased to 14.0597% after ozonation. This increase was even more pronounced in the acetylated ozonated samples, with A-OS 8 showing the highest value of 16.835%. The enhanced solubility is likely due to the ozonation process, which breaks down starch molecules into smaller, more soluble fragments, and the acetylation process further disrupts the crystalline structure of starch by incorporating ester groups **(Yadav Prakruthi *et al.*, 2025)**. In contrast, WAI decreased after ozonation from 36.605 in native starch to 30.586 in ozonated starch. This reduction could be attributed to structural changes in starch, which reduced its capacity to absorb water. While acetylated ozonated starches also showed a decrease in WAI compared to native starch, the effect was less pronounced, with A-OS 7 having the highest WAI of 33.692. These results indicate that, while acetylation slightly mitigates the effect of ozonation on water absorption, it still leads to a more ordered starch structure that is less prone to water uptake **(Cahyono *et al.*, 2024)**. The phenomenon of increasing WSI and decreasing WAI after treatment is supported by the XRD profile (Figure 3), which shows an increase in the number of sections. The increase in WSI is due to the partial depolymerization of amylopectin and increased availability of short-chain polysaccharides, while the decrease in WAI can be attributed to the reduction in starch granule swelling due to structural reorganization and partial cross-linking. These outcomes are consistent with previous reports on oxidation and acetylation modifying the water interaction capacity of starch systems **(Calderón-Castro *et al.*, 2019; Wardhani *et al.*, 2019)**.

The oil absorption capacity (OAC) of sago starch undergoes distinct changes after different modification treatments. Native sago starch exhibited a baseline OAC of 0.319 g oil/g starch, which increased by 18.7% to 0.3796 g/g after ozonation treatment ($p<0.05$). This significant enhancement in oil retention capacity can be attributed to the structural modifications induced by ozonation. According to He *et al.* (2023), oxidative cleavage of glycosidic bonds during ozonation creates a more porous granule architecture and increases surface roughness, as evidenced by our

SEM observations (Figure 2b) **(He *et al.*, 2023)**. These morphological changes provided additional void spaces and a greater surface area for the physical entrapment of oil molecules. Furthermore, the introduction of carbonyl and carboxyl groups through oxidation may alter the surface hydrophobicity of starch granules, potentially enhancing their affinity for nonpolar lipid molecules **(X. Wang *et al.*, 2020)**.

Interestingly, subsequent acetylation of ozonated starch resulted in OAC values (0.308-0.318 g/g) that were statistically similar to those of native starch ($p>0.05$) but significantly lower than those of ozonated starch alone ($p<0.05$). This suggests that acetylation effectively counteracted the enhancement in oil absorption achieved by ozonation. As demonstrated by **Zhang *et al.* (2023)**, the introduction of acetyl groups (-$COCH_3$) creates steric hindrance and increases the hydrophilic character of starch molecules through the esterification of hydroxyl groups **(Wardhani *et al.*, 2018; C. Zhang *et al.*, 2023)**.

Viscosity Properties

As shown in Table 3, native starch showed the highest Peak Viscosity (5344 ± 43.13 cP), which decreased significantly after ozonation (4474 ± 11.31 cP). Acetylation restored the Peak Viscosity, particularly at 4 g acetate (5214 ± 24.85 cP), but the values gradually declined as the acetylation level increased. The substantial reduction in Peak Viscosity after ozonation reflects the partial degradation of starch granules, leading to a weakened swelling capacity and disrupted granular integrity. Acetylation introduces acetyl groups, enhances water absorption, and restores viscosity. The gradual decline at higher acetylation levels (e.g., A-OS 7 and 8) may have resulted from excessive substitution, which reduced the intergranular bonding strength. This behavior is consistent with studies on corn starch modified by **Sun *et al.* (2023)**, which demonstrated similar viscosity patterns upon chemical modification **(Sun *et al.*, 2023)**.

Ozonation increased the trough viscosity (2510 ± 28.28 cP) compared to native starch (1520 ± 55.86 cP), while drastically reducing the breakdown viscosity. Acetylation further enhanced the Trough and Breakdown viscosities, with the highest breakdown observed at 8 g of acetate (2451 ± 33.23 cP). Higher Trough viscosities in ozonated starch indicate improved stability during shearing, potentially owing to structural rearrangements that enhance gel stability **(Maqbool *et al.*, 2024)**. The subsequent increase in breakdown viscosity after acetylation suggests that the acetylated starch granules regained some swelling capacity, albeit with a higher tendency to disintegrate under shear stress. This observation aligns with the findings of **Bello-Pérez *et al.* (2010)**, who reported increased breakdown in acetylated starches owing to enhanced hydrophilicity **(Bello-Pérez *et al.*, 2010)**.

Table 3 Viscosity Properties of Native, Ozonated, and Acetylated-Ozonated Sago Starches with Various Ratios of Acetylation Agents

Sample code	Peak viscosity (cP)	Through viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Set Back viscosity (cP)	Peak Time (minutes)	Pasting Temperature (°C)
S	5344 ± 43,13^{c}	1520 ± 55,86^{a}	3824 ± 12,73^{c}	2626 ± 43,13^{a}	1106 ± 10,01^{a}	5,93 ± 0,01^{a}	74,55 ± 0,11^{a}
OS	4474 ± 11,31^{a}	2510 ± 28,28^{b}	1964 ± 16,97^{a}	3664 ± 28,28^{b}	1154 ± 56,57^{a}	5,98 ± 0,01^{a}	74,88 ± 0,32^{ab}
A-OS 4	5214 ± 24,85^{bc}	2844 ± 21,36^{c}	2370 ± 12,21^{b}	4138 ± 7,11^{e}	1294 ± 9,22^{b}	6,00 ± 0,02^{a}	75,05 ± 0,07^{ab}
A-OS 5	5193 ± 30,41^{bc}	2827 ± 24,75^{c}	2366 ± 15,66^{b}	4118 ± 28,99^{de}	1291 ± 4,24^{b}	6,00 ± 0,01^{a}	75,30 ± 0,28^{ab}
A-OS 6	5187 ± 22,03^{b}	2789 ± 25,01^{c}	2398 ± 11,36^{b}	3949 ± 16,76^{cd}	1160 ± 15,96^{a}	5,87 ± 0,04^{a}	75,50 ± 0,07^{ab}
A-OS 7	5133 ± 16,37^{b}	2759 ± 43,13^{b}	2375 ± 14,19^{b}	3917 ± 45,25^{c}	1159 ± 2,12^{a}	5,90 ± 0,03^{a}	75,60 ± 0,21^{ab}
A-OS 8	5111 ± 33,23^{b}	2660 ± 18,23^{b}	2451 ± 33,23^{b}	3885 ± 33,23^{c}	1225 ± 11,98^{ab}	5,87 ± 0,05^{a}	75,68 ± 0,17^{b}

Note: Values are presented as means ± standard deviations. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

The final viscosity increased after ozonation (3664 ± 28.28 cP) compared to that of native starch (2626 ± 43.13 cP) and peaked at 4 g acetylation (4138 ± 7.11 cP). However, the setback values remained relatively stable, indicating minimal retrogradation. The increase in the Final Viscosity post-modification indicates an improved gel-forming ability, particularly in acetylated-ozonated starches. Higher values at lower acetylation levels suggest optimal structural integrity and flexibility balance. The relatively stable setback values indicate minimal retrogradation, making these starches suitable for applications requiring extended storage stability **(Scott & Awika, 2023)**. **Xu *et al.* (2022)** also reported reduced retrogradation in modified potato starches, thus reinforcing this finding **(Xu *et al.*, 2022)**.

The peak Time and Pasting Temperature parameters remained broadly consistent across the treatments, with slight increases observed at higher acetylation levels. The consistent Peak Time across treatments reflects the retention of gelatinization kinetics despite modifications. Slight increases in Pasting Temperature at higher acetylation levels suggest restricted water diffusion into starch granules owing to increased acetyl group substitution. Similar trends were observed in acetylated chickpea starch by **Zhang *et al.* (2023) (C. Zhang *et al.*, 2023)**.

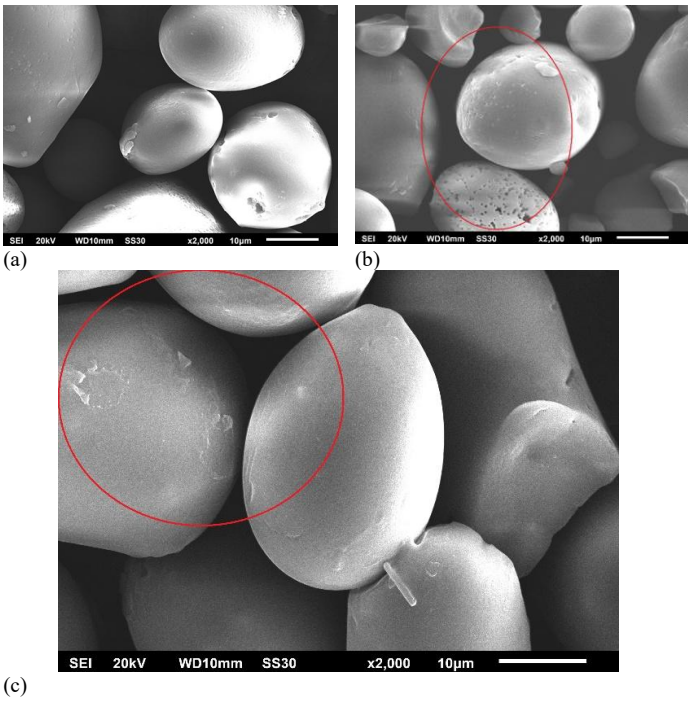
The observed trends in viscosity behavior are broadly consistent with the findings of other studies on modified starches. Acetylation improved the viscosity and gel stability of yam starch. However, this study's relatively stable setback values contrast with the increased retrogradation reported in some acetylated starches, highlighting the unique impact of ozonation **(Thakur *et al.*, 2023)**. These results demonstrated the potential of combined ozonation and acetylation for producing starches with tailored pasting properties.

Scanning electron micrographs (SEM)

The morphological changes observed in the SEM images of native, ozonated, and acetylated-ozonated sago starch granules (Figure 2) align with previously reported findings, offering further insights into the effects of ozonation and acetylation treatments.

Native starch granules, characterized by smooth surfaces and uniform shapes, resemble the granule structure described in the first article. Native sago starch also exhibited spherical shapes and smooth surfaces, some with truncated or flake-like structures. The similar clustering of granules observed in our native sago starch SEM images is consistent with these findings, as clustering is a common feature of starch granules in their native state.

Upon ozonation, the surface morphology of the sago starch granules changed significantly, showing increased roughness and fibrous texture. These alterations align with the observations in the second article, in which ozonation was reported to cause surface roughness and irregularities in corn and potato starch granules. The ozonation process likely partially depolymerizes the amylopectin chains, exposing the hydrophilic groups and promoting swelling. Similarly, the ozonated sago starch in our study may have experienced oxidative cleavage of glycosidic bonds, leading to surface disruptions without complete disintegration, maintaining the granule's overall integrity **(Thakur *et al.*, 2023)**.



(a) (b) (c)

Figure 2 Scanning electron micrographs (SEM) of (a) Native, (b) Ozonated, and (c) Acetylated-Ozonated Sago Starches with magnification 2000x

Acetylation of ozonated sago starch further modifies its morphology, introducing shallow fissures and rougher surfaces, indicative of structural changes due to the incorporation of acetyl groups. These findings are consistent with those of the first article, which highlighted that acetylation at higher degrees of substitution (DS)

can cause surface roughness, cluster formation, and even loss of granule structure under more severe reaction conditions. While the acetylation reaction in our study appears less drastic, the observed morphological transformations support the notion that the esterification process reduces intermolecular hydrogen bonding, thereby increasing granule volume and surface irregularities **(Figueroa Flórez *et al.*, 2016)**.

X-ray diffraction (XRD)

Figure 3 shows the XRD patterns of native starch (S). The diffraction pattern of native starch exhibited distinct crystalline peaks at approximately 15°, 17°, 18°, and 23°, which are characteristic of the A-type crystalline structure commonly found in sago starch. After the ozonation treatment (OS), the peaks remained visible at similar positions; however, there was a slight broadening of the peaks, particularly at 17° and 18°, suggesting a reduction in the ordered crystalline regions. This broadening indicates a partial disruption of the molecular arrangement of the starch granules due to the oxidative effects of ozonation. In the case of acetylated-ozonated starch (A-OS), the XRD patterns still showed peaks corresponding to the A-type crystalline structure, but a notable shift and slight merging of peaks were observed at approximately 15° and 17°.

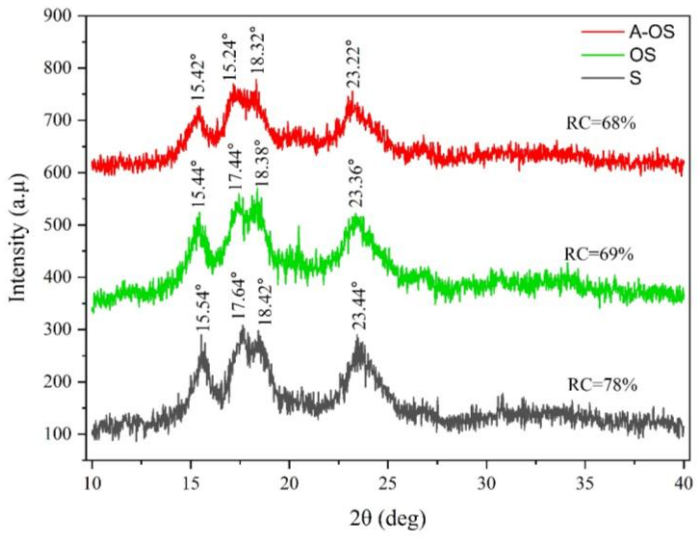


Figure 3 X-ray diffraction (XRD) of (a) Native, (b) Ozonated, and (c) Acetylated-Ozonated Sago Starches

The persistence of the peaks in all three samples confirms that the A-type crystalline structure of sago starch is retained despite the treatments, although with varying degrees of structural reorganization. The broadening and shifting of the peaks observed for the OS and A-OS samples indicate that the treatments introduced amorphous regions within the starch granules. This observation aligns with the findings of **Akhila *et al.* (2009)**, who reported similar peak broadening in oxidized starches, which they attributed to the partial degradation of crystalline regions **(Akhila *et al.*, 2024)**.

The ozonation process likely causes the oxidative cleavage of glycosidic bonds, which weakens the crystalline structure without eliminating it, as evidenced by the 9% reduction in RC (from 78% to 69%). Subsequent acetylation treatment further disrupts the molecular order by introducing substituent acetyl groups **(Chi *et al.*, 2023)**, Though the marginal 1% RC difference between OS and A-OS suggests acetylation's impact is less pronounced than ozonation. These results are consistent with the findings of **Ashogbon (2021)**, who observed that chemical modifications, such as acetylation, could introduce structural irregularities without destroying the native crystalline pattern of starch. Oxidative treatments broaden the XRD peaks owing to the formation of amorphous zones **(Ashogbon, 2021)**.

FTIR

The FTIR spectra in Figure 4 clearly illustrate the structural modifications of native sago starch (S), ozonated sago starch (OS), and acetylated ozonated sago starch (A-OS-6). For native sago starch (S), characteristic peaks are observed, including a broad absorption band around 3450 cm^{-1}, attributed to O-H stretching vibrations, and a peak near 1645 cm^{-1}, associated with angular O-H bending of water molecules. A weaker peak at approximately 2960 cm < re > ¹ corresponds to C-H stretching vibrations, typical of the native starch structure.

These characteristic peaks remained prominent upon ozonation (OS), indicating that the primary starch structure was retained, but with potential minor alterations. The peak at 1645 cm < ss > ¹ exhibited a slight reduction in intensity, suggesting partial modification of the hydroxyl groups during the ozonation process.

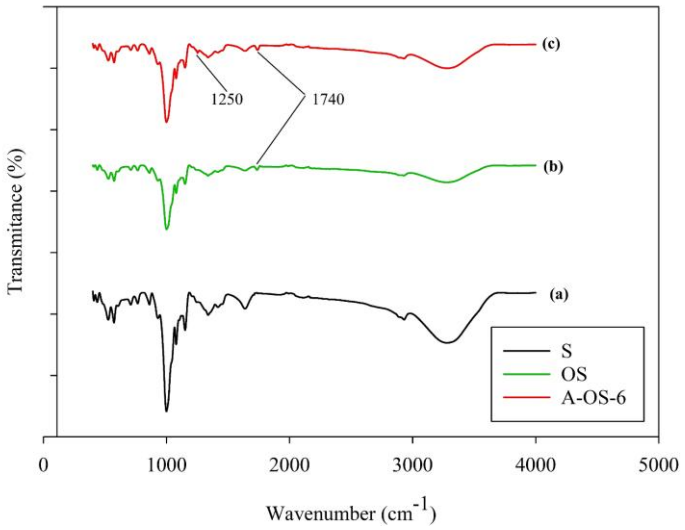


Figure 4 FTIR of (a) Native, (b) Ozonated, and (c) Acetylated-Ozonated Sago Starches

The spectrum of acetylated-ozonated sago starch (A-OS-6) revealed significant changes, highlighting successful acetylation. New absorption bands emerged at 1250 cm^{-1} and 1740 cm^{-1}. The band at 1250 cm^{-1} corresponds to C-O stretching, while the peak at 1740 cm^{-1} is attributed to the C=O stretching of carbonyl groups, indicating carbonyl ester formation. The intensity and area of these peaks are significantly higher than those of native and ozonated starch, reflecting the introduction of acetyl groups during the esterification process. Simultaneously, the peak at 1645 cm^{-1} is further reduced, corroborating the substitution of hydroxyl groups by carbonyl groups from the acetic anhydride.

These observations are consistent with those of previous studies, which reported the emergence of characteristic absorption bands at 1250 cm^{-1} and 1740 cm^{-1} for acetylated starches derived from potatoes, corn, barley, and **oats (Trela *et al.*, 2020; Zhao *et al.*, 2018)**. The spectral changes confirmed the successful incorporation of acetyl groups into the starch matrix, with the degree of substitution (DS) influencing the extent of these modifications. The prominent peaks in the A-OS-6 sample indicate a higher DS than that of unmodified or ozonated starch, thereby validating the effectiveness of the acetylation process in producing modified sago starch with enhanced properties.

Noodle texture and cooking performance

The data in Table 4 show that native sago starch exhibited the highest hardness (593.46 ± 4.78 g), which significantly decreased with ozonation (331.37 ± 41.19 g). Hardness showed a downward trend among the acetylated-ozonated samples as acetylation levels increased, with the lowest value recorded at 8 g acetylation (302.37 ± 1.61 g). The significant reduction in hardness following ozonation reflects the oxidative weakening of the starch granules, likely due to partial chain cleavage and structural reorganization. Acetylation at lower concentrations partially reversed this effect by introducing acetyl groups, which promoted limited crosslinking and increased internal bonding strength **(Handarini *et al.*, 2020; Lima *et al.*, 2021)**. However, higher acetylation levels (e.g., 8 g) may have oversaturated the structure, further reducing the hardness owing to decreased granular integrity. This behavior aligns with findings from Trela *et al.* (2020), who observed reduced hardness in acetylated cassava starch at higher acetylation levels **(Trela *et al.*, 2020)**.

Ozonation reduced adhesiveness (-30.16 ± 16.14 g.sec) compared to native starch (-53.68 ± 6.48 g.sec). Acetylation further varied adhesiveness without a clear trend. The lowest adhesiveness was observed in A-OS 6 (-60.78 ± 6.6 g.sec). Ozonation reduced starch adhesiveness, indicating a decrease in stickiness, potentially owing to the destruction of surface hydrogen bonds. Acetylation at specific levels (e.g., 6 g) disrupted the gelatinized starch network, further reducing adhesiveness. The recovery observed at lower acetylation levels may be attributed to enhanced hydrophilic interactions due to the acetyl group introduction. This phenomenon aligns with the findings of **Wang (2020)**, who emphasized that modifications influencing hydration properties impact adhesiveness **(X. Wang *et al.*, 2020)**.

Springiness and Cohesiveness: Minimal variation was observed in these parameters, with springiness ranging from 0.69 to 0.76, and cohesiveness increasing slightly in ozonated and acetylated samples (0.77–0.80). These parameters remained relatively stable, suggesting that ozonation and acetylation primarily influenced structural integrity without substantially altering the elastic properties **(Pandiselvam *et al.*, 2019; Wei *et al.*, 2024)**. The slight increase in cohesiveness may reflect enhanced inter-chain interaction post-modification **(Mehboob *et al.*, 2020; Sindhu *et al.*, 2021)**.

Table 4 Textural Properties of Native, Ozonated, and Acetylated-Ozonated Sago Starches with Various Ratios of Acetylation Agents

Sample code	Hardness (g)	Adhesiveness (g.sec)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)
S	593,46 ± 4,78^{d}	−53,68 ± 6,48^{a}	0,71 ± 0,02^{a}	0,74 ± 0,00^{a}	436,44 ± 3,55^{c}	310,39 ± 8,45^{a}
OS	331,37 ± 41,19^{ab}	−30,16 ± 16,14^{a}	0,69 ± 0,06^{a}	0,77 ± 0,05^{a}	252,15 ± 15,50^{ab}	172,80 ± 15,80^{a}
A-OS 4	500,05 ± 18,52^{c}	−31,39 ± 5,38^{a}	0,73 ± 0,03^{a}	0,75 ± 0,03^{a}	373,15 ± 0,54^{b}	270,67 ± 11,04^{a}
A-OS 5	455,99 ± 0,29^{bc}	−41,43 ± 0,20^{a}	0,76 ± 0,00^{a}	0,77 ± 0,01^{a}	351,29 ± 3,75^{b}	268,16 ± 4,13^{a}
A-OS 6	430,51 ± 10,58^{b}	−60,78 ± 6,60^{a}	0,73 ± 0,01^{a}	0,79 ± 0,01^{a}	339,09 ± 5,57^{b}	248,86 ± 0,47^{a}
A-OS 7	430,51 ± 19,46^{b}	−26,44 ± 5,15^{a}	0,76 ± 0,01^{a}	0,79 ± 0,00^{a}	277,91 ± 14,29^{ab}	210,86 ± 12,24^{a}
A-OS 8	302,37 ± 1,61^{a}	−40,99 ± 20,57^{a}	0,71 ± 0,05^{a}	0,80 ± 0,00^{a}	241,16 ± 1,26^{a}	170,18 ± 11,51^{a}

Note: Values are presented as means ± standard deviations. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Gumminess and Chewiness: Both parameters decreased significantly with ozonation, reflecting softer and less cohesive textures. Acetylation restored some gumminess and chewiness, peaking at 4–6 g acetylation but diminishing again at higher concentrations. The drastic reduction in gumminess and chewiness after ozonation highlights the softened texture caused by granular damage. Moderate acetylation levels restore gumminess by increasing intermolecular cohesion, a trend supported by studies of acetylated rice starch **(Nawaz *et al.*, 2020)**. However, higher acetylation levels (A-OS 7–8) likely hinder gel network formation and reduce gumminess and chewiness.

These findings suggest that ozonation and acetylation induce significant modifications in starch texture, with acetylation levels playing a critical role in tailoring mechanical properties. The findings of this study are consistent with those of previous research on modified starches, particularly regarding the textural effects of combined ozonation and acetylation. For instance, **Sindhu *et al.* (2021)** reported reduced hardness and gumminess of oxidized and acetylated starches **(Sindhu *et al.*, 2021)**. However, the observed decline in adhesiveness contrasts with studies on acetylated potato starch, in which increased stickiness was noted **(Kolarič *et al.*, 2020).** This discrepancy may stem from differences in the starch source and modification conditions. The results demonstrate the versatility of acetylation-ozonation as a dual modification strategy for tailoring starch properties to specific applications.

Table 5 Cooking Performance of Native, Ozonated, and Acetylated-Ozonated Sago Starches with Various Ratios of Acetylation Agents

Sample code	Colour cooked noodles (L/a/b)	Cooking time (minutes)	Water absorption (%)	cooking loss (%)
S	52.4/-0.6/2.8	5,00 ± 0,76^{a}	90,22 ± 3,39^{d}	19,39 ± 0,58^{d}
OS	48.9/13.1/27.6	7,00 ± 0,29^{b}	89,62 ± 2,56^{d}	17,12 ± 0,18^{c}
A-OS 4	70.9/3.2/2.6	7,00 ± 0,31^{b}	86,31 ± 1,10^{b}	17,22 ± 0,32^{c}
A-OS 5	73.1/3.5/2.3	7,00 ± 0,27^{b}	86,31 ± 1,10^{b}	16,76 ± 0,40^{c}
A-OS 6	72.8/3.4/2.5	7,00 ± 0,50^{b}	83,31 ± 1,70^{b}	15,55 ± 0,38^{bc}
A-OS 7	71.5/3.3/2.6	6,00 ± 0,48^{a}	81,01 ± 1,16^{ab}	14,01 ± 0,47^{b}
A-OS 8	73.3/3.4/2.4	5,00 ± 0,32^{a}	77,29 ± 2,00^{a}	12,85 ± 0,56^{a}

Note: Values are presented as means ± standard deviations. Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

The cooking performance of the noodles is presented in Table 5. The cooked noodles, native sago starch (NS), had a duller appearance (L = 52.4). In contrast, ozonated starch (OS) showed a darker coloration (L = 48.9) with increased red and yellow hues (a = 13.1, b = 27.6). In contrast, noodles prepared using acetylated ozonated starches showed improved lightness, with values ranging between 70.9 and 73.3 for A-OS 4 to A-OS 8, indicating enhanced visual quality. The enhanced lightness (L values) of acetylated-ozonated starch noodles can be explained by chemical modifications that reduce the susceptibility of starch to browning reactions. Acetylation can improve the visual quality of starch-based products by stabilizing their structural integrity under thermal processing conditions **(Subroto *et al.*, 2023)**.

The cooking time increased slightly after ozonation, where OS noodles required 7 min compared with 5 min for S noodles. However, acetylated-ozonated samples (A-OS) retained a cooking time of approximately 6–7 min, with A-OS 8 requiring the shortest time at 5 min. The water absorption values decreased significantly with increasing acetylation-agent concentration. The S and OS samples exhibited the highest water absorption (90.22% and 89.62%, respectively), while the A-OS samples, particularly A-OS 8, showed the lowest water absorption of 77.29%. This trend indicates a modification in starch hydration capacity due to acetylation. The differences observed in the cooking performance can be attributed to the effects of ozonation and acetylation on the physicochemical properties of sago starch. Ozonation disrupts the crystalline structure of starch granules, leading to an increase in the amorphous regions and subsequent changes in water absorption and cooking time. This phenomenon is consistent with the findings of **Subroto *et al.* (2009)** and Kolarič *et al.* (2020), who reported that oxidative treatment could weaken starch granule integrity, resulting in longer cooking times and higher water absorption **(Kolarič *et al.*, 2020; Subroto *et al.*, 2023)**.

Native starch noodles experienced the highest cooking loss (19.39%), followed by the OS noodles (17.12%). A progressive reduction in cooking loss was observed in the A-OS samples, with A-OS 8 demonstrating the most significant improvement (12.85%). These findings suggest that acetylation improves starch stability during cooking, likely because of structural modifications that enhance the resistance of starch to thermal and shear forces. Acetylation introduces acetyl groups into starch molecules, reducing their hydrophilicity and modifying their water-binding capacity. As acetylation levels increase (A-OS 4 to A-OS 8), starch becomes less prone to swelling and leaching during cooking, as evidenced by the lower water absorption and cooking loss values. Similar trends have been reported by **Gałkowska *et al.* (2023)**, where acetylated starches exhibited improved cooking stability owing to their modified molecular structure **(Gałkowska *et al.*, 2023)**. The reduced cooking loss and water absorption observed in A-OS 8 suggest that higher acetylation levels result in a more compact and stable starch network, minimizing the release of solids into cooking water. This observation aligns with the results of **Wang *et al.* (2024)**, who highlighted the importance of acetylation in improving starch-based noodle quality by enhancing thermal stability and reducing molecular degradation **(J. Wang *et al.*, 2024)**. One noodle preparation method was applied in this study, which may limit the generalizability of the findings to other noodle types and processing methods. Further studies are recommended to evaluate the modified starch in various formulations and conditions to confirm its broader applicability.

Prospect for the industrial application

Figure 5 illustrates how ozonated–acetylated sago starch offers key functional benefits that enhance its suitability for industrial wet noodle production. The improved water and oil absorption enhance noodle texture, elasticity, and flavor retention, while reduced setback viscosity prolongs freshness by minimizing retrogradation. Lower peak viscosity and minimal cooking loss improve processability, yield, and consistency during production, and the brighter color and softer texture increase visual appeal and palatability. These attributes align with industry demands for high-quality, stable, and appealing noodle products, making the modified starch a promising ingredient for future large-scale applications and product diversification.

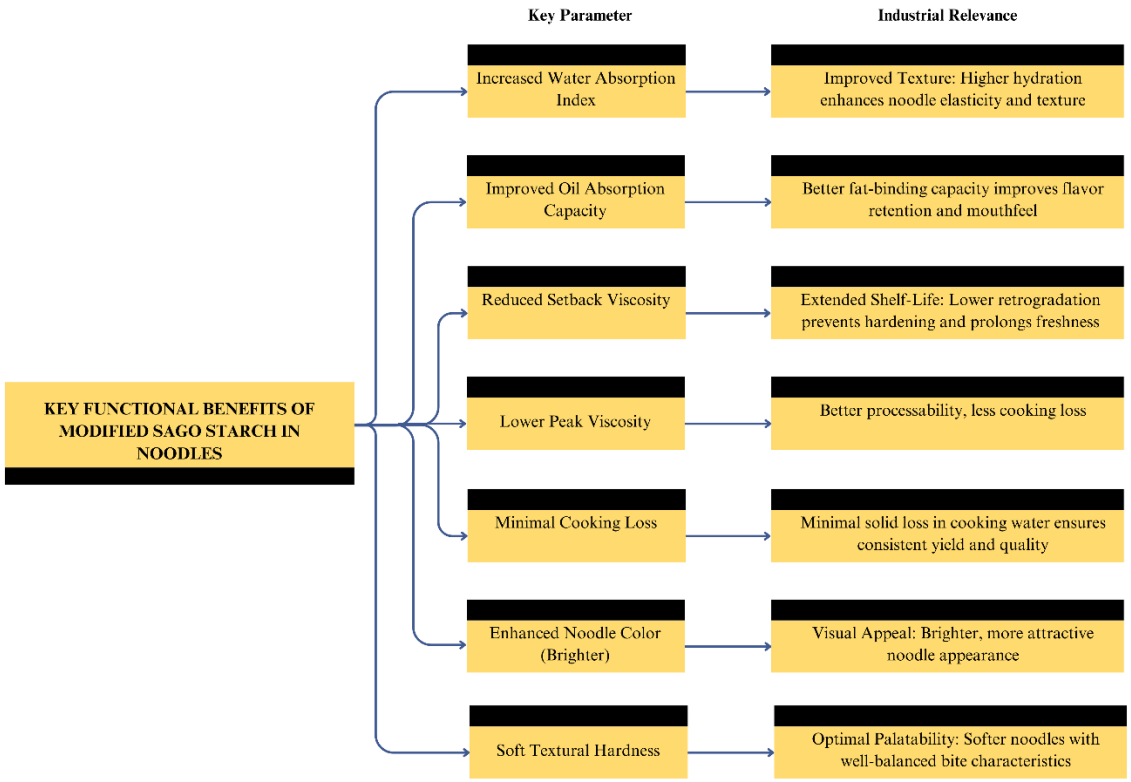


Figure 5 Key Functional Benefits of Modified Sago Starch in Noodles

CONCLUSION

This study established sequential ozonation acetylation as an effective strategy for enhancing sago starch functionality in wet noodle applications. The dual-modification approach capitalizes on the synergistic effects of mild ozonation and controlled acetylation, creating starch with optimized structural and functional properties for high-moisture food systems. The introduced acetyl groups (confirmed by FTIR spectroscopy) and modified granule morphology (observed via SEM) collectively contribute to improved hydration capacity and paste stability while simultaneously inhibiting retrogradation, addressing key challenges in noodle texture maintenance. Importantly, this modification protocol demonstrates how targeted physicochemical alterations can transform native sago starch into a specialized ingredient for quality improvement in starch-based foods. These findings highlight the potential of ozonation-coupled acetylation as a viable processing route for developing starch ingredients with enhanced performance for wet noodle production. Optimization of processing parameters, scale-up of production, other noodle preparation methods and formulations, and more extensive sensory testing are recommended for future research to determine commercial feasibility and consumer acceptability.

Acknowledgments: The authors would like to thank the Department of Chemical Engineering for providing support and facilities for this study. We also acknowledge Universitas Diponegoro for funding this research through World Class Research Undip (WCRU) scheme with contract number 357-34/UN7.D2/PP/IV/2024.

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