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Effects of Processing Conditions on Type 4 Resistant Starch Content from Jackfruit Seed Starch

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Abstract: Resistant starch type 4 (RS4) is produced from many types of starch by chemical methods. In this study, jackfruit seed starch was mixed with citric acid, lactic acid and acetic acid with an acid: starch ratio of 2:1, incubated for 20 hours and heated for 6 hours, the starch content was antagonistic. The sample using acetic acid had the highest RS of 84.83%, an increase of more than 21% compared to the initial amount of RS in the starch sample (63.47%), followed by the sample using citric acid with RS of 71.36. %. However, for samples treated with lactic acid, the amount of RS decreased to 33.44%. The results showed that the RS4 content increased or decreased depending on the nature of the acid. SEM of modified starch granules for citric acid has a rougher surface and larger average size of starch granules, while for the other two acids there is no obvious change compared to the starch granule configuration original powder.
Keywords: Jackfruit seed starch, starch, resistant starch, type 4 resistant starch, modified starch

I. Introduction

Jackfruit (Artocarpus heterophyllus) is a tropical fruit, commonly grown in Southeast Asian countries and Brazil. In Vietnam, jackfruit brings great economic value, mainly eaten fresh or processed into dried products. However, jackfruit seeds (accounting for about 10 to 15% of the total weight of the fruit) have no use and are considered a waste product, even though they are rich in slowly digested starch, protein, crude fiber, and phytonutrients. such as lignans and isoflavones have anti-cancer, anti-aging, diabetes and obesity treatment effects [1].In particular, resistant starch (RS - resistant starch) in jackfruit seeds has the same function as soluble fiber, they pass through the small intestine into the large intestine without being digested by human enzymes. Therefore, this starch fraction acts as a carbon substrate for beneficial bacteria to ferment prebiotics and produce short-chain fatty acids (SCFAs), mainly acetic acid, propionic acid and butyric acid. This process inhibits the growth of certain pathogenic bacterial strains and supports mucin production in the colon, helping to prevent gastrointestinal diseases including colon cancer [2].Because of the above benefits, the demand for RS consumption has increased, leading to many studies being carried out to increase RS concentrations in foods in general and jackfruit seeds in particular. A number of methods have been studied including physical effects (temperature treatment, hydrothermal treatment, pressure), chemical (acidification), biological effects (enzymatic treatment, genetics), ... [3], [4]. There are currently 5 different forms of RS that have been classified (RS1 - RS5) based on how they resist digestion. RS4 is a chemically modified starch produced by a number of different methods, including the decomposition of ester cross-links between starch molecules, the addition of chemical groups, or by hydrolysis and acid heat treatment [5].

The objective of this study will focus on investigating the conditions for obtaining digestive resistant starch type 4 (RS4) from Thai jackfruit seed starch - one of the most popular jackfruit varieties today in Vietnam. Thereby contributing to opening up new directions in utilizing jackfruit seed by-products of the food industry, choosing acids under appropriate survey conditions to create starch with the highest RS4 content.

II. Materials and Methods

2.1. Materials

Raw materials: Fresh jackfruit seeds of the Thai jackfruit (*Artocarpus heterophyllus*) when collected can be refrigerated to facilitate the research process because this material depends on the harvest season.

Chemicals: 99.7% alcohol, KOH, Resistant starch Assay Kit (Product code K-RSTAR of Megazyme); C_2H_5OH 99.99%; Potassium hydroxide 85% (Merck), citric acid, acetic acid, lactic acid and some other common chemicals used in the laboratory.

Instruments: UV-VIS Spectrophotometer (Jasco V-630, Japan); Scanning Electron Microscope (SEM) JEOL JSM-5410LV (JEOL, USA); Hettich EBA20 centrifuge, Germany; STUART CB162 Hotplate Stirrer, UK; Vortex Mixer GEMMY-VM-300, Taiwan; EMM20K22B microwave oven, China; Thermostatic Water Tank Bluepard DK-8AD, China; Autoclave HYSC AC-100, Korea.

2.2. Methods

2.2.1. Preparation of jackfruit seed starch

Jackfruit seed starch was obtained by chemical methods according to the research group of Wong et al. After removing the hard outer shell, jackfruit seeds were ground and diluted in distilled water (1:2), with continuous magnetic stirring (500 rpm) for 3 hours at room temperature. Then filter the residue using a sieve or filter cloth. The resulting milk suspension was centrifuged at 8000g for 5 minutes, and the supernatant was decanted. The lower slurry layer added 0.1M sodium hydroxide to dissolve the remaining proteins. The suspension was kept at room temperature for 18 hours with continuous stirring. Next, the suspension underwent centrifugation at 8000g for 10 minutes (25°C) and was subsequently washed with 0.1M sodium hydroxide solution twice.The supernatant was decanted, and the remaining brown layer was also scraped off. The sample was then rinsed with water and neutralized with 0.1M hydrochloric acid until a pH of approximately 6.5 to 7.0 was achieved. The powder sample was washed three more times with distilled water to remove excess salt and centrifuged again at 8000g for 10 minutes (25°C). The moisture content of the starch suspension was adjusted to 70% before it was dried in an oven at 40°C for 18 hours. The dry starch sample was ground using a flour mill. Starch recovery efficiency from jackfruit seeds is calculated by dividing the final starch mass by the initial mass of jackfruit seeds utilized in the extraction process[6].

2.2.2. Survey of conditions for obtaining type 4 resistant starch

According to the research of Hung et al [7], there are slight changes, the experiment is arranged according to the diagram in Figure 1.Prepare mixed acid: dissolve 21 grams of citric acid ($C_6H_8O_7.H_2O$)/9 grams of lactic acid ($C_3H_6O_3$)/6 grams of acetic acid ($C_2H_4O_2$) in 20 ml of water, then use 10 M NaOH to adjust the pH of solution to 3.5; Add water to a final volume of 50 ml. The resulting acid solutions have a concentration of 2M.Weigh 2 grams of jackfruit seed starch into a petri dish and add the prepared volumes of 2M acid corresponding to the acid: starch ratio (v:m) of 1:2; 1:1 and 2:1. After mixing, the plates were equilibrated at room temperature for 12, 14, and 20 hours then heated at 120°C for 3, 6, and 9 hours. After heat treatment, the starch samples were thoroughly washed with distilled water several times to neutral pH. When washing starch, add 20ml of distilled water to the plate and stir well, centrifuge the solution at 1500 rpm for 2 minutes and decant the sediment. Repeat the distilled water washing - centrifuging step about 3-4 times until the solution pH reaches neutral. The processed starch was recovered by centrifugation at 1500 rpm for 10 minutes and then dried at 45°C for 24 hours, ground finely and sieved through a 0.3 mm sieve (Figure 1).

* Experimental arrangement:

- Experiment 1: Investigating the acid: starch mixing ratio

+ Fixed parameters: incubation time 16 hours, heating at 120^{o}C for 6 hours, drying at 45^{o}C for 24 hours, grinding into fine powder.

+ Survey parameters: citric acid, acetic acid, lactic acid with acid: starch mixing ratio of 1:1 respectively; 1:2 and 2:1.

+ Monitoring criteria: RS4 content formed.

- Experiment 2: Investigation of incubation time

+ Fixed parameters: acid: starch mixing ratio of experiment 1, heating at 120^{o}C for 6 hours, drying at 45^{o}C for 24 hours, grinding into fine powder.

+ Survey parameters: incubation time 12 hours, 16 hours, 20 hours.

+ Monitoring criteria: RS4 content formed.

- Experiment 3: Investigation of heating time

+ Fixed parameters: acid: starch mixing ratio of experiment 1, incubation time of experiment 2, heating at 120^{o}C, drying at 45^{o}C for 24 hours, grinding into fine powder.

+ Survey parameters: heating time is 3 hours, 6 hours, 9 hours.

+ Monitoring criteria: RS4 content, SEM of RS4 for 3 acids used.

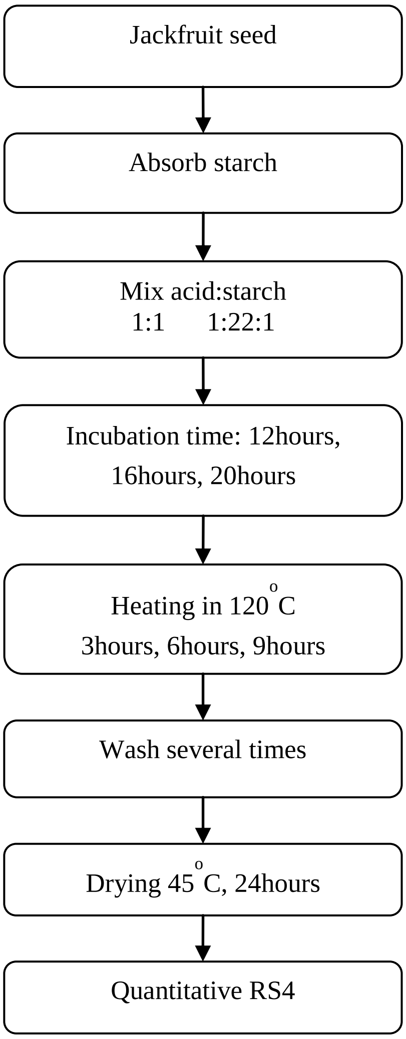

Figure 1. Method for obtaining type 4 resistant starch

2.2.3. Determining the resistant starch content

The resistant starch content is analyzed using AOAC 2002.02 method. It involves weighing 0.1 grams of starch sample into a test tube with 0.1M sodium acetate buffer (pH=4.5), adding porcine pancreatic α-amylase and amyloglucosidase from Aspergillus Niger, vortex mixing, and enzymatic hydrolysis in a shaking water bath at 37°C for 16 hours. The reaction is stopped by adding ethanol, and the resistant starch fraction is recovered via centrifugation. The combined liquid portions are quantified to determine the digested starch (DS). The residue is dried, dissolved in 2M KOH in an ice-water bath, adjusted to pH ~4.5 with acetate buffer, and the undigested starch is quantified, considered as the amount of RS hydrolyzed by amyloglucosidase in a shaking water bath at 37°C for 30 minutes. This is further diluted to 100ml, and glucose absorption is measured using a glucose oxidase-peroxidase (GOPOD) assay at 510nm wavelength against a standard sample to determine the RS starch content, calculated as glucose content × 0.9. The total starch content of the sample is determined by combining the contents of digestible starch (DS) and resistant starch (RS)[8].

2.2.4. Scanning electron microscopy (SEM) analysis.

Experimental SEM to analyze particle surface, shape and size using a JEOL JSM-5410LV instrument (JEOL, USA) equipped with a large field detector. The acceleration voltage is 15 kV in low vacuum mode (0.7-0.8 torr). The sample was affixed onto a copper stub using adhesive tape and then coated with gold in a vacuum environment. Images were taken at 2250x magnification [9].

2.2.5. Statistical Analysis

The statistical analysis was conducted under the following conditions: The experimental results were carried out with 5 replicates and statistically processed using Microsoft Excel 2016 and Statgraphic Centurion 19.1.2 software. The numerical data represent the mean values of 3 replicates ± standard deviation with a significance level of p ≤ 5%.

III. Results and Discussion

3.1. Investigation of acid: starch mixing ratio

From the statistical results, it shows that the RS values of the samples according to the 3 mixing ratios have a statistically significant difference (p<0.05) and are shown in Table 1 and Figure 2. The RS content increases when increasing the acid: starch ratio and reaching the highest value with a ratio of 2:1, from 7.3% (citric acid) to 20.3% (acetic acid) higher than the control starch sample (63.47%). Particularly, samples treated with lactic acid recorded a significant reduction in RS content, from about 36.1% to 44.7% lower.

Table 1. Effect of acid: starch mixing ratio on RS4 content

Type of acid	Mixing ratio of acid: starch			Control sample (starch)
	1:2	1:1	2:1	
Citric acid	$64{,}41^{A} \pm 0{,}087$	$67{,}04^{B} \pm 0{,}759$	$70{,}75^{C} \pm 0{,}312$	63,47±1,025
Lactic acid	$27{,}34^{x} \pm 0{,}496$	$23{,}83^{y} \pm 0{,}834$	$18{,}66^{z} \pm 0{,}826$	
Acetic acid	$67{,}41^{a} \pm 0{,}745$	$70{,}71^{b} \pm 0{,}675$	$83{,}72^{c} \pm 0{,}294$	

A, B, C; x, y, z; a, b, c: Different letters represent statistically significant differences (p < 0.05) for the same monitored parameter.

According to P. Raigond et al., during the incubation process, acid will hydrolyze the amorphous parts of starch granules and then hydrolyze the crystalline region. This will result in the production of shorter chains, which will then be reorganized through autoclaving and rearranged to form a more ordered double helix structure (forming bonds). cross) can prevent the hydrolysis of digestive enzymes such as α-amylase and amyloglucosidase (AMG), thus causing the amount of RS to increase [10]. Furthermore, during the heating process, the acids are dehydrated to form acid anhydrides, which will partially gelatinize the starch to form RS3, thereby increasing the total RS content. The remaining starch under the influence of heat and moisture has its structure stretched, swelled, increased the replacement of hydroxyl radicals of the starch chain by previously created acid anhydrides, the resulting starch configuration is more stable, thus enzymes are more difficult to hydrolyze.

Another explanation for the increased RS of starch could be a decrease in the swelling coefficient of starch. The swelling ability of starch when treated with acid-moist heat is reduced due to the presence of short-chain molecules produced by acid hydrolysis, which in turn make it difficult to access of digestive enzymes with starch granules and thereby increasing RS content [7]. According to research by Kim et al., when treated with heat and citric acid at a ratio of 30% (w/w), the swelling coefficient of rice starch decreased significantly from 16.29 to 4.07 corresponding to the rice starch content RS increased from 11.29% to 63.77% [11].

Among the three types of acids surveyed, sample RS4 treated with acetic acid had the highest efficiency in increasing RS. This resulting RS4 is a mixture of type 3 and 4 resistant starch. As evidence, according to research by Kapelko-Zeberska et al. conducted on a sample of potato starch (gelatinized and cooled) that was esterified. with acetic acid and adipic acid, arguing that they can be considered a preparation of resistant starch types 3 and 4, and that the level of RS formed is proportional to the degree of esterification (corresponding to the amount of reactive acid). response) [12].

Sample RS4 treated with citric acid also showed an increase in RS content when increasing the acid:starch ratio. This is similar to Li and Hu's study when using rice starch as raw material. The study showed that when rice starch was treated with citric acid at rates of 0%, 1%, 10% and 30% (w/w). Obtained RS contents of 11.3%, 17.4%, 35.4% and 63.8%, respectively. The largest sample had an RS4 value 5.6 times higher than the control rice starch sample [13].

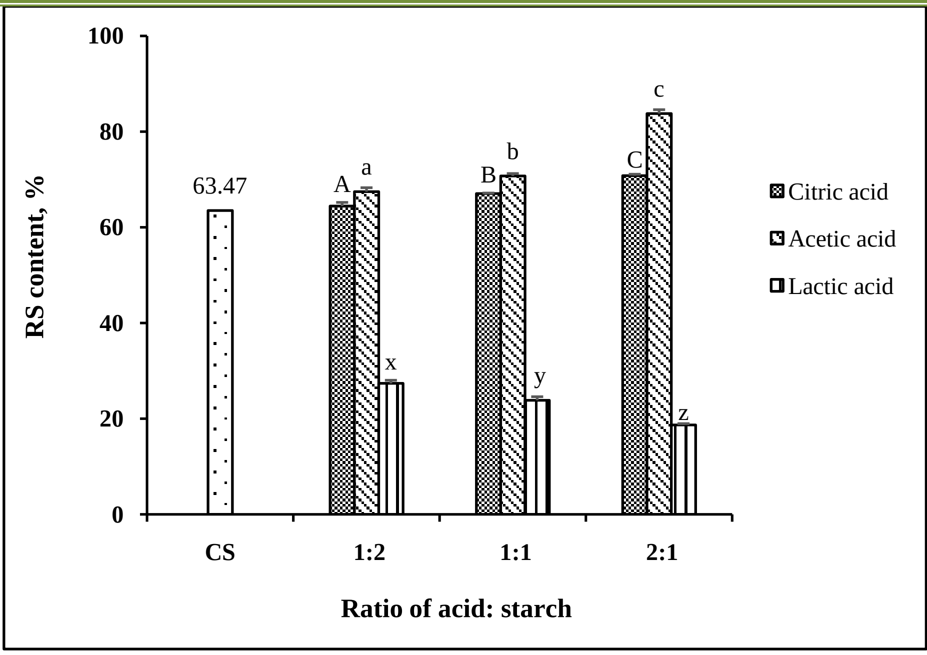

Figure 2. Graph showing the effect of acid: starch mixing ratio on RS4 content

A, B, C; x, y, z; a, b, c: Different letters represent statistically significant differences (p < 0.05) for the same monitored parameter.

However, this result is not similar to the study Pham Van Hung and colleagues conducted on raw materials such as sweet potato starch and yam starch, heat-denatured combined with 3 types of organic acids: citric acid, lactic acid. and acetic acid. Specifically, Pham Van Hung's research concluded that citric acid has the greatest impact on RS formation, followed by lactic acid and finally acetic acid, while this study with jackfruit seed starch showed that acid lactic has no effect on increasing RS, on the contrary, it tends to decrease sharply when increasing the amount of acid [7].

Another study by Aaliya Basheer and colleagues on the effects of organic acids on starch from palm tree trunks *(Corypha umbraculifera L.)* also showed different results. Specifically, starch from the Talipot palm stem is esterified with two organic acids, acetic acid and lactic acid. Lactic acid showed a higher impact on RS formation than acetic acid (conventional starch: 36.71%RS; acetic acid-treated starch: 41.22%; lactic acid-treated starch: 44.70%) [14].

The inconsistency between the results of the studies may be due to differences in the origin and characteristics of the starches and the conditions used to transform the starch. From the above results, it shows that the starch sample has the highest RS4 content when treated with acetic acid and citric acid respectively, while lactic acid is reduced compared to the control with the acid: starch mixing ratio times the turn is 2:1; 1:1 and 1:2. Therefore, this study chose an acid: starch mixing ratio of 2:1 to conduct the next experiment.

3.2. Investigation of incubation time

According to the results obtained in the above experiment, determine the acid incubation time at the intervals: 12 hours, 16 hours and 20 hours. Fixed parameters include: mixing ratio 2:1 (acid: starch), heating at 120^{o}C for 6 hours. The experiment was repeated 3 times, the results are presented in Table 2 and Figure 3. Effect of acid incubation time on the amount of RS4 formed.

Table 2. Effect of acid incubation time on the amount of RS4 formed

Type of acid	**Incubation time**			**Control sample (starch)**
	12 hours	**16 hours**	**20 hours**	
Citric acid	67,55^{a} ± 0,788	70,48^{b} ± 0,361	72,64^{c}±0,265	63,47±1,025
Lactic acid	33,46^{A}± 0,372	26,89^{B} ± 0,923	24,85^{C} ± 0,901	

Acetic acid	66,55^{x}±0,765	83,48^{y}± 0,741	85,38^{z} ± 0,528	

A, B, C; x, y, z; a, b, c: Different letters represent statistically significant differences (p < 0.05) for the same monitored parameter.

From the survey results, it shows that the RS4 values of the samples according to the 3 incubation time points have a statistically significant difference (p<0.05). It was found that for acetic acid and citric acid, the RS4 content increased with increasing incubation time and reached the highest value with incubation time of 20 hours (RS rate increased with incubation time), higher from 9% (citric acid) to 21.9% (acetic acid) compared to the control starch sample. Particularly for samples treated with lactic acid, a decrease in RS content was still recorded, from about 30.0% to 39.6% lower. Thus, incubation time has an effect on the increase in RS4 content (for acetic acid and citric acid) in the acid-heat method. The longer the acid incubation time, the more acid molecules are created to penetrate the starch granules and thereby create more substrates for subsequent reactions such as cleavage reactions, starch chain rearrangements, replacement reactions of hydroxyls by acid anhydrides, starch swelling, gelatinization reactions, etc. Take place during the heating stage, as stated above.

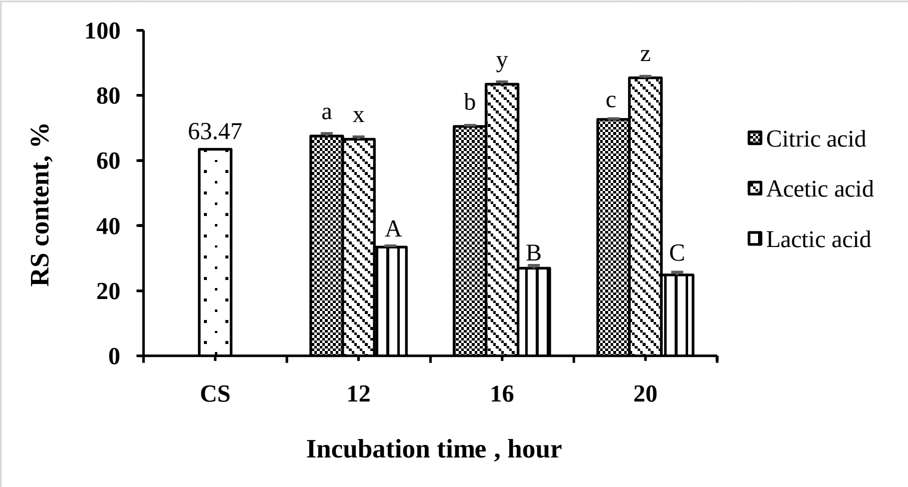

Figure 3. Graph showing the effect of acid incubation time on the amount of RS4 formed

A, B, C; x, y, z; a, b, c: Different letters represent statistically significant differences (p < 0.05) for the same monitored parameter.

The above statement is consistent when comparing two similar studies by Wepner et al and Xie & Liu. Specifically, the above two studies created RS4-resistant starch from corn starch by treating starch with 40% (w/w) citric acid at different reaction times and temperatures. Wepner reported the best reaction time was 5 hours at 140^{o}C, yielding 50.8% resistant starch, while Xie and Liu's study obtained up to 78.8% RS corresponding to 7 hours and temperature 140°C. The difference in RS4 content is attributed to the different acid incubation time at room temperature (16 hours in the study by Xie and Liu, compared to 12 hours in Wepner et al.)[15],[16].

From the above results, it was found that starch samples had the highest RS4 content when treated with acetic acid and citric acid, respectively, with incubation time of 20 hours. However, with lactic acid, RS decreased much compared to the original RS. Therefore, choose an incubation time of 20 hours to conduct the next experiment.

3.3. Investigation of heating time

Survey the RS content of samples treated with citric acid, lactic acid and acetic acid, heating times were 3, 6 and 9 hours respectively. Conditions such as acid: starch ratio were chosen in experiment 1, incubation time was chosen in experiment 2, heating at 120^{o}C. The experiment was repeated 3 times, the results are presented in Table 3 and Figure 4.

Table 3. Effect of heating time on the amount of RS4 formed

Type of acid	**Heating time**			**Control sample (starch)**
	3 hours	**6 hours**	**9 hours**	

Citric acid	63,963^{a}± 1,175	71,357^{b} ± 0,552	71,85^{b}± 0,342	63,47±1,025
Lactic acid	49,343^{x} ± 0,201	33,437^{y} ± 1,166	21,037^{z} ± 3,415	
Acetic acid	64,12^{A}±0,966	84,833^{B} ± 0,716	85,977^{B} ± 0,647	

A, B, C; x, y, z; a, b, c: Different letters represent statistically significant differences (p < 0.05) for the same monitored parameter.

From the survey results, it shows that the RS values of the samples heated for 6 hours and 9 hours do not have a statistically significant difference (acetic acid and citric acid have p>0.05). The sample with the highest RS is the sample using acetic acid with a heating time of 9 hours, reaching 85.98%, 22.52% higher than the control starch sample. Particularly for samples treated with lactic acid, a decrease in RS content was still recorded, from about 13.1% to 42.4% lower.

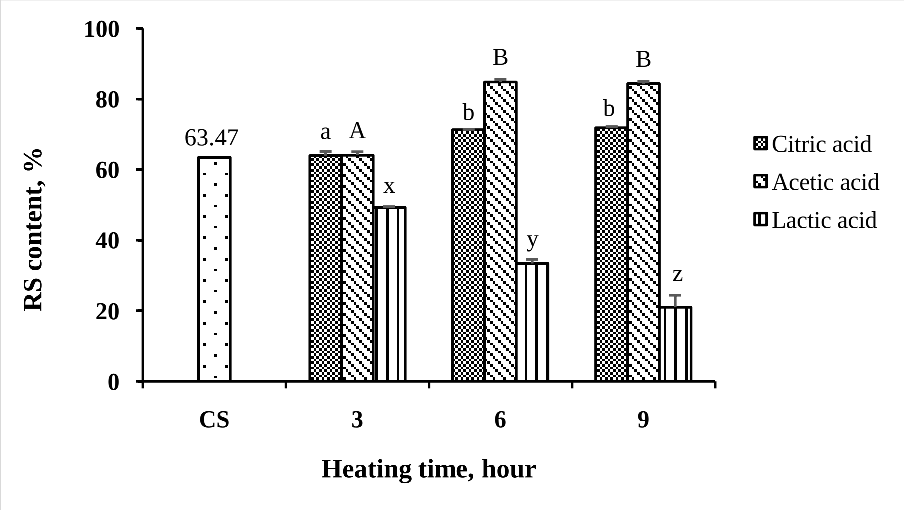

Figure 4. Graph showing the effect of heating time on the amount of RS4 formed

A, B; a, b ; x, y, z: different letters represent statistically significant differences (p<0.05) for the same monitoring parameter.

This result is also consistent with the conclusion of Xie & Liu when treating regular corn starch with 40% (w/w) citric acid at different temperatures (120°C-150°C) for different periods of time. Different reaction times (3 hours and 9 hours). The results showed that the resistant starch content gradually increased with temperature and reaction time, highest at 140°C for 7 hours and 78.8% RS4 was obtained. After the acid molecules penetrate into the starch granules at the tempering stage, the substrates are ready for further reactions that occur during heating. At high temperatures, the starch chains continue to swell, becoming more susceptible to acid attack by replacing the hydroxyl glucans of the starch chains with acid anhydride, then rearranging the starch chains with bonds. horizontal, helps resist digestion by amylase enzymes, thus increasing RS content [16]. However, in this study with jackfruit seed starch, it was suggested that when increasing the incubation time beyond 20 hours, the RS content may decrease due to the dissociation of substituents from the starch molecule. In general, the heat treatment stage will create favorable conditions for the formation of RS, but high temperature treatment/long reaction time can destroy the digestion-resistant structure and reduce the efficiency of RS formation [17].

From the results of the three experiments above, it shows that, with an acid: starch ratio of 2:1, an incubation time of 20 hours and a heating time of 6 hours, the RS4 content obtained from the initial jackfruit seed starch is the highest. when using acetic acid (84.84%), followed by citric acid (71.36%). However, lactic acid does not have the potential to create digestive resistant starch compared to jackfruit seed starch.

3.4. SEM measurement results

When observed from the SEM scanning electron microscope in Figure 5.a, it shows that the initial jackfruit seed starch configuration has a round, domed, polygonal shape with a smooth surface, consistent with the research of Mahanta and Kalita [18].

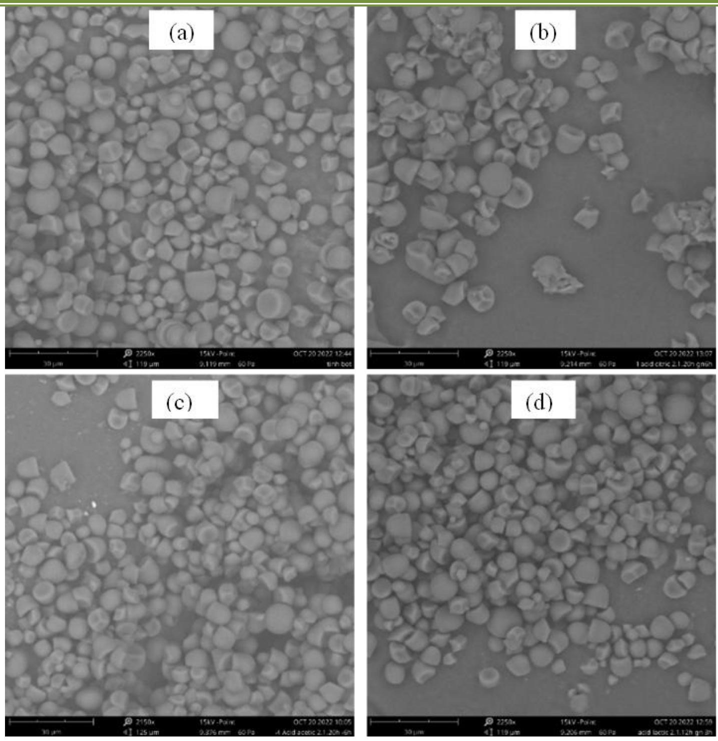

Figure 5. SEM measurement results of jackfruit seed starch (a), RS4 with citric acid (b), RS4 with acetic acid (c), RS4 with lactic acid (d)

Besides, the surface of RS4 starch using citric acid (Figure 5.b) has a rougher surface and a larger average size of starch granules. These morphological changes may indicate that citric acid has penetrated into the existing pores on the starch surface and reached the core (amorphous part) through channels connecting the pores to the core of the granule [11], from which the acid corrodes the amorphous part and increases the proportion of crystalline part, leading to an increase in RS content.

RS4 starch treated with acetic acid (Figure 5.c) generally has a smaller average size than the control sample (but not significantly) and no clear difference in grain surface is seen like the sample treated with citric acid. This may be because the attack and reactivity of acetic acid is lower than that of citric acid, and thus no difference can be seen at 2250x magnification. At the same time, the rearrangement of the structure in a more orderly and compact way can also partially reduce the size of starch granules.

SEM images of lactic acid-treated starch (Figure 5.d) showed no visible differences compared to the original starch sample. Our above survey results also show that lactic acid has no effect in increasing the RS content of jackfruit seed starch, thereby leading to no changes in the morphology of starch granules. However, it cannot be ruled out that there are very small changes that the current resolution is not enough to see.

IV. Conclusion

Type 4 resistant starch can be produced through a number of different methods and ingredients. The amount of RS4 formed depends on the nature of the starch, type of acid, and processing conditions. For jackfruit seed starch, when using acetic acid with a mixing ratio of 2:1 (acid: starch) and incubation for 20 hours, then heating at 120^{o}C for 6 hours, the RS4 content formed is the highest, reaching 84.83%. Under the same survey conditions as above, when using citric acid, the RS4 formed is lower than acetic acid, and for lactic acid, the RS4 is even lower than the initial RS of the sample. The results of this study are the basis for producing RS4 from jackfruit seed starch as well as the direction for continued research on many different RS-rich starch sources.

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