



Enhancing Resistant Starch Content and Functional Properties of Corn Flour via Dual Modification with Organic Acids and Annealing

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Abstract. Corn flour holds a potential for functional food through chemical and thermal modification. Nevertheless, the modification of corn flour to enhance the content of resistant starch (RS) has been performed to a limited extent. The present study examines the impact of dual modification—esterification catalyzed by organic acid (citric, acetic, or lactic acid) followed by annealing—on the physicochemical and morphological properties of corn flour. The combination of citric acid treatment and annealing resulted in the lowest swelling power (504.66) and the highest clarity (0.054), indicating improved stability and transparency. Acetic acid modification produced the highest RS values, with an increase from 5.64% to 8.42%. Furthermore, dual modification led to a reduction in peak viscosity (from 2,931 cP to 446 – 2,409 cP), thereby improving heat and shear resistance. These structural alterations, as confirmed by FTIR and SEM analysis, are indicative of ester bond formation and granule reorganization. The findings highlight the potential of modified corn flour as a functional ingredient.

Keywords: Corn flour; resistant starch; esterification; annealing; functional properties.

Type of the Paper: Regular Article.



1. Introduction

Corn (*Zea mays* L.) is one of the main food commodities in Indonesia with the potential to be developed as a raw material for functional food products. Corn contains complex carbohydrates that, in addition to being an energy source, can be modified to increase its added value. Given its composition of starch, fiber, and protein, corn flour can serve as a material for the development of carbohydrate-based functional foods [1,2].

One widely studied approach in functional food development is increasing the content of resistant starch (RS), particularly RS3 and RS4. Resistant starch (RS) is known to have the potential to function as a prebiotic, to help regulate blood glucose levels, and to improve digestive health [3,4]. The process of chemical modification, using organic acids such as citric, acetic, and lactic acids, can form ester bonds with hydroxyl groups on starch, thereby increasing RS content and concomitantly altering the functional properties of the flour [5,6]. Moreover, physical treatments such as annealing can enhance crystallinity and thermal stability without damaging the granular structure [7,8].

Despite the numerous studies conducted on the modification of pure starch, the number of studies focusing on whole corn flour, especially in the context of combined chemical and physical modifications, remains limited [8]. A dual modification involving acetylation and annealing has been applied by Sitanggang *et al.* [9] and Egodage [10] to mung bean, waxy potato, waxy rice, waxy barley, and waxy corn starches. The results showed that the granules became weaker, their surfaces rougher, and the distance between granules became more tenuous. In addition, Yaqoob *et al.* [11] reported that starch modification via fermentation using lactic acid bacteria increased granule porosity and reduced water-retaining capacity. Esterification using lactic acid through fermentation even improved the textural, thermal, and pasting profile of corn dough. In a separate study, Yaqoob *et al.* [12] stated that fermentation altered the structure and function of starch, as the enthalpy required for gelatinization was lower in fermented corn flour than in unfermented flour. It can be hypothesized that dual modification provides better effects in comparison to single modification, while annealing is beneficial for improving the thermal stability of acid-modified starch.

The presence of non-starch components, such as protein and fiber, in flour has been demonstrated to influence the efficiency of modification and the final product characteristics [13,14]. As posited by Fonseca *et al.* [13], protein acts as a physical barrier to enzymes, heat, or other physical processes. This implies that the presence of protein in flour may hinder the modification process. However, protein can offer certain advantages in this context, due to its capacity to enhance thermal stability and resistance to shear. Modifying flour rather than pure starch offers practical advantages, including enhanced ease of supplementation with other ingredients and better applicability in industrial settings. In contrast, the modification of pure starch focuses more on granule structure changes and is generally more suitable for specific food formulations as an additive. A further advantage of modifying corn in flour form as opposed to starch form is that it has the potential to reduce processing waste.

This study aims to evaluate the effects of combined chemical modification, using citric, acetic, and lactic acids, and physical annealing treatment on the physicochemical and structural characteristics of Indonesian local corn flour. The analyses include amylose content, resistant starch, granular morphology (SEM), chemical structure (FTIR), and pasting properties. The results of this study are expected to contribute to the development of efficient and applicable corn flour modification technologies for functional food industries.

2. Materials and Methods

2.1. Materials

The corn flour utilized in this study was produced by CV. Nawasena Pangan Kreatif, Kediri, East Java, Indonesia. The corn flour had a yellow color and was sieved to an 80-mesh particle size. It was produced from a local corn variety that is commonly cultivated in East Java, Indonesia. Citric acid, lactic acid, acetic acid, HCl, and NaOH employed in the treatments were obtained from Merck, Germany.

2.2. Esterification

The esterification process with citric or lactic acid was conducted in accordance with the method described by Shaikh *et al.* [3], while the esterification process with acetic acid was executed based on the method outlined by Khurshida *et al.* [15], with minor adjustments. Sixty grams of citric or lactic acid were dissolved in 60 ml of distilled water. The pH of the solution was adjusted to 3.5 using 10 M NaOH if the pH was too acidic. The volume was then increased to 150 ml. Subsequently, 150 g of corn flour was mixed with the acid solution. The mixture was stirred using a magnetic stirrer at low speed and left at room temperature for 12 hours. The separation process was performed by centrifugation at 6000g for a duration of 15 minutes. The precipitate was then dried at a temperature of 45°C until the moisture content reached 10%. Following this, the flakes were ground and sieved using an 80-mesh sieve.

The esterification process with acetic acid began by the preparation of a 25% (v/v) acetic acid solution. This solution was obtained by mixing acetic acid with distilled water at a 1:4 (v/v) ratio. Corn flour was then mixed with the acetic acid solution at a proportion of 1:4 (b/v). Subsequently, the mixture was placed in a water bath shaker for a period of 2 hours at 45°C. It was then centrifuged at 6000g for a duration of 15 minutes. Following this, the precipitate was separated from the supernatant and dried at a temperature of 45°C until the moisture content reached 10%. The acetic acid-modified corn flour was subsequently ground and sieved using an 80-mesh sieve.

2.3. Annealing

The three types of esterified flour were modified using the annealing method described by Ariyantoro *et al.* [16] with some modifications. A total of 62.5 g of modified flour was mixed with 125 ml of distilled water and heated in a water bath shaker at 55°C for 24 hours. The mixture was then centrifuged at 1665g for a duration of 15 minutes. The resulting precipitate was subjected to a drying process at a temperature of 45°C, continuing until the moisture content reached 10%. Subsequently, the material was ground and sieved using an 80-mesh sieve.

2.4. Analytical Parameters

The modified flour was subjected to a series of analytical procedures: the assessment of amylose and resistant starch content according to AOAC methods [17], the determination of degree of substitution [18], the evaluation of water solubility, the determination of clarity, and the measurement of swelling power as outlined by Wang *et al.* [19]. The pasting properties were analyzed using the method from Wang *et al.* [19]. The SEM analysis followed the method outlined by Ratnaningsih *et al.* [20], while the FTIR analysis was conducted using the approach detailed by Fashi *et al.* [21]. Analyses were conducted on native corn flour (N), acetic acid-modified flour (AA), lactic acid-modified flour (LA), and citric acid-modified flour (CA), all of which were subsequently subjected to annealing.

2.5. Statistical Analysis

All data were analyzed using one-way ANOVA with IBM SPSS version 25. Results are presented as the mean \pm standard deviation.

3. Result and Discussion

The amylose content of the modified corn flour increased in comparison to native (unmodified) flour (Table 1). The content of amylose in native corn flour was 19.03%, which increased to 21.76% (AA), 21.02% (CA), and 20.69% (LA). This increase is attributed to the cleavage of α -1,6-glycosidic bonds by organic acids, resulting in shorter and more linear chains (amylose) [13]. Additionally, the annealing process following esterification process promotes molecular reorganization, reinforcing the formation of amylose double helices. These helices are identified as amylose in iodine-based analysis. In native flour, amylose tends to form complexes with proteins and lipids, making it undetectable. The esterification process disrupts these complexes, increasing the amount of detectable amylose. Similar findings were reported by Feng *et al.* [22], who investigated the effects of enzymatic and physical modification on rice flour, as well as by Yaqoob *et al.* [12], who examined the effects of fermentation combined with thermal cycling on corn flour.

Table 1. Physicochemical characteristics of native flour and modified flour

| Treatment | Amylose (%) | Resistant starch (%) | Degree of substitution (DS) | Clarity | Swelling power | Water solubility |
|-----------|-------------------------------|------------------------------|------------------------------|-------------------------------|---------------------------------|------------------------------|
| Native | 19.03 \pm 0.04 ^a | 5.64 \pm 0.01 ^a | 0.00 ^a | 0.020 \pm 0.00 ^a | 1023.11 \pm 0.21 ^d | 2.29 \pm 0.05 ^d |
| AA | 21.76 \pm 0.02 ^d | 8.42 \pm 0.01 ^d | 0.07 \pm 0.00 ^c | 0.039 \pm 0.00 ^c | 998.56 \pm 1.90 ^c | 0.25 \pm 0.01 ^a |
| CA | 21.02 \pm 0.02 ^c | 7.92 \pm 0.01 ^c | 0.02 \pm 0.00 ^b | 0.054 \pm 0.00 ^d | 504.66 \pm 3.39 ^a | 1.44 \pm 0.02 ^c |
| LA | 20.69 \pm 0.02 ^b | 6.88 \pm 0.01 ^b | 0.08 \pm 0.00 ^d | 0.034 \pm 0.00 ^b | 662.31 \pm 3.36 ^b | 0.43 \pm 0.00 ^b |

AA: esterification with acetic acid followed by annealing; CA: esterification with citric acid followed by annealing; LA: esterification with lactic acid followed by annealing.

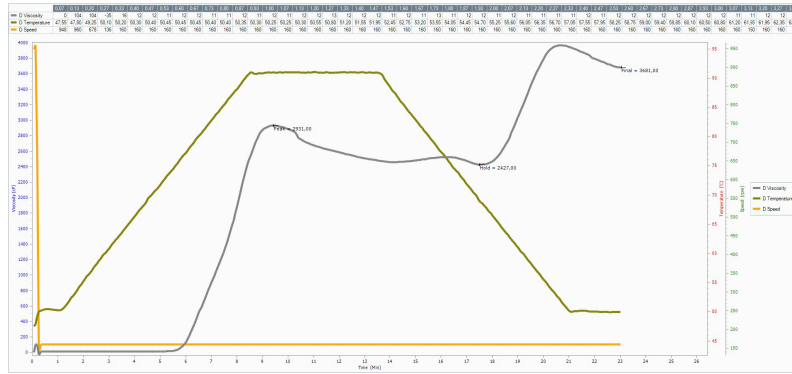
One of the main goals of flour modification is to increase the content of resistant starch (RS). In this study, dual modification was found to significantly enhance RS levels. Acetic acid esterification followed by annealing yielded the highest increase, from 5.64% to 8.42%. According to the findings of previous studies, citric acid has been demonstrated to be more effective than lactic acid in increasing RS [3]. Conversely, acetic acid has been shown to produce RS levels that approximate 8.19% [23]. In its pure form, starch is more reactive to esterification than it is in its flour form, likely due to the interference of non-starch components such as proteins and lipids.

The presence of acetic acid resulted in a high DS due to the acid's simple chemical structure and high dissociation rate, facilitating the conversion of hydroxyl groups to carboxyl groups. It likely cleaves amylopectin chains, forming shorter chains that reorganize more easily. Lactic acid, which contains one carboxyl group, yielded a higher DS than citric acid, which contains three carboxyl groups. The complex structure of citric acid may hinder its mobility within starch granules and promote cross-linking between starch molecules, reducing esterification efficiency. Annealing reorganizes the internal starch structures, stabilizing them and allowing more accurate amylose measurement, although it does not directly increase amylose quantity. The combination of increased DS and granular restructuring contributes to RS enhancement.

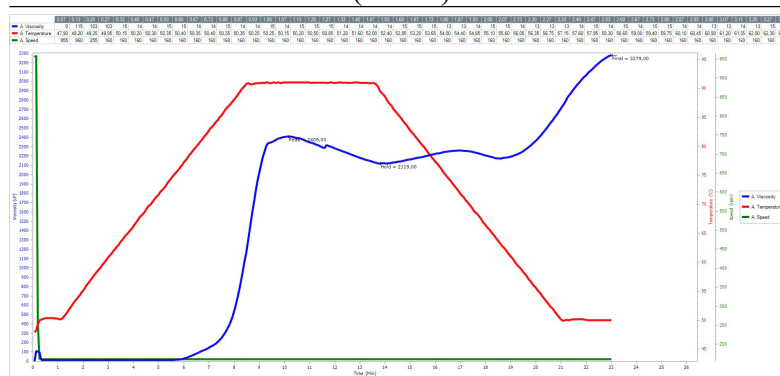
The esterification and annealing processes reduced swelling power and solubility. Citric acid treatment resulted in the lowest swelling power due to its three carboxyl groups acting as cross-linking agents, forming rigid networks that resist water absorption and expansion [24]. Annealing further densified starch structure, limiting swelling [25]. In contrast, AA and LA primarily resulted in monoester substitutions, which, while reducing chain flexibility and disrupting hydrogen bonding, did not lead to extensive crosslinking. Annealing further promoted molecular rearrangement and crystalline perfection, increasing structural order within the starch matrix. The reduction in amorphous regions and the improvement of molecular packing decreased water penetration and suppress granule swelling.

Water solubility decreased markedly after dual modification. The water solubility of native flour was 2.29%, which decreased to 0.25% (AA), 1.44% (CA), and 0.43%. The significant decrease in water solubility that was observed following dual modification can be attributed to a combination of ester bond formation and annealing-induced structural reorganization. Esterification partially substitutes hydroxyl groups with ester functionalities, reducing the number of hydrophilic sites available for hydrogen bonding with water. Annealing further promotes molecular packing and crystalline rearrangement, decreasing the proportion of amorphous regions that are typically more soluble. The reduced swelling power observed in the modified samples supports this interpretation [9,26,27]. Decreased solubility can prove advantageous in food systems requiring thermal and shear stability. A more structurally stable flour matrix may improve

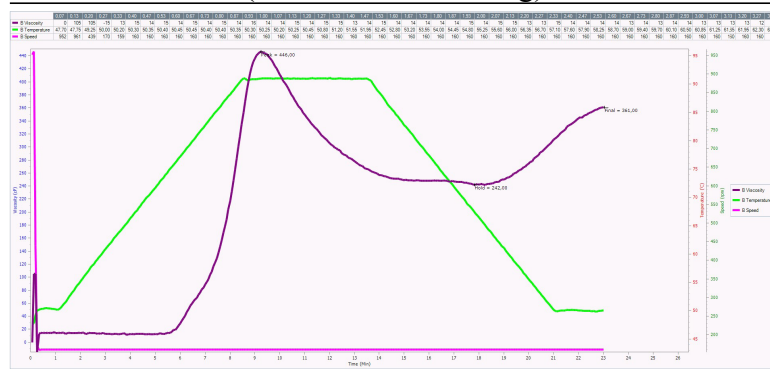
viscosity retention during heating, reduce excessive thickening breakdown, and enhance resistance to enzymatic hydrolysis. These properties are advantageous for products such as sauces, batters, and functional foods targeting controlled digestibility or lower glycemic response.



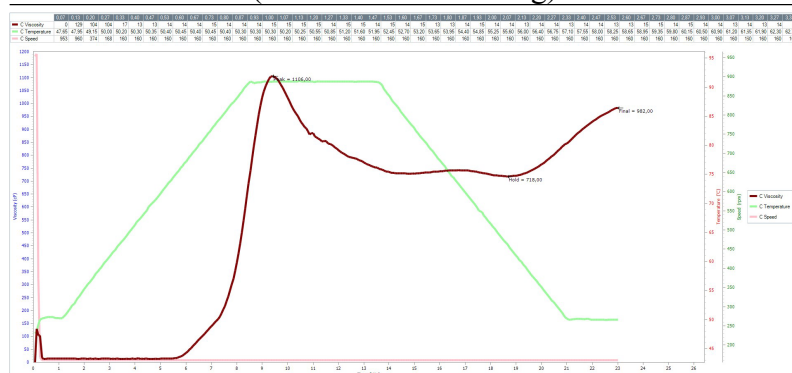
(native)



(Acetic acid-annealing)



(Citric Acid-Annealing)



(Lactic Acid-Annealing)

Fig. 1. Pasting properties of corn flour treated with dual modification

Dual modification was found to reduce viscosity values [13,28,29]. A different result was reported by Ariyantoro *et al.* [16], where the combination of succinylation and annealing treatments increased the viscosity of corn starch. The native flour exhibited a peak viscosity (PV) of 2,931 cP, while the modified samples demonstrated a range from 446 to 2,409 cP (Fig. 1). The lowest PV was observed in citric acid–annealed flour, indicating strong structural stabilization. Breakdown viscosity (BV) decreased from 504 cP to a range of 204–388 cP, showing improved heat and shear resistance. Setback viscosity (SV) also declined from 1,254 cP to a range of 119–1,160 cP, suggesting reduced retrogradation and better cold stability. Yao *et al.* [30] posit that starch with low BV and SV is suitable for food products such as ice cream or starch-based beverages.

The final viscosity and setback values were lower in the modified samples, with citric acid treatment yielding the lowest setback. These alterations reflect disrupted amylose–lipid complexes and ester bond formation that prevent starch chain reassociation. These findings are consistent with previous reports on acid-treated and annealed starches, which showed improved paste stability and reduced retrogradation. From functional standpoint, these alterations offer advantages in food processing. Lower PV and breakdown viscosity improve tolerance to heating and mixing. Furthermore, reduced setback prolongs shelf-life and improves texture. Modified corn flour is thus deemed suitable for sauces, batters, and instant products that require thermal stability.

The dual modification influences several functional parameters that are closely related to RS formation. The observed changes in amylose content, swelling power, solubility, and pasting properties suggest that both chemical substitution and structural rearrangement contribute to starch resistance. The formation of RS in dual-modified corn flour appears to be governed by a combination of chemical substitution and thermally induced molecular reorganization. Although the degree of substitution (DS) provides evidence of ester bond formation, RS formation is also governed by structural reorganization, reaction conditions, and molecular packing, which may influence enzymatic accessibility [31,32].

The significant reduction in swelling power observed in CA-treated samples indicates restricted water penetration and enhanced network formation, which are commonly associated with reduced enzymatic accessibility [33,34]. Despite the relatively low DS, steric hindrance and limited molecular mobility in flour systems may restrict extensive substitution. Thus, RS formation in CA-treated flour is likely attributable to the combined effects of partial chemical crosslinking (RS4-type contribution) and annealing-induced molecular reorganization leading to recrystallization (RS3-type contribution), rather than substitution level alone [32]. In the AA-treated samples, the highest RS content was observed. However, this was not accompanied by the highest DS when compared to the other treated samples. It can be deduced that acetic acid primarily promoted partial depolymerization of amylopectin branches, forming shorter linear

chains that were more prone to realignment during the annealing process. The increased, measurable amylose content supports this mechanism. Upon annealing, these linear chains presumably reorganized into more stable double helices and crystalline domains, a characteristic of RS3 formation. The moderate reduction in swelling power and viscosity further indicates restricted granular expansion, which may have limited enzymatic penetration and enhanced RS retention [35,36]. LA-treated samples exhibited the highest DS, yet not the highest RS level. This finding indicates that substitution density alone does not directly determine RS formation. Lactic acid may predominantly form monoester substitutions without extensive crosslinking, leading to structural modification but not to sufficient network rigidity to maximize the restriction of enzymatic hydrolysis [37].

The morphological characteristics of corn starch granules were significantly altered by dual modification involving esterification with organic acids (acetic, citric, and lactic) followed by annealing (Fig. 2). Scanning electron microscopy (SEM) micrographs revealed distinct changes in surface texture, granule integrity, and structural compactness across treatments. The morphology of the native corn starch granules was found to be smooth and rounded with minimal surface disruption, consistent with previous reports on unmodified starches [16]. This baseline structure reflects the natural organization of amylose and amylopectin chains, which are susceptible to modification under chemical and thermal treatments.

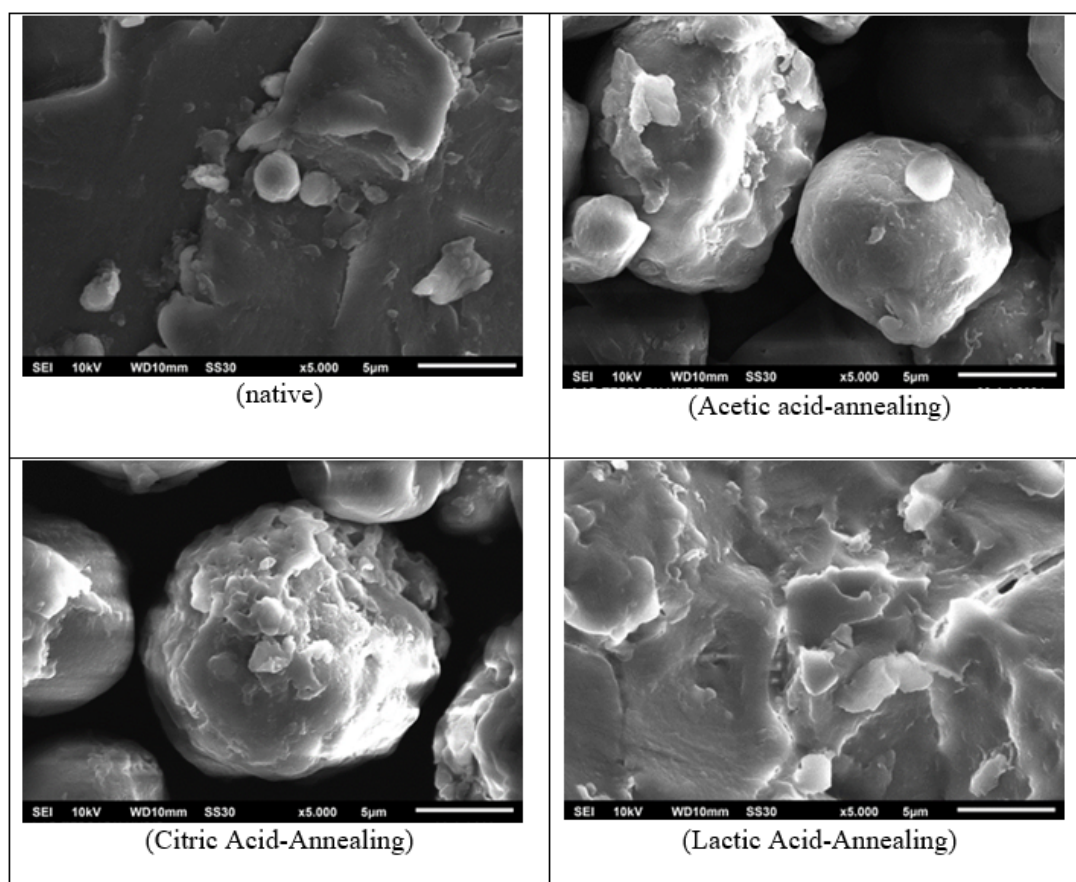


Fig. 2. SEM micrographs of corn flour

The modification of corn flour using organic acids, followed by annealing, induced significant changes in granule structure. The initial smooth and rounded granules observed in native corn flour transformed, becoming rougher, denser, and exhibiting surface fissures (Fig. 2). These findings are consistent with those of Kaur *et al.* [38], which reported that esterification with citric acid significantly affects granule morphology. Dolas *et al.* [39] also noted similar observations regarding the effects of acetylation on starch granules. In contrast, Ariyantoro *et al.* [16] reported that dual modification involving succinylation and annealing did not result in noticeable morphological changes in starch granules.

Corn flour modified with acetic acid and subsequent annealing (AA) exhibited granules with roughened surfaces and fine fissures. These alterations suggest a partial disruption of crystalline regions and a reorganization of amorphous zones, likely due to acetyl group substitution and subsequent molecular rearrangement during annealing. Similar morphological transitions were reported by Imre and Vilaplana [40]. Their findings indicate that esterification with short-chain organic acids enhances moisture resistance and thermal stability by altering granule compactness and surface porosity.

Citric acid-modified starch (CA) displayed the most pronounced morphological transformation. The granules manifested increased density, with deeper fissures and rigid surfaces, indicative of extensive cross-linking. Citric acid, which contains three carboxyl groups, facilitates multi-point esterification, reinforcing the granule matrix and reducing its swelling capacity. This observation aligns with the findings of Ariyantoro *et al.* [16], which reported that dual modification with succinylation and annealing increased granule rigidity and reduced susceptibility to shear stress.

Lactic acid-modified starch (LA) exhibited a more porous and less compact surface morphology in comparison to starch subjected to CA and AA treatments. The presence of widespread micro-cracks suggests that lactic acid promotes a less rigid molecular arrangement, which could lead to an increase in water absorption and solubility. The present findings are consistent with those reported by Sumardiono *et al.* [41], i.e., morphological changes in sago starch modified with lactic acid.

Overall, the findings of SEM analysis confirms that the type of organic acid used in the esterification process plays a critical role in determining the extent and nature of the ensuing morphological alterations. These structural modifications are closely linked to functional properties such as swelling power, paste clarity, and thermal stability.

The structural changes in corn flour resulting from dual modification via organic acid esterification and annealing were examined using Fourier-transform infrared (FTIR) spectroscopy.

The FTIR spectra revealed distinct shifts and variations in absorption band intensities, indicating successful chemical modification and molecular reorganization [42].

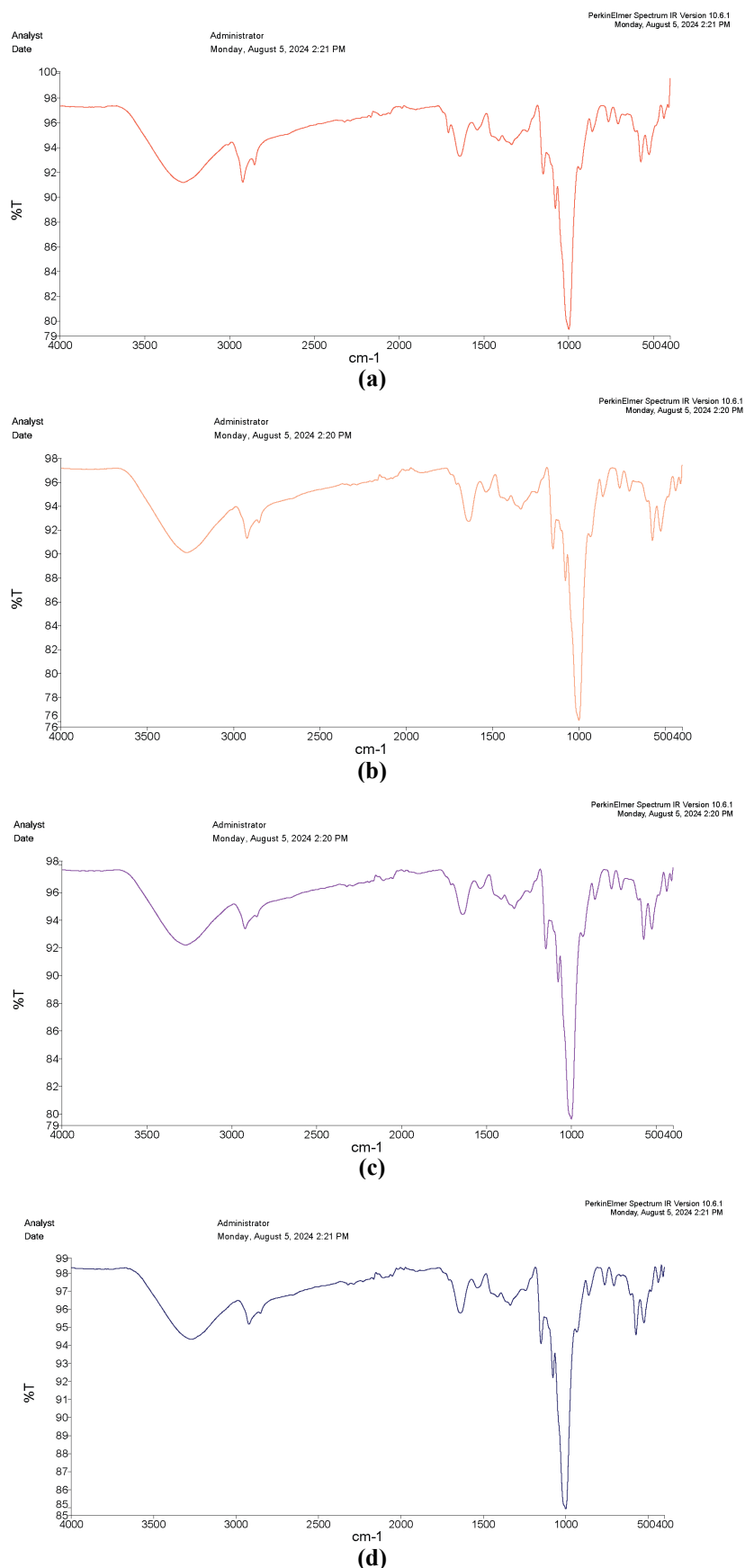


Fig. 3. FTIR analysis on native corn flour (a), as well as corn flour subjected to acetic acid-annealing (b), citric acid-annealing (c), and lactic acid-annealing (c)

In native corn flour, prominent absorption bands were observed around $3,400\text{ cm}^{-1}$ (Fig. 3), corresponding to O–H stretching vibrations, and near $2,930\text{ cm}^{-1}$, attributed to C–H stretching of aliphatic chains. The presence of these bands was observed in all samples, although their intensity was reduced in modified flours. This finding suggests that there has been a partial disruption of hydrogen bonds and an increase in molecular mobility [42]. The region near $1,640\text{ cm}^{-1}$, which is typically associated with H–O–H bending of absorbed water, showed slight shifts and narrowing in modified samples. This reflects changes in water-binding capacity due to structural rearrangement [43].

Esterification led to the emergence of new absorption bands or the intensification of existing ones within the range of $1,730\text{--}1,750\text{ cm}^{-1}$, corresponding to the C=O stretching vibrations of ester groups. These bands were most pronounced in acetic acid-treated samples, confirming the formation of ester linkages between starch hydroxyl groups and carboxylic acid moieties [42]. Furthermore, citric acid- and lactic acid-treated samples exhibited similar features, albeit with lower intensity, consistent with their molecular size and reactivity.

The fingerprint region ($1,200\text{--}900\text{ cm}^{-1}$), which represents C–O stretching and C–C skeletal vibrations of polysaccharides, displayed subtle shifts and intensity changes across the treatments. These variations are indicative of alterations in glycosidic linkages and backbone conformation of starch molecules. Modified samples showed broader and less defined peaks in this region, indicating an increase in amorphous character and a decrease in crystallinity.

As demonstrated in Fig. 3, these spectral changes are consistent with the findings of Yaqoob *et al.* [12]. The latter, via FTIR analysis, revealed significant alterations in chemical attributes and molecular structure of modified corn flour. The presence of ester bands and diminished O–H stretching intensity confirms the success of the dual modification and its impact on the architecture of starch.

4. Conclusion

This study demonstrates that dual modification using organic acids (acetic, citric, and lactic acids) combined with annealing effectively enhances resistant starch (RS). The results have shown that RS content increased from 5.64% in native flour to 6.88–8.42% after modification, with AA treatment producing the highest RS. The degree of substitution (DS) exhibited a range of 0.02 to 0.08, with LA treatment demonstrating the most significant DS, indicating the successful formation of ester bond. The swelling power exhibited a significant decrease, ranging from 1023.11 (native) to 504.66–998.56, accompanied by a reduction in solubility and PV, reflecting structural reinforcement and restricted granule expansion. Despite the increased DS, the formation of RS was governed by the combined effects of the chemical substitution, reduced swelling, altered pasting properties, and molecular reorganization during annealing.

Abbreviations

| | |
|------|------------------------------|
| AA | acetic acid |
| BV | breakdown viscosity |
| CA | citric acid |
| DS | degree of substitution |
| LA | lactic acid |
| Fig | figure |
| FTIR | fourier transform infrared |
| PV | peak viscosity |
| RS | resistant starch |
| SEM | scanning electron microscopy |
| SV | setback viscosity |

Data Availability Statement

All data has been presented in this article

CRediT Authorship Contribution Statement

Akhmad Mustofa: conceptualization, methodology, funding acquisition, research coordination, article drafting, corresponding author. **Vivi Nuraini:** coordinating research, data analysis, project administration. **Eri Wahyu Riadi:** conducting tests with acetic acid, data analysis, article draft. **Kelvin Matthew Richardo:** conducting tests with citric acid, data analysis, article draft. **Dinda Fitria:** conducting tests with lactic acid, data analysis, article draft.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

Declaration of Use of AI in the Writing Process

The authors used chat-gpt during the preparation of this manuscript. After using the tool, the authors carefully reviewed and edited the content and take full responsibility for the content of the publication.

Acknowledgement

The research was supported by Universitas Slamet Riyadi

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