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Characterization of Sago Starch Complex with Ferulic Acid Isolated from Corn Cob and Evaluation of Antioxidant Activity

Edi Suryanto[1]*, Mercy Irda Riantiny Taroreh[2], Alexander Sam Leonard Bolang[3], Imam Jayanto[4]

[1]Department of Chemistry, Faculty of Mathematics and Natural Science, Sam Ratulangi University, Manado, Indonesia
[2]Department of Food and Science Technology, Faculty of Agriculture, Sam Ratulangi University, Manado, Indonesia
[3]Department of Nutrition, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia
[4]Department of Pharmacy, Faculty of Mathematics and Natural Science, Sam Ratulangi University, Manado, Indonesia

*Corresponding author email: edisuryanto@unsrat.ac.id

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ABSTRACT. Corncob is one of agro-food by-products, a potential source of natural antioxidants and functional food ingredients, rich in ferulic acid and dietary fibre. In this study, ferulic acid was isolated from corncobs after alkaline pretreatment using a multi-step extraction process and structurally verified, providing a suitable bioactive compound for baruk sago (SB) starch complexation. FTIR analysis showed characteristic absorption peaks at 3379-3387 cm^{-1}, indicating interactions between FA and starch. XRD results revealed a transformation of the native SB starch crystalline structure and a marked decrease in crystallinity from 40.31% to 8.60% after complexation. SEM observations demonstrated morphological changes from predominantly smooth, oval, and compact granules to a looser gel structure in the starch–FA complex. In vitro antioxidant evaluation using the DPPH radical scavenging assay showed that the SB starch–FA complex exhibited higher scavenging activity compared to native SB and pre-gelatinized (SBG) starch. These findings confirm the successful incorporation of FA into SB starch and demonstrate that complexation significantly modifies physicochemical properties and enhances antioxidant capacity, highlighting its potential application as a functional food ingredient and nutraceutical material.

Keywords: Antioxidant, characterization, sagu baruk, starch-ferulic acid complex

INTRODUCTION

Starch is a major carbohydrate in staple foods and serves as a primary energy source. However, its rapid digestibility and high glycemic response are associated with increased risk of metabolic disorders. Consequently, recent research has focused on strategies to reduce starch digestibility by modifying its structure to slow glucose release and enhance enzyme resistance. One promising approach is the formation of starch inclusion complexes with bioactive compounds.

Starch consists of amylose and amylopectin fractions, with amylose playing a key role in complex formation. Amylose is known to form inclusion complexes with various ligands, including fatty acids and phenolic acids, resulting in a characteristic V-type crystalline structure (Babaoglu et al., 2024; Igoumenidis et al., 2018). These V-type complexes have been reported to limit starch digestion and exhibit properties similar to dietary fiber (Hernández et al., 2022). Among phenolic acids, ferulic acid has attracted particular attention due to its antioxidant capacity and potential to interact with starch molecules, thereby modifying physicochemical and functional properties.

Polyphenol-starch complexation has emerged as a strategy to simultaneously improve the functional and health-related properties of starch. Despite increasing interest in polyphenol-modified starch systems, studies specifically addressing starch–ferulic acid interactions remain limited, particularly for underutilized starch sources.

Sago baruk starch (SB) from *Arenga microcarpha*, is a local starch source with potential functional applications; however, its structural characteristics and modification behavior have not been extensively studied. At the same time, corn cobs represent an abundant agricultural by-product rich in phenolic compounds, including ferulic acid. Although previous studies have reported the extraction, characterization, and antioxidant properties of phenolic compounds from corn cobs (Suryanto et al., 2018; Suryanto et al.,

2020; Suryanto & Taroreh, 2022a; Suryanto & Taroreh, 2022b), research exploring their application in starch modification systems remains scarce.

Therefore, this study aimed to characterize sago baruk starch and enhance its functionality through modification with an isolated phenolic compound from corn cobs, with particular emphasis on evaluating changes in physicochemical properties and antioxidant activity. This work seeks to address the existing research gap in starch–ferulic acid systems using SB as a novel starch source.

EXPERIMENTAL SECTION

Materials

The baruk sago (SB) starch was purchased in the local supermarket, made by Manganitu, Sangihe Islands Regency, whereas corn cobs variety Manado kuning obtained from Tompaso, Minahasa, North Sulawesi. The chemicals used were ethanol, hydrochloric acid, sodium hydroxide, sodium acetate, iron(II) sulfate, iron(III) chloride, potassium ferricyanide, trichloroacetic acid, sodium carbonate, Folin-Ciocalteou reagent from Merck (Darmstadt, Germany). The 2,4,6-tri-pyridyl-s-triazine (TPTZ), ferulic acid, and gallic acid were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Amylase (100000 U/g) and glucoamylase (≥100,000 U/g) were obtained from Shanxy Biotechnology Co., Ltd.

Preparation of Sagu Baruk Starch

The wet sagu baruk (SB) was dried in an oven at 50 °C for 2 x 24 hours. After drying, micronization was performed using a grinding tool and sieving with a 100-mesh sieve. The flour yield was calculated based on dry weight, and the result was stored in plastic bags before analysis.

Preparation of Corn Cobs

Corn cobs with a moisture content of less than 10% were ground and sieved through a 40-mesh sieve. Subsequently, 100 g of the 40-mesh corn cobs were milled using a Fomac milling machine (model FCT-Z200) to produce a powder that passed through a 100-mesh sieve. The powder was then stored in plastic bags before analysis.

Extraction and Isolation of Ferulic Acid from Corn Cobs

Corn cobs were processed to extract and isolate ferulic acid (FA) using a method described by Sibhatu et al (2021). Twenty-five grams of corn cob powder were mixed with 500 mL of 1 M sodium hydroxide solution in a round-bottom flask, heated to 60-80 °C for 6 hours. After cooling, the mixture was filtered to collect the filtrate and residue, which was washed with distilled water. The pH of the combined solution was adjusted to 2 using 6 M HCl. Ethyl acetate was used to extract the compounds from the solution using a separatory funnel. The resulting supernatant was evaporated under vacuum to concentrate the phenolic compounds. After that, the extract was then mixed with 95% ethanol to obtain a ferulic acid concentrate, centrifuged, and further purified. The final product was analyzed using FTIR, HPLC, and LC–MS/MS.

Production of SB Starch-FA Isolate Complex

Five grams of SB starch and 100 mL of distilled water were mixed in a beaker and stirred for 10-15 minutes. The mixture was heated to 70 °C while stirring for 15 minutes. Then, ferulic acid with concentrations of 0, 4, 8, and 16% was added, and the mixture was stirred at 70 °C for 30 minutes. After this, the mixture was poured into a petri dish and cooled for 24 hours in a refrigerator until a starch gel formed. The gel was cut into small pieces and frozen for 12 hours. It was then filtered, dried at 50 °C for 24 hours, ground, and sieved for further assessment. The native starch (SB) and SB gelatinisation (SBG) were used to compare.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of starch and complexes were obtained with an FTIR Spectrophotometer (IR Prestige-21 SHIMADZU, Japan) using a transmission mode. The starch sample (2 mg) was blended with potassium bromide (KBr) powder (150 mg) by grinding for 10 min and pressed the mixed power into sheets with a vacuum compressor before measurement. The FTIR spectrophotometer was used to determine the spectral data of all samples in a band with a texture number range of 400-4000 cm^{-1}.

X-ray Diffraction (XRD)

An X-ray diffractometer (MiniFlex II, Rigaku, Japan) was used to determine the crystal structures of starch and complexes. The diffractometer worked at 40 kV and 100 mA with scanning radiation in the angular range of 4°-40° (2θ) at a scanning speed of 0.1°/s. The XRD data were analyzed by Origin, and used to analyze the relative crystallinity of each powder.

Scanning Electron Microscopy (SEM)

Morphological characteristics of starch and complexes were analyzed through SEM (Jeol/MP) at 15.0 kV. The sample was stabilized on the surface of copper stubs before being thinly coated with gold, then observed under 20 kV.

In Vitro Starch Hydrolysis Analysis

In vitro starch hydrolysis analysis was prepared according to the method of Hao et al. (2024) with minor modifications. Starch sample (200 mg) was dispersed ini sodium acetate buffer (15 mL, 0.1 M, pH 5,2) was added, and the mixture was boiled for 30 min. The mixture was then cooled to about 37 ◦C, 10 mL of enzyme solution (α-amylase: 290 U/mL and glucosidase: 15 U/mL). The mixture was enzymatically hydrolyzed at 37 °C with continuous stirring at 160 rpm for 120 min. After that, 1 mL aliquot was taken, 2 mL of DNS reagent (3,5-dinitrosalicylic acid) was added, and heated in a boiling water bath for 10 minutes. The absorbance of the solution was measured at a wavelength of 520 nm and decrease in starch hydrolysis (DSH) was calculated using the following equations:

DSH(%)= $\left(\frac{\text{control absorbance of starch}-\text{sample absorbance of starch}}{\text{control absorbanceo of starch}}\right)$ x100%

Determination of Total Phenolic Content

Determination of total phenolic content: Total phenolic content (TPC) of ethanol extracts was estimated using the Folin-Ciocalteu reagent based colorimetric assay (Suryanto & Taroreh, 2022b). Each sample solution (0.1 mL) was added to Folin-Ciocalteu reagent (0.1 mL, 50%) in a test tube and then this mixture was vortexed for 3 min. After intervals of 3 min, 2 mL of Na_2CO_3 2% solution was added. After the incubation at room temperature for 30 min, the mixture was kept in the dark for 30 min. The supernatant was measured using a spectrophotometer at 760 nm. The standard curve was prepared using different concentrations of gallic acid and the results were expressed as gallic acid equivalents in milligrams per milligram extract.

Determination of DPPH radical scavenging

DPPH radical scavenging activity of ethanol extracts was evaluated according to the method of Suryanto & Momuat (2017) with slightly modifications. A total of 2 mL solution of 92 μM 1,1-diphenyl-2picrylhydrazyl (DPPH) in ethanol was added 0.5 mL ethanol extract and a solvent fraction. A level of colour reduction of the solution shows the efficiency of radical scavenger. The last five minutes of the 30 min, the absorbance was measured using a spectrophotometer at 517 nm. Free radical scavenger activity was calculated as a percentage reduction of DPPH color using the equation:

Free radical scavenging activity (%) $= \left(1-\frac{\text{sample absorbance}}{\text{control absorbance}}\right)$ x100%

RESULTS AND DISCUSSION

Isolation and Identification

Ferulic acid (FA) was isolated from corn cobs through alkaline hydrolysis followed by ethyl acetate extraction. Under alkaline conditions, ester linkages between lignin–carbohydrate complexes and ferulic acid were cleaved, releasing FA monomers from their bound ester form. Sodium hydroxide hydrolyzed the ester bonds, liberating ferulic acid from lignocellulosic matrices. Twelve hydrolysis repetitions yielded 0.86, 1.57, 0.42, 1.65, 0.64, 0.55, 0.40, 0.29, 0.82, 0.64, 0.44, and 0.56 g of FA, with a total yield of 8.83 g (7.36%). The relatively consistent recovery across repetitions indicates effective ester bond cleavage and solvent partitioning. The alkaline hydrolysate was extracted with ethyl acetate to recover free FA for purification and quantification. HPLC analysis (**Figure 1**) showed a dominant peak at 9.370 min at 320 nm and 30 °C, corresponding to the FA standard under identical conditions. The high linearity of the calibration curve ($Y = 0.1277x + 0.0085$; $R^2 = 0.9969$) confirmed reliable quantification, and the FA concentration in the extract was 1.89 µg/mL. The single sharp peak and absence of major secondary peaks suggest high purity of the isolate.

FTIR analysis (**Figure 2**) further confirmed the chemical identity. The isolate exhibited characteristic absorption bands at 3417 cm^{-1} (phenolic O–H stretching), 2924 cm^{-1} (aromatic C–H stretching), 1697 cm^{-1} (C=O stretching of carboxylic acid), and 1327 cm^{-1} (C–O stretching). The similarity between isolate and standard spectra confirmed structural integrity and purity.

LC–MS/MS analysis (**Figure 3**) showed a molecular ion at m/z 193 ($[M–H]^-$), consistent with the molecular formula $C_{10}H_{10}O_4$ (MW 194 g/mol). Fragment ions at m/z 178 (loss of CH_3), 149 (loss of CO_2), and 134 (sequential losses) correspond to typical fragmentation pathways of ferulic acid, confirming structural identity.

The agreement among HPLC retention time, FTIR functional group analysis, and LC-MS/MS fragmentation patterns confirms that the isolated compound is ferulic acid with high purity, suitable for complexation with starch.

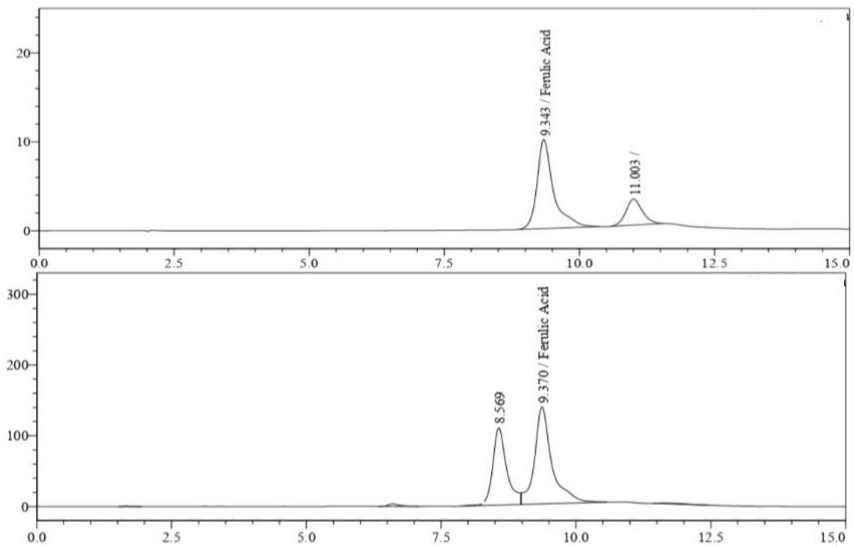


Figure 1. HPLC chromatogram of ferulic acid standard and ethyl acetate fraction of corn cobs.

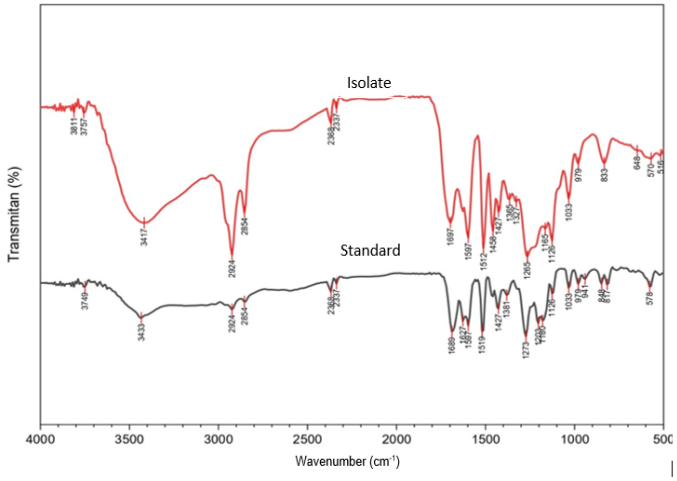


Figure 2. FTIR spectra of ferulic acid (isolate and standard)

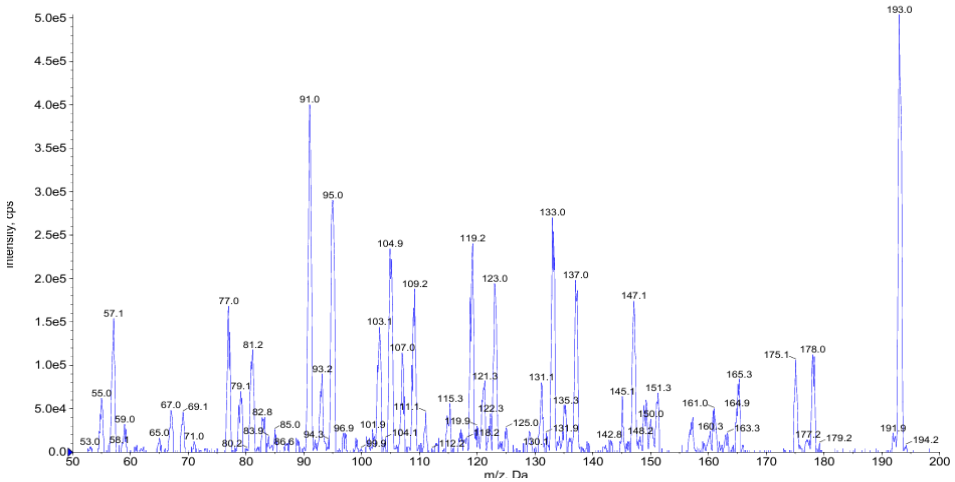


Figure 3. ESI MS/MS for mass ion fragmentation spectra of ferulic acid isolate

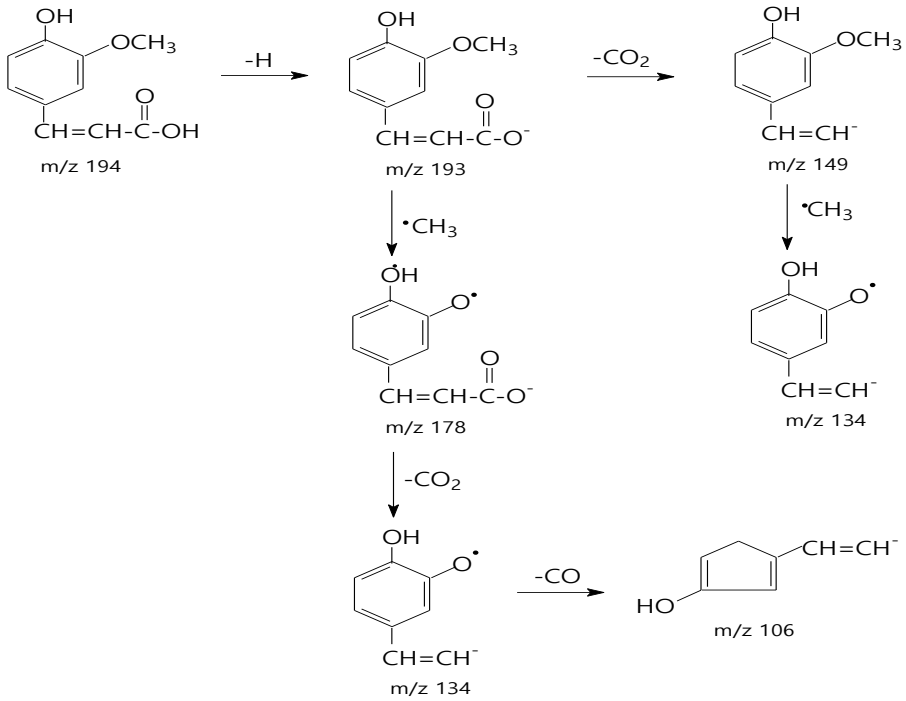


Figure 4. Proposed fragmentation mechanisms for deprotonated ferulic acid

FTIR Characterization of SB Starch-Ferulic Acid Complex

FTIR spectra of native SB starch, gelatinized SB starch (SBG), and SB-FA complexes are shown in **Figure 5.** Native SB starch exhibited characteristic bands at 3387 cm^{-1} (O–H stretching), 2924 cm^{-1} (C–H stretching), and 1149 cm^{-1} (C–O–C stretching of anhydroglucose units). Upon complexation with FA, a shift of the O–H stretching band from 3387 cm^{-1} to 3379 cm^{-1} was observed. This shift indicates the formation of new hydrogen bonds between hydroxyl groups of starch and phenolic/carboxyl groups of FA, confirming intermolecular interactions (Han et al., 2020). The reduction in intensity near 1643 cm^{-1} suggests altered water–starch interactions in the amorphous region, likely due to FA molecules occupying hydrophobic cavities within amylose helices (Hao et al., 2024). Additionally, the presence of aromatic ring vibrations (1450–1600 cm^{-1}) in the complex spectra confirms the incorporation of FA into the starch matrix. The changes in the 800–1200 cm^{-1} region indicate modifications in short-range molecular order, suggesting structural rearrangement of starch chains (Chi et al., 2017). Collectively, these FTIR changes substantiate hydrogen bonding and possible inclusion-type interactions between FA and amylose helices, supporting complex formation rather than simple physical mixing. These results suggest a possible change in the main interaction between ferulic acid isolate and SB starch.

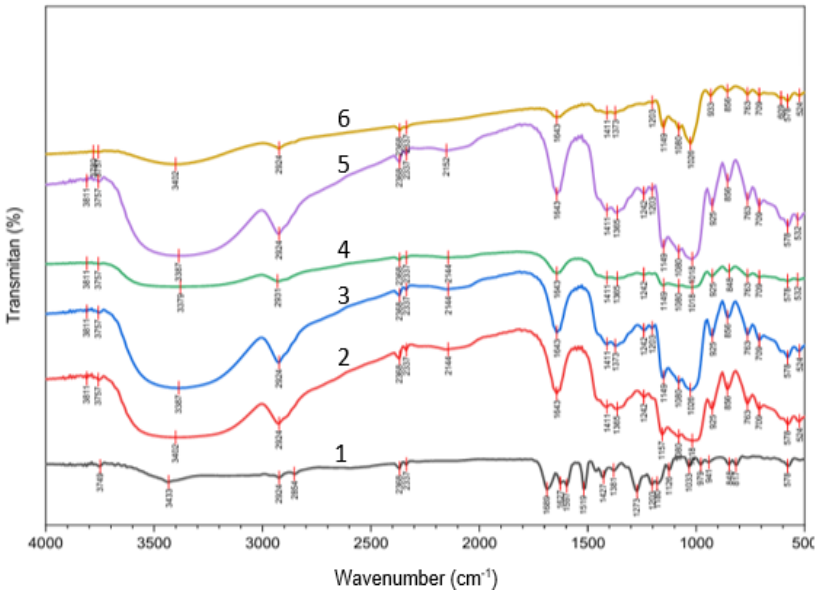


Figure 5. FTIR spectra of SB starch, SBG starch, and SB–FA complexes at various concentrations. 1. Ferulic acid, 2. SB starch, 3. SBG starch, 4. SB starch-IAF 0.02%, 5. SB starch- IFA 0.04%, 6. SB starch-FA 0.08%.

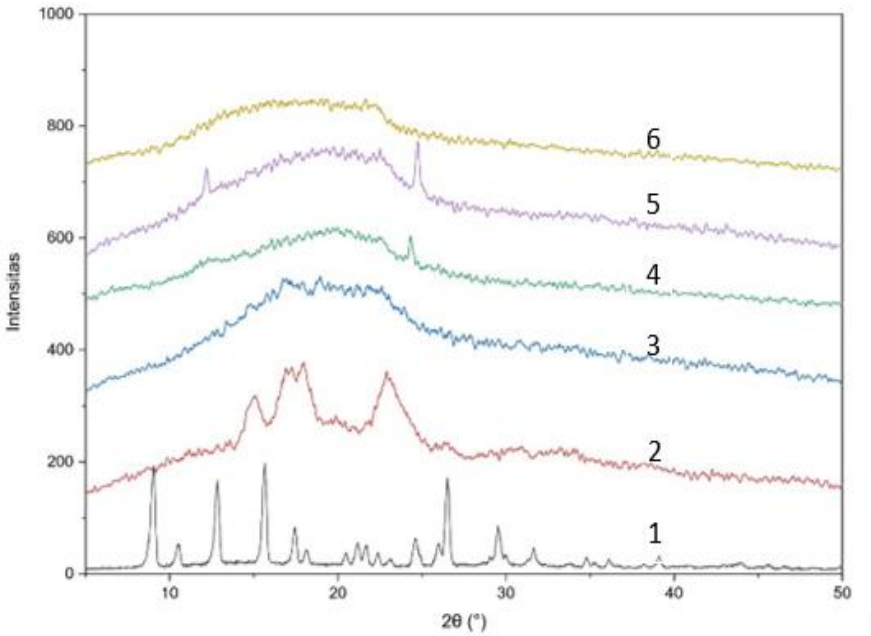


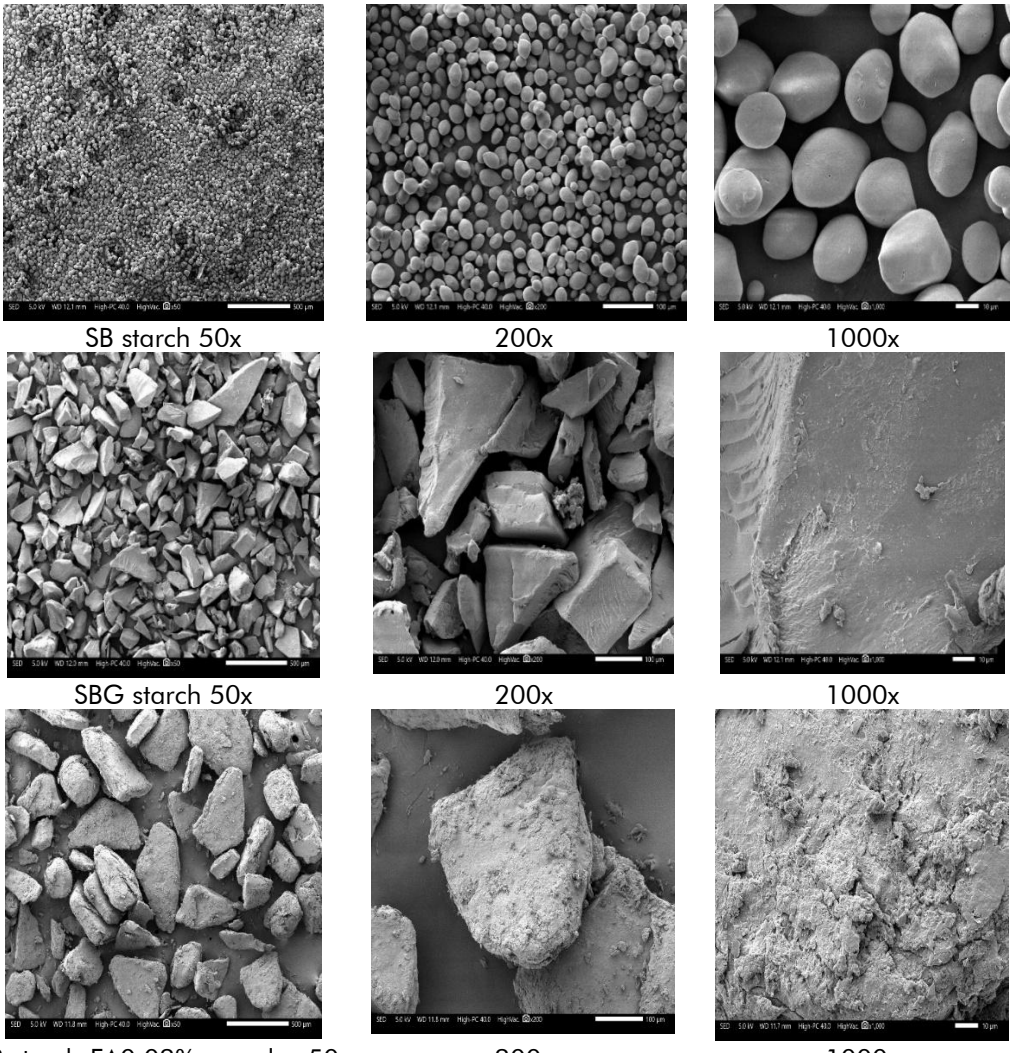
Figure 6. X-ray pattern of SB starch, SBG and SB starch-FA complexes with various concentrations. Abbreviations as in **Figure 5**.

XRD Characterization of SB–FA Complex

The XRD patterns are presented in **Figure 6**. Native SB starch exhibited a typical A-type crystalline structure with strong peaks at 15.10°, 17.24°, 17.98°, and 22.90°. After gelatinization (SBG), the pattern became predominantly amorphous due to disruption of crystalline lamellae. The relative crystallinity decreased significantly from 40.31% (native SB) to 15.27% (SBG) and further to 8.60–16.51% in SB–FA complexes, indicating that FA incorporation disrupted ordered double-helical arrangements. Importantly, the appearance of a weak peak near 19.74° suggests the formation of a V-type crystalline structure, typically associated with amylose inclusion complexes (Gao et al., 2021). This indicates that FA molecules may be partially embedded within amylose single helices, forming host–guest complexes. The reduction in overall crystallinity and emergence of V-type characteristics confirm that FA alters starch molecular packing. Such structural reorganization explains the increased resistance to enzymatic hydrolysis, as reduced crystallinity combined with inclusion complex formation limits enzyme accessibility. Similar results have been reported, where a new peak at $2\theta \approx 24°$ appeared in the starch-ferulic acid complex (Hao et al., 2024).

SEM Morphological Analysis

The morphological changes in the modified SB-starch granules after ferulic acid treatment are exhibited in **Figure 7.** SEM micrographs show that native SB starch granules were smooth, oval to irregular, and 21.30–25.09 µm in size. These granules were larger than those of rice, corn, and tapioca starch (Grace & Henry, 2020). After gelatinization without complexation, the starch granules were disrupted, forming irregular amorphous aggregates. In contrast, SB–FA complexes formed porous, rough gel matrices with irregular surface cavities. This morphological transformation indicates structural reorganization during complexation, suggesting many reactive sites between SB starch and FA due to interactions. The increased surface roughness and porous network suggest stronger intermolecular interactions and partial retrogradation, consistent with FTIR and XRD findings. The denser and less ordered matrix likely restricts enzymatic penetration and substrate accessibility, contributing to reduced digestibility.



SB starch 50x 200x 1000x

SBG starch 50x 200x 1000x

SB starch-FA0,02% complex 50x 200x 1000x

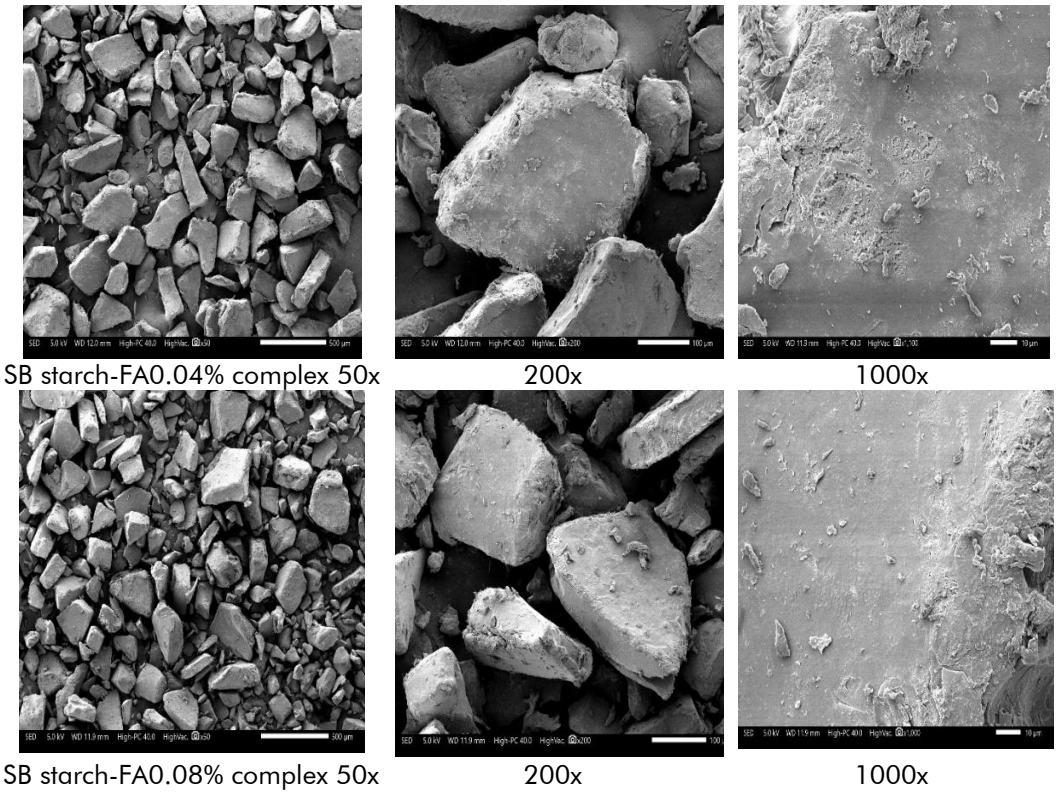


Figure 7. Scanning electron microscopic (SEM) images of SB starch-FA complex at different magnifications.

In Vitro Starch Hydrolysis

In vitro starch hydrolysis is a measurement of the level of starch digestibility that mimics the human digestive system using the enzymes α-amylase and amyloglucosidase. Both enzymes have the ability to hydrolyze starch into simple sugars such as maltose, glucose, and dextrin. Based on starch hydrolysis, health problems can occur due to the rapid rate of digestion and absorption, which can lead to metabolic disorders such as increased blood glucose after meals, insulin resistance, and obesity (Alatrach et al., 2019; Zheng et al., 2020). Figure 8 shows that all SB-AF starch complexes treated with various concentrations produced modified starch that was resistant to digestion. The percentage of reduction in the digestibility of SB-AF starch complex with various concentration treatments (0.02-0.08%) at 70 °C ranged from 93.87-98.68%.

This data indicates that SB-AF starch complex with various concentrations have lower digestibility than SB starch and SBG starch. Lower digestibility was indicated by a greater decrease in digestibility (**Figure 8**). This shows that the complexation of ferulic acid isolates in SB starch can produce digestible starch in the form of gelatinization. The results of this study support the results of previous studies which noted that the starch-ferulic acid complex produces a denser structure and increases resistance to enzymatic hydrolysis and slows the rate of complex starch hydrolysis and causes conformational changes in digestive enzymes, thereby inhibiting starch digestibility (Romero et al., 2022; Sun & Miao, 2020). In other words, ferulic acid isolates bind to starch by occupying its hydrophobic helical domain, resulting in a more compact structure and slowing the rate of starch hydrolysis. Several studies have reported that ferulic acid reduces the rate of starch hydrolysis by altering the secondary structure of digestive enzymes (Zheng et al., 2020). In other words, polyphenols can not only form complexes with starch molecules and increase their structural density, but also inhibit the activity of digestive enzymes, thereby slowing the starch digestion process (Karunaratne & Zhu, 2016).

The structural evidence from FTIR, XRD, and SEM confirms the formation of starch–ferulic acid complexes. These structural modifications directly explain the reduced digestibility, as FA binding reduces available hydroxyl groups for enzyme interaction, inclusion complexes hinder α-amylase access to glycosidic bonds, and lower crystallinity and compact reorganization slow hydrolysis. In vitro hydrolysis results showed a significant reduction in digestibility in SB starch–FA complexes compared to native and gelatinized starch, indicating enhanced resistant starch formation. This reduction can be mechanistically explained by hydrogen bonding between FA and starch hydroxyl groups, reducing enzyme-accessible sites, formation of amylose–FA inclusion complexes (V-type), limiting α-amylase binding, and decreased crystallinity and denser matrix formation, hindering enzyme diffusion.

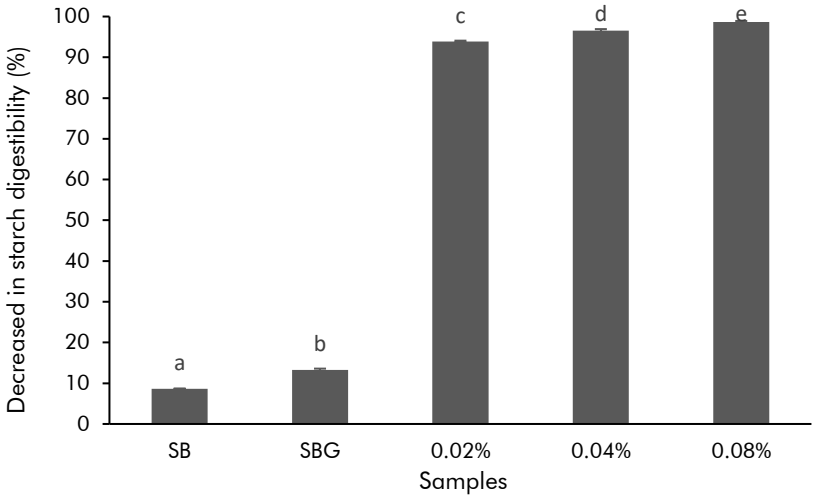


Figure 8. Percentage of decreased in starch digestibility of SB-IAF starch complexes.

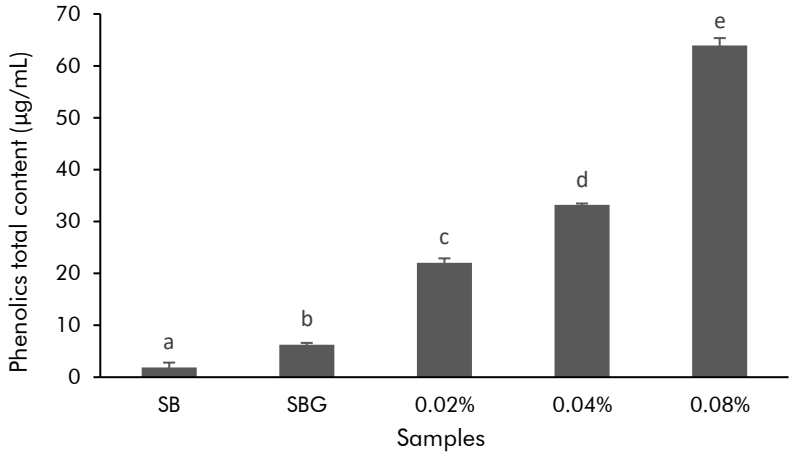


Figure 9. Total phenolic content of SB–FA complexes.

Total Phenolic Content of SB Starch-FA Complex

The study analyzed total phenolic content (TPC) as an antioxidant in various SB starch-FA extracts. It found that the SB starch-FA complex extract had the highest TPC compared to SB and SBG extracts. The higher TPC in SB-FA is due to better bioavailability of phenolic compounds and more effective extraction methods. TPC levels ranged from 22.05 to 63.93 μg GAE/mL (**Figure 9**). TPC in extracts was increased with increasing proportion of SB-FA starch complex concentrations. Hao et al. (2024) and Liu et al. (2023) also reported FA content of the complexes in corn and rice starch extract, respectively 6.31 μg/mL and 28.96 mg FAE/g extract. Phenolic compound such as ferulic acid was considered to be major contributors to the antioxidant activity of plants extract (Suryanto, 2017)

DPPH free Radical Scavenging Activity of SB-FA Starch Complex

Figure 10 shows the ability of SB starch-FA complexes, SB and SBG starch to scavenge DPPH radicals. The presence of hydroxyl groups in starch and phenolic substances corresponds to the radical inhibition of the formed complexes. The result of the radical scavenging activity indicates that SB starch-FA complex has the highest activity than SB and SBG starch. According to Zhou et al. (2021), starch has negligible reducing power because the strong intramolecular and intermolecular hydrogen bonds interactions between the hydroxyl groups in the starch molecules limit the hydrogen donating capacity.

The highest DPPH free-radical scavenging capability was found in the 0.08% sample, while the lowest was exhibited by the 0.02%. The scavenging potential of SB starch-AF complex increased with increasing FA concentrations from 0.02% (80.56%) to 0.08% (90.18%), which of significantly improved capacity depending on the phenolic compounds structure and concentration of the complexes. The contribution of the phenolic hydroxyl groups bound to the starch must be responsible for contributing antioxidant properties of the prepared complexes. One possible explanation is that ferulic acid may enhance free radical scavenging through electron transfer and donate hydrogen atoms. Key structural element responsible for ferulic acid antioxidant action is the hydroxyl group. Yong et al. (2020) revealed that phenolic compound bound to the starch with hydroxyl groups that might neutralize free radicals, terminating free radical chain reactions.

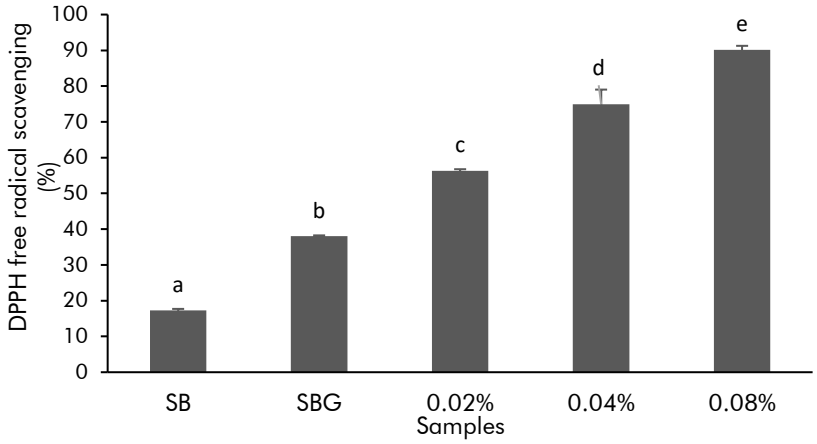


Figure 10. DPPH radical scavenging activity of SB starch-AF complexes.

CONCLUSIONS

The results of this study demonstrate that ferulic acid was successfully isolated from corncobs and structurally confirmed, providing a reliable bioactive compound for starch complexation. Starch-ferulic acid complexation is an effective approach to simultaneously modulate starch digestibility and introduce bioactive functionality. Complexation of SB starch with FA decreased starch digestibility and increased phenolic content and antioxidant activity. Therefore, these results suggest that the SB starch-FA complex has antioxidant activity and is a potential raw material for the production of functional food ingredients and nutraceuticals.

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