

Modified starch with resistant starch content and antioxidant activity from unripe *Carica papaya* fruits

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Unripe *Carica papaya* fruits are often discarded during cultivation, contributing to environmental waste despite their rich fibre and nutritional value. This study aimed to modify starch from unripe papayas by using different physical methods, including steaming, drying, and microwaving. The starch samples were evaluated for their amylose content, resistant starch levels, phenolic content, and antioxidant activity based on DPPH radical scavenging activity. Among the methods tested, the combination of autoclaving and microwaving yielded the best results, with an amylose content of 2.76 ± 0.02 mg/g, resistant starch at $2.82 \pm 0.08\%$, polyphenols at 45.97 ± 0.19 mg GAE/g, and an IC_{50} value for DPPH radical scavenging of $95.12 \mu\text{g/mL}$, which showed slight antioxidant activity compared to the positive control (Trolox, $IC_{50} = 10.07 \mu\text{g/mL}$). Furthermore, the modified starch samples were assessed for physical properties, including water solubility index (31.16 ± 0.63 – $44.04 \pm 0.80\%$), water absorption capacity (6.70 ± 0.38 – 9.83 ± 0.22 g/g), swelling power (20.27 ± 0.42 – 27.60 ± 0.29 g/g), and light transmittance (37.59 ± 0.21 – $49.32 \pm 0.25\%$ on day 1). The findings provide valuable data on modified starch from unripe *Carica papaya* fruits and offer a solution to reduce the annual disposal of unripe papayas, thereby mitigating their environmental impact.

Keywords: Amylose, *Carica papaya*, DPPH radical scavenging activity, Phenolic content, Resistant starch

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Introduction

Starch is a major carbohydrate in the human diet and an important energy source. It is classified into two main types of polysaccharides: amylose and amylopectin. Amylopectin, a branched polymer, is responsible for the gelatinous properties of starch and is commonly found in waxy starches. In contrast, with its linear structure, amylose contributes to starch's firm texture and is used as a form of resistant starch¹. Resistant starch (RS) resists digestion in the small intestine and ferments in the colon to produce short-chain fatty acids². It includes five types: RS₁, encapsulated in fibre; RS₂, ungelatinized granules; RS₃, retrograded starch; RS₄, chemically modified starch; and RS₅, starch complexes with lipids³. Based on its resistance to digestion in the small intestine, resistant starch consumption lowers glycemic index values, which can help with obesity and type II management. It also shows promise in preventing gallstone formation, reducing the risk of

cardiovascular disease, and enhancing mineral absorption⁴.

Polyphenols are a group of natural compounds found in plants, known for their antioxidant, antibacterial, and antitumor properties. Polyphenols have phenolic hydroxyl groups that can interact with starch through non-covalent bonds such as hydrogen bonding, electrostatic, and ionic interactions. These interactions can lead to forming two types of complexes: V-type inclusion complexes and non-inclusion complexes. Besides, polyphenols in starch have been shown to slow or inhibit the activity of amylolytic enzymes, pancreatic amylase, and glucosidase, which hinders starch breakdown and lowers post-meal blood glucose levels^{5,6}. Therefore, starch modification is carried out to enhance the content of resistant starch and polyphenols by physical, chemical, and biological methods. However, physical methods (drying, steaming, microwave treatment) are advantageous because they avoid potentially hazardous chemicals^{7,8}. Previous studies have shown that the unripe papaya starch heated in a hot air oven at 70°C for 12 hours had an amylose

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content of $1.44 \pm 0.33\%$ ⁹ or microwave treatment of rice at a power level between 90 and 500 W reduces amylograph viscosity⁹.

Carica papaya fruits are commonly grown in tropical climate regions. They are rich in fibre, vitamins, and essential minerals. Many studies have reported that unripe fruits have lower sugar content and higher fibre than ripe papaya. During cultivation, unripe papayas are often removed to enhance the quality of ripe fruit, leading to many unripe papayas being discarded annually¹⁰. The surplus of unripe papayas contributes to global food waste and causes economic losses, particularly for farmers, as it leads to market price reductions and income instability. Moreover, improper management of these surplus papayas may result in their decomposition, releasing methane, a potent greenhouse gas, which exacerbates negative impacts on the environment and climate change. One of the ways to handle them is to use them as ingredients for salads, or one study has explored their use as excipients in drug delivery systems¹¹. Though unripe papaya powder has been generated and evaluated the amylose, phenolic content, antioxidant activity, and bile acid binding¹⁰, no studies have reported its resistant starch or the preparation of modified starch for amylose, resistant starch, and polyphenol enrichment. Therefore, using various physical methods, this study produced different modified starches enriched with resistant starch, amylose, phenolic compounds, and antioxidant capacity from unripe papayas. The optimal procedure may address the issue of discarded immature fruits each year and offer significant health benefits, particularly for diabetic patients. Specifically, resistant starch helps regulate postprandial blood glucose levels by slowing carbohydrate digestion, while polyphenols protect pancreatic beta cells from oxidative stress and inflammation, enhancing their function and viability.

Materials and Methods

Materials

Unripe *Carica papaya* fruits were purchased in Ho Chi Minh City, Vietnam. α -Amylase from porcine pancreas (PAA) (75000 U/g), amyloglucosidase (AMG) from *Aspergillus niger* (3260 U/mL), the gluco-oxidase-peroxidase (GOPOD) test kit was purchased from Megazyme (Wicklow, Ireland). Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Merck KGaA (Darmstadt, Germany).

Preparation of starch

The unripe fruits of *C. papaya* (2 kg) were peeled, rinsed 2–3 times with distilled water (sample to distilled water ratio of 1:2), and soaked in distilled water at room temperature for 24 hours, with the water being replaced every 6 hours. Then, they were homogenized into a uniform slurry using a high-speed blender (Philips HR3760/01) for 2 minutes (sample to distilled water ratio of 1:2). This slurry was kept for 6 hours to allow sedimentation and filtered through a double layer of muslin cloth. Finally, the sediment obtained was washed with distilled water for 2 minutes and dried at 45°C until its moisture content reached 12% to obtain starch.

Preparation of starch with high-resistant starch content and antioxidant capacity

Starch modification using the autoclave (Biobase, model BKM-K18N)

A 20% (w/v) starch solution was prepared by dissolving 5 g of starch in 20 mL distilled water. This mixture was autoclaved for 120 minutes and stored in the refrigerator for 24 hours at 4°C. This cycle of autoclaving and cooling was repeated daily for 5 days. The sample was dried at 45°C until its moisture content reached 12% to yield resistant starch. Factors such as autoclaved time (30, 60, 90, and 120 minutes) and the number of autoclaving and cooling cycles (3, 4, and 5 days) were researched to identify the optimal procedure.

Starch modification using the microwave

A household microwave oven (Sharp R-678VN(S/W)), a pulsed variable microwave rated power output from 240 to 720 W by a timer, and an inner cavity volume 20 L with sample rotation during measurement were used in this experiment. The microwave oven was preheated before each measurement by heating 2 L of water for 5 minutes.

Starch (5 g) with 20% moisture content was performed by microwave-assisted heat treatment at 400 W for 30 minutes. To attain the homogenous radiation distribution on the starch samples, samples were placed in the centre of the turntable and regularly stirred every 2 minutes to ensure even energy and temperature distribution upon treatment. The study investigated different microwave power levels (240, 400, and 600 W) and treatment durations (20, 25, 30, and 35 minutes) to identify the optimal processing conditions.

Starch modification using the drying oven

The drying oven (Kenton KH-75A), with temperatures ranging from 30 to 200°C, has an inner chamber volume of L and is equipped with 5 trays. The drying oven was preheated before each measurement by heating the oven for 5 minutes.

Starch (5 g) with 20% moisture content was dried at 80°C for 20 hours to produce resistant starch. Samples were placed in the centre of tray 3, among five trays in the drying oven, and stirred every 2 hours to ensure consistent heat distribution. The study examined different temperatures (70, 80, 90, and 100°C) and drying durations (16, 20, and 24 h) to obtain the optimal procedure.

Determination of resistant starch

The resistant starch (RS) content was determined based on Vietnamese Standard TCVN 13287:2021¹². In brief, 4 mL of an enzyme mixture containing PAA (30 U/mL) and AMG (3 U/mL) was added to 100 mg of modified starch. The mixture was incubated in a shaking water bath at 37°C for 16 hours. Exactly 4 mL of absolute ethanol (EtOH) was added to stop the enzyme activity. Resistant starch was separated by centrifugation (1500 g, 10 minutes). The pellet was resuspended in 2 mL of 50% EtOH. To eliminate digested starch, 50% ethanol (6 mL × 2) was used to add to the tube. The remaining sediment was dissolved in 2 mL of KOH 2 M, stirred in an ice bath for 20 minutes, and neutralized with 8 mL of CH₃COONa buffer 1.2 M. The mixture was added with 0.1 mL of AMG (3300 U/mL), incubated at 50°C for 30 minutes, and centrifuged again (1500 g, 10 minutes).

With samples (having >10% resistant starch), the entire contents of the tube were transferred to a 100 mL volumetric flask, diluted with distilled water to 100 mL, and centrifuged (1500 g, 10 minutes) to obtain the solution. For samples with <10% resistant starch, centrifugation was directly performed. A 0.1 mL aliquot of the supernatant was mixed with 3 mL GOPOD reagent and incubated at 50°C for 20 minutes. Reagent blank solution was prepared by combining 0.1 mL of CH₃COONa buffer 0.1 M (pH = 4.5) with 3.0 mL of GOPOD reagent, while glucose standard solutions were prepared by mixing 0.1 mL of D-glucose (1 mg/mL) with 3.0 mL of GOPOD reagent. Absorbance at 510 nm was measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The resistant starch, expressed as % of weight, was calculated by using the formula:

$$\text{RS content (\%)} = \Delta A \times F \times \frac{\text{EV}}{W} \times 0.9 \quad \dots(1)$$

Where: ΔA : Absorbance of sample solution read against reagent blank.

F: Factor to convert absorbance values to μg glucose

EV: Sample extraction volume

W: Sample weight in mg

0.9: Factor to convert from free glucose to anhydroglucose as occurs in starch.

Amylose content

The amylose content was measured based on the formation of the amylose-iodine complex¹³. A 1 g starch sample was heated at 105°C for 1 day, mixed with 0.1 mL of EtOH 95% and 1 mL of NaOH 1 N. The mixture was stored at 4°C for 1 day, diluted to 10 mL with distilled water, and refrigerated again for 16–18 hours to obtain a starch stock solution (1000 $\mu\text{g}/\text{mL}$). 5 mL of starch stock was adjusted to pH 6 using HCl (1 M), followed by adding 0.1 mL of a 0.2% I₂/KI solution. The absorbance was measured at 620 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The amylose content was calculated based on the standard curve generated from potato amylose and expressed as mg amylose/g starch. To prepare the calibration standards (concentration range from 1 to 10 $\mu\text{g}/\text{mL}$), 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 mL of stock solution, which was adjusted to pH 6 using HCl (10 $\mu\text{g}/\text{mL}$), was added to 6 separate volumetric flasks, then 0.1 mL of iodine reagent solution was added to each calibration level.

Determination of total phenolic content

The total phenolic content of each extract was measured by using the Folin-Ciocalteu assay¹⁴. Briefly, diluted samples were mixed with 1 mL 10% Folin-Ciocalteu reagent. After a 5-minute reaction, add 1.25 mL Na₂CO₃, 10%, and water to bring the total volume to 5 mL. The mixture was shaken and incubated in the dark at room temperature for 30 minutes. The absorbance was measured using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 737 nm. The total phenolic content was quantified using the standard curve of gallic acid and expressed as gallic acid equivalents (mg GAE/1 g sample extract).

DPPH radical scavenging assay

The antioxidant activity of samples was evaluated by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay¹⁵. A 1.5 mL aliquot of 0.1 mM DPPH[•] in 90% EtOH was added to 1.5 mL of each sample at concentrations of 200, 100, 50, and 20 µg/mL in 90% ethanol. After 30 minutes of incubation in the dark at room temperature, the absorbance was recorded at 517 nm by Shimadzu UV-1800 spectrophotometer. The percentage of inhibition was calculated using the formula:

$$I\% = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100\% \quad \dots(2)$$

Each experiment was conducted in triplicate. The IC₅₀ value (µg/mL) was determined as the test sample concentration needed to neutralize 50% of the DPPH free radicals. Trolox was used as a positive control with IC₅₀ = 10.07 µg/mL.

Physicochemical properties

Water solubility index, water absorption, and swelling power

These measurements were performed following the method described by Joymaket *et al.*¹⁰. Briefly, 50 mg of starch was dissolved in 1 mL of distilled water, heated at 90°C for 10 minutes, and cooled to room temperature before being centrifuged (1500 g) for 5 minutes. The supernatant was collected and dried at 105°C.

$$\text{Water solubility index (WSI) (\%)} = (\text{weight of dried supernatant}/\text{weight of initial starch}) \times 100 \quad \dots(3)$$

$$\text{Water absorption (WA) (g/g)} = \text{weight of supernatant after centrifuging}/\text{weight of initial starch} \quad \dots(4)$$

$$\text{Swelling power (SP) (g/g)} = \text{weight of dried supernatant}/(\text{weight of initial starch} - \text{weight of supernatant after centrifuging}) \quad \dots(5)$$

Light transmittance is determined based on the Wani *et al.* method¹⁶. A 1% (w/v) starch suspension was heated in a water bath at 90°C for 30 minutes and cooled to room temperature. Samples were stored at 4°C for 5 days, and transmittance was measured daily at 640 nm by Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

MPI values

To determine optimal procedure, the amylose AM_i, resistant starch RS_i, total phenolic content TPC_i, and antioxidant activity DPPH_i values were normalized by scaling them between their minimum values AM_{min},

RS_{min}, TPC_{min}, DPPH_{min}, and the maximum values AM_{max}, RS_{max}, TPC_{max}, DPPH_{max} to calculate the relative amylose \overline{AM}_i , and relative resistant starch content \overline{RS}_i , relative total phenolic content \overline{TPC}_i , and antioxidant activity \overline{DPPH}_i (Eq. 6–9). The mobile phase performance index (MPI_i) was calculated by combining the relative amylose, resistant starch, total phenolic content, and antioxidant activity into one value¹⁷. The contribution of each part to the sum was 0.25 (Eq 10). Experiments with higher MPI values are chosen in process optimization, as they demonstrate better amylose, resistant starch, polyphenol content, and antioxidant activity.

$$\overline{AM}_i = \frac{AM_i - AM_{\min}}{AM_{\max} - AM_{\min}} \quad \dots (6)$$

$$\overline{RS}_i = \frac{RS_i - RS_{\min}}{RS_{\max} - RS_{\min}} \quad \dots (7)$$

$$\overline{TPC}_i = \frac{TPC_i - TPC_{\min}}{TPC_{\max} - TPC_{\min}} \quad \dots (8)$$

$$\overline{DPPH}_i = 1 - \frac{DPPH_i - DPPH_{\min}}{DPPH_{\max} - DPPH_{\min}} \quad \dots (9)$$

$$MPI_i = 0.25 \times (\overline{AM}_i + \overline{RS}_i + \overline{TPC}_i + \overline{DPPH}_i) \quad \dots (10)$$

Statistical analysis

All the samples were analyzed in triplicate. The results were expressed as a mean ± SD (standard deviation). Data were analyzed using an independent sample t-test to compare the means of two groups with equal variance.

Results and Discussion

Starch modification using the autoclave

Effect of autoclaving time

Table 1 exhibited that extending the autoclaving time from 30 to 120 minutes led to increased content of amylose, resistant starch, and total phenolic compounds and decreased IC₅₀ values in the DPPH radical scavenging activity. Amylose content ranged from 0.09 to 0.17 mg/g, with no significant difference between samples autoclaved for 30, 60, and 90 minutes, (*p*-value > 0.05). Resistant starch and total phenolic content increased from 0.07 to 0.23% (*p*-value < 0.05), from 11.47 to 13.34 mg GAE/g (*p*-value < 0.05), respectively, while IC₅₀ values decreased from 530.08 to 273.72 µg/mL. Polyphenols

Table 1 — Effect of autoclaving time on starch modification of *C. papaya*

Autoclaving time (minutes)	Amylose content (mg/g)	Resistant starch content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
30	0.09±0.02	0.07±0.01	11.47±0.11	530.08
60	0.10±0.02	0.12±0.02	12.12±0.08	417.28
90	0.11±0.02	0.15±0.02	12.68±0.07	398.07
120	0.17±0.01	0.23±0.01	13.34±0.10	273.72

link with the amorphous hydrophobic regions of the single helices in amylose. An increase in the amylose content encourages the structured formation of polyphenol-amylose complex crystallites, which leads to an increase in the resistant starch content^{5,6}. Besides, short autoclaving duration influences the retrogradation of starch, which means that amylose does not have enough time to reorganize into crystalline formations, which decreases resistant starch content². Based on the MPI values in Fig. 1a, the MPI value for the 120-minute autoclaving time was higher than that of the others. Heating above the gelatinization temperature breaks hydrogen bonds in amylopectin, melts crystallites, and releases amylose, which then forms double helices and recrystallizes into RS₃. Additionally, longer autoclaving time generates more boiling water, which could enhance the extraction of bound phenolics from the food matrix. Therefore, the effective autoclaving time was 120 minutes.

Effect of heating-cooling cycles

The amylose, resistant starch, and total phenolic content showed a slight increase with the number of heating-cooling cycles, with values ranging from 0.18 to 0.32 mg/g (p -value < 0.05), from 0.21 to 0.31% (with no significant difference between 4 and 5 cycles, p -value > 0.05), from 13.38 to 20.77 mg GAE/g (p -value < 0.05), respectively (Table 2). However, IC₅₀ values obtained from the DPPH assay exhibited a minor fluctuation with the increase in these cycles (Table 2). Previous studies have indicated that the repeated heating-cooling cycles produce higher resistant starch content. Specifically, heating helps gelatinize the starch and release amylose molecules through the presence of water in the starch. Cooling promotes retrogradation, which means that the linear amylose molecules undergo rearrangement, forming hydrogen bonds and double-helix structures, which may inhibit digestive enzymes^{18,19}. According to MPI values, five heating-cooling cycles were selected for the optimal result (Fig. 1b).

Starch modification using the microwave

Effect of microwave power

When the microwave power was increased from 240 to 400 W, there was an increase in the amylose, resistant starch, and total phenolic content, with values rising from 0.11 to 0.16 mg/g (p -value < 0.05), from 0.09 to 0.20% (p -value < 0.05), from 14.03 to 15.83 mg GAE/g (p -value < 0.05). Furthermore, the IC₅₀ values for DPPH radical scavenging activity reduced, ranging from 221.85 to 192.89 µg/mL (Table 3). However, the above content declined with the rise in microwave power from 400 to 600 W (p -value < 0.05) (Table 3). Many studies have reported that microwave heating causes rapid vibrations of the starch molecules and breaks the glycosidic linkages in the amylopectin, increasing the amylose content and polyphenol-amylose complex crystallites^{19,20}. Nevertheless, if the microwave power is excessively increased, it may cause a rise in temperature and a reduction in moisture content, which complicates the gelatinization process⁹. Additionally, excessively high temperatures can lead to the degradation of phenolic compounds. Based on MPI values (Fig. c), the optimal microwave power for further research was 400 W.

Effect of microwave time

Table 4 shows that when the microwave treatment time was extended from 20 to 35 minutes, the content of amylose, resistant starch, and polyphenols rose, while the IC₅₀ value for DPPH radical scavenging activity declined. Specifically, the values changed as follows: from 0.17 to 0.53 mg/g for amylose content (p -value < 0.05), from 0.19 to 0.72% for resistant starch content (p -value < 0.05), from 16.21 to 22.26 mg GAE/g for phenolic content (p -value < 0.05), and the IC₅₀ value dropped from 190.17 to 173.26 µg/mL. However, with the extension of treatment time from 30 to 35 minutes, the content of amylose and resistant starch dropped from 0.61 to 0.53 mg/g (p -value > 0.05), from 0.86 to 0.72% (p -value < 0.05), respectively. This may be because the longer

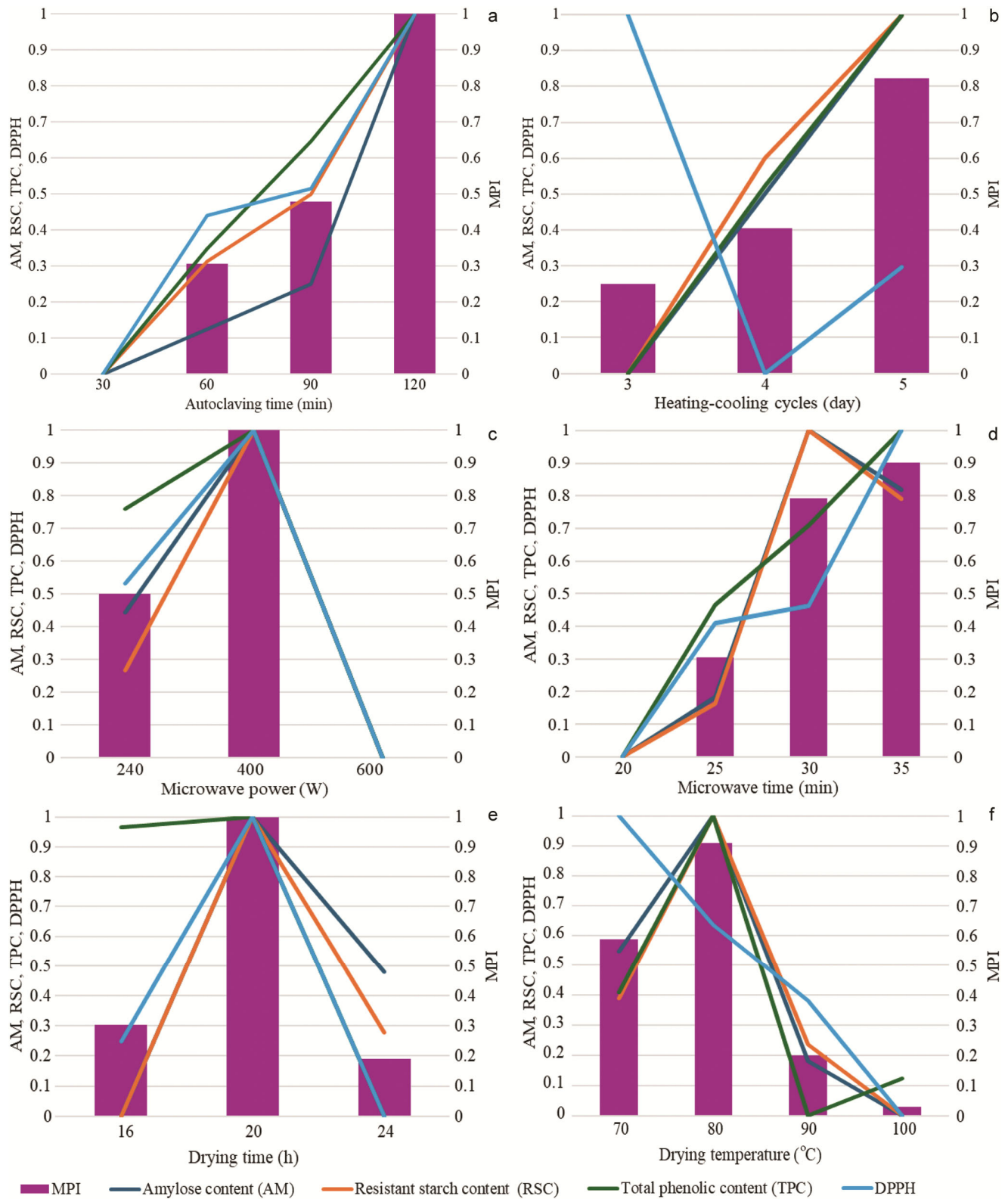


Fig. 1 — MPI plot of selected a) autoclaving time, b) heating-cooling cycles, c) microwave power, d) microwave time, e) drying time, and f) drying temperature.

Table 2 — Effect of heating-cooling cycles on starch modification of *C. papaya*

Heating-cooling cycles (day)	Amylose content (mg/g)	Resistant starch content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
3	0.18±0.03	0.21±0.02	13.38±0.11	295.59
4	0.25±0.04	0.27±0.03	17.25±0.15	301.22
5	0.32±0.03	0.31±0.02	20.77±0.13	299.55

Table 3 — Effect of microwave power on starch modification of *C. papaya*

Microwave power (W)	Amylose content (mg/g)	Resistant starch content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
240	0.11±0.03	0.09±0.01	14.03±0.08	221.85
400	0.16±0.02	0.20±0.02	15.83±0.14	192.89
600	0.07±0.01	0.05±0.01	8.37±0.05	254.87

Table 4 — Effect of microwave time on starch modification of *C. papaya*

Microwave time (minutes)	Amylose content (mg/g)	Resistant starch content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
20	0.17±0.01	0.19±0.02	16.21±0.10	190.17
25	0.25±0.06	0.30±0.04	19.02±0.14	183.24
30	0.61±0.08	0.86±0.04	20.51±0.12	182.35
35	0.53±0.09	0.72±0.05	22.26±0.06	173.26

Table 5 — Effect of drying time on starch modification of *C. papaya*

Drying time (hours)	Amylose content (mg/g)	Resistant starch content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
16	2.27±0.03	1.85±0.10	19.25±0.10	362.65
20	2.50±0.04	2.46±0.07	19.41±0.20	137.01
24	2.38±0.02	2.02±0.06	14.71±0.07	436.92

Table 6 — Effect of drying temperature on starch modification of *C. papaya*

Drying temperature (°C)	Amylose content (mg/g)	Resistant starch content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
70	2.47±0.03	2.14±0.06	21.45±0.09	70.04
80	2.52±0.02	2.58±0.07	25.84±0.08	193.44
90	2.43±0.03	2.03±0.05	18.41±0.14	279.40
100	2.41±0.04	1.86±0.06	19.32±0.06	408.80

treatment time disrupts the structure of amylose and amylopectin, which reduces their recrystallization ability and limits the formation of resistant starch^{9,19,20}. Based on MPI values (Fig. 1d), the MPI value at 35 minutes was the highest; however, its amylose and resistant starch content were not statistically significantly different from those at 30 minutes. To avoid the damaged starch and take into account the visual colour of the starch, 30 minutes was selected as optimal.

Starch modification using the drying oven

Effect of drying time

The impact of drying time on three content and IC₅₀ values mirrored the changes observed with extended microwave treatment. Specifically, increasing the drying time from 16 to 20 hours significantly improved the amylose by about 10%

(p -value < 0.05), resistant starch by 33% (p -value < 0.05), phenolic content by 0.8% (p -value > 0.05), and DPPH inhibition from 362.65 to 137.01 µg/mL (Table 5). However, when the drying time was further extended for more 4 hours, these values decreased (Table 5) (p -value > 0.05). Based on the MPI values (Fig. 1e), the optimal drying time was 20 hours.

Effect of drying temperature

When the temperature increased from 70 to 80°C, the content of resistant starch and polyphenols rose by 20% (p -value < 0.05) (Table 6). Nevertheless, when the temperature was further increased by an additional 20°C, these values decreased (p -value < 0.05) (Table 6). This can be explained by the rapid loss of moisture in the sample due to the high temperature, which complicates the processes of gelatinization and recrystallization. Furthermore,

excessive heating may lead to the degradation of compounds. According to MPI values (Fig. 1f), the optimal drying temperature was 80°C.

Types of starch

This study prepared modified starch using physical treatment methods such as drying, steaming, and microwaving. Table 7 demonstrates that the levels of amylose, resistant starch, polyphenols, and DPPH radical scavenging activity have significantly improved in the modified starch compared to the untreated starch. Among individual methods, drying at 80°C for 20 h yields the optimal values for amylose, resistant starch, phenolic content, and the IC₅₀ value, with the values of 2.52 mg/g, 2.58%, 25.84 mg GAE/g, and 193.44 µg/mL (Table 7). When combining steaming and drying or microwave methods, these values in modified starch show a more significant improvement than using a single method for starch modification (Table 7). This is because, during autoclaving, hydrated starch gelatinizes, releasing amylose chains as random coils, which then recrystallize into tightly packed double helices stabilized by hydrogen bonds during cooling²¹. Microwave treatment improves the crystalline structure, enhancing its resistance to hydrolysis by digestive enzymes while also breaking down the α-1,6-glucoside bonds of amylopectin, increasing amylose content and reducing enzyme attack sites²². As previously mentioned, a large quantity of immature papaya fruits is annually discarded to enable the plant to allocate more nutrients to the remaining fruits, which enhances their quality.

Though this practice benefits the yield of ripe fruits, it contributes to a significant waste of immature green papayas. The findings of this study could offer a solution to repurpose these discarded fruits, reducing annual waste.

Physical properties

Table 8 shows that starch modified using three methods with high amylose content (drying, autoclave-drying, autoclave-microwave) had higher water absorption capacity (WA) than starch prepared using microwave and autoclave methods. In starch with higher amylose content, there are more available sites for water molecules to link with starch, which increases the starch's water absorption capacity. Gelatinization can increase the amount of resistant starch and enhance the presence of hydrophilic hydroxyl groups. Under the influence of heat, a large amount of bound water is formed due to covalent interactions between hydroxyl groups and starch molecular chains²⁰. However, the water solubility index (WSI) of modified starch samples with high amylose content (drying, autoclave-drying, autoclave-microwave) was lower than that of starch prepared by the remaining methods (Table 8). The solubility of starch in water is influenced by the ratio of amylose and amylopectin. Generally, starch samples with higher amylose content have lower solubility because the linear amylose chains tend to form strong hydrogen bonds and retrograde, making them less soluble.

Conversely, higher amylopectin content typically leads to higher solubility due to its branched

Table 7 — Different types of *C. papaya* modified starch

Method	Amylose content (mg/g)	RS content (%)	Total phenolic content (mg GAE/g sample extract)	DPPH (IC ₅₀ , µg/mL)
Starch	0.13±0.01	0.15±0.02	12.60±0.08	534.18
Starch modification				
Autoclave	0.32±0.03	0.31±0.02	20.77±0.13	299.55
Microwave	0.61±0.08	0.86±0.04	20.51±0.12	182.35
Drying	2.52±0.02	2.58±0.07	25.84±0.08	193.44
Autoclave + drying	2.62±0.02	2.76±0.10	40.27±0.16	199.03
Autoclave + microwave	2.76±0.02	2.82±0.08	45.97±0.19	95.12

Table 8 — Water solubility index, water absorption, swelling power, and light transmittance of different types of starch

Physical properties	WSI (%)	WA (g/g)	SP (g/g)	Light transmittance (%)				
				1	2	3	4	5
Day								
Autoclave	40.25±0.51	6.70±0.38	27.60±0.29	47.97±0.11	47.86±0.25	47.10±0.18	46.56±0.29	43.35±0.27
Microwave	44.04±0.80	6.72±0.43	27.42±0.20	37.59±0.21	37.24±0.39	36.81±0.36	36.39±0.29	36.06±0.33
Drying	37.14±0.46	7.78±0.35	23.67±0.48	49.32±0.25	49.09±0.22	48.75±0.31	47.53±0.26	44.97±0.24
Autoclave + drying	31.16±0.63	9.15±0.55	26.14±0.53	41.69±0.33	40.93±0.28	40.37±0.39	40.09±0.28	39.72±0.23
Autoclave + microwave	35.78±0.62	9.83±0.22	20.27±0.42	42.07±0.35	40.93±0.26	40.09±0.42	39.72±0.22	39.08±0.27

structure⁸. The results on the swelling power (SP) of *C. papaya* starch showed high values, ranging from 17.42 to 26.14 g/g, with no significant difference between samples prepared by an autoclave and microwave (p -value > 0.05). The high SP can be attributed to their relatively high levels of carbohydrates and dietary fibre, as well as their size and chemical composition, all of which contribute to their capacity to absorb water molecules¹⁰. Besides, the decrease in light transmittance of starch samples after 5 days was observed in Table 8. This may be due to the stabilization of starch molecules interacting with other substances during fermentation, which affects light passage through samples⁸.

Conclusion

Five modified starch samples were successfully prepared using physical methods (drying, steaming, and microwaving). Their content of amylose, resistant starch, polyphenols, and antioxidant capacity was significantly enhanced compared to untreated starch. Among the evaluated methods, the combination of autoclaving and microwaving demonstrated the most effective results. The results of the physical properties of *C. papaya*, such as water solubility index, water absorption, swelling power, and light transmittance, provided a valuable database for Vietnamese food. The findings of this study present a potential solution to address the annual surplus of unripe papayas while also offering an opportunity to utilize this surplus as a nutritious food source for diabetic patients. This approach provides an affordable alternative to current dietary options, using readily available raw materials.

Conflict of interest

All authors declare no conflict of interest.

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