

## Effect of various heat processing methods on resistant starch level in jackfruit (*Artocarpus heterophyllus* Lam.) seed starch

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### Abstract

The content of resistant starch (RS) can increase or decrease depending on materials, processing methods, moisture levels of product, and processing times. In the present work, the RS content of jackfruit seed starch sample boiled for 25 min increased to 78.23% compared to the original sample of 63.48%, then steadily decreased over the boiling time. The RS content of the steamed sample decreased remarkably initially, then steadily increased, yet still lower than the control, reaching 49.98% at 35<sup>th</sup> min. The RS content of the deep-fried sample increased by  $4.92 \pm 10.89\%$ , varying by the period of frying. The RS content of the starch sample with high moisture and processed by steam sterilisation increased by 4.38%, whereas the ones with low moisture decreased by  $2 \pm 3\%$ ; while the baked sample considerably increased by  $0.8 \pm 11.43\%$ . The RS content of microwaved sample steadily decreased over cooking time but was negligible, reaching 60.83% at 40<sup>th</sup> s. These findings could serve as background data for further research and development of jackfruit seed starch-supplemented food and processing methods in order to obtain both sensory and health values.

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### Introduction

Resistant starch (RS) is a functional food ingredient that has the ability to resist enzymatic digestion in the small intestine, and intestine fermentation is carried out in the large intestine. Resistant starch has many benefits for human health by promoting blood sugar balance and beneficial gut bacteria (Ho and Wong, 2020). RS has been widely acknowledged for its beneficial effects, for instances, improving the insulin resistance and glucose balancing (Johnston *et al.*, 2010; Bodinham *et al.*, 2014), anti-colitis (Zhao *et al.*, 2011), weight control (Harazaki *et al.*, 2014), increase of colonic short-chain fatty acid (SCFA) content (Regina *et al.*, 2006), and especially, lowering blood lipids (Nichenametla *et al.*, 2014; Xu *et al.*, 2020). Natural sources of RS are green banana, jackfruit seed, green bean, rice, *etc.*; the jackfruit seed starch has received significant interest from researchers (Åkerberg *et al.*, 1998). Jackfruit seeds make up 10 - 15% weight of a

jackfruit, and released during consumption and production; the starch content in jackfruit seeds accounts for about  $79.34 \pm 0.06\%$  of the total dry matter content in seeds (Mahanta and Kalita, 2015). It is reported that RS content of jackfruit seed starch varies between 24 and 74%, depending on jackfruit varieties and planting zones (Chen *et al.*, 2016). The RS of jackfruit seed starch is type II RS due to its relatively high amylose content of 24 - 32% (Åkerberg *et al.*, 1998). Jackfruit seed starch has been widely used in a number of food products such as baked food (Gebre-Mariam *et al.*, 1996).

Nowadays, the percentage of diabetes patients is rapidly increasing worldwide. According to the International Diabetes Federation (IDF) in 2021, there were 537 million people suffering from diabetes, corresponding with the rate of one out of ten adults at the ages 20 - 79 suffering from diabetes; one out of six children born is affected by diabetes during foetal growth. There are also up to 50% adults suffering from diabetes without being diagnosed. In

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Vietnam, the investigation made by the Ministry of Health in 2021 found that the percentage of diabetes adult patients was estimated at 7.1%, where the diagnosed number made up only 35%, and the number being administered and treated at medical facilities made up 23.3%. According to forecast, the number of diabetes patients around the world including Vietnam will continue to rapidly increase in coming years.

Therefore, it is urgent and necessary to pay attention to research and development of starch-based food with high RS content. It can be seen from a number of studies that RS can reduce the released glucose amount, and avoid the state of hyperglycaemia after meals. This is one of outstanding advantages of RS used in diets for diabetes or obesity patients. At the same time, RS also increases the sensitivity of insulin, which is significantly important in diabetes patients, especially type 2 diabetes (Shi and Maningat, 2013). In addition to the role as a fibre supporting digestion, it also prevents constipation as when entering intestines, it stimulates bowel movement; RS also helps regulate intestinal bacteria and reduce the risk of colon cancer (Fuentes-Zaragoza *et al.*, 2011). The research question of the present work is that which methods and conditions of heat processing can increase RS content in food products.

The amount of RS in starchy foods during processing has been reported to be influenced by factors such as moisture content, temperature, and added supplementary agents (Vaidya and Sheth, 2011). Besides, RS content also depends on rate of amylose/amylopectin, interaction with starchy protein, lipid-amylose complexes, and starch retrogradation process (Mahadevamma and Tharanathan, 2004). According to Thuy and Van Tai (2022) regarding the research on changes in RS in processed macaroni made from wheat flour and various combined sources of RS through boiling, steaming, microwaving, stir-frying, and deep-frying, it was observed that the RS content decreased from 66.66% to 3.37%.

Another research on four different varieties of rice (jasmine, long-grain, medium grain, and short grain) cooked by three methods (baking, normal electric cooker, and pressure cooker) revealed that cooking methods had negligible effect on RS content in those rice varieties. Pressure cooking considerably decreased RS content in jasmine rice compared to electric cooker and baking. In the conditions of

cooking by pressure cooker and baking, the rice variety had significant effect on RS content in rice. It can be seen that cooking methods had remarkable effect on RS content, especially cooking by electric cooker which yielded the highest RS content compared to the three other methods (Chiu and Stewart, 2013).

The present work evaluated the effect of heating processing methods such as boiling, steaming, frying, steam sterilisation, baking, and microwaving on RS content in jackfruit seed starch. The obtained data would be beneficial for research and development of healthy food added with jackfruit seed starch.

## Materials and methods

### Samples

The fresh jackfruit seeds from the Thai jackfruit variety (*Artocarpus heterophyllus* Lam.) were preserved in a cold environment upon harvesting to facilitate the research process, as this raw material is seasonal and dependent on the harvest time.

### Chemicals

NaOH, > 97% (AR, Fisher, CAS 1310-73-2); HCl,  $\geq$  99% by HPLC (Merck); resistant starch assay kit (product code K-RSTAR of Megazyme); C<sub>2</sub>H<sub>5</sub>OH, 99.99%; KOH, 85% (Merck); and other common chemicals.

### Instruments

UV-VIS spectrophotometer (Jasco V-630, Japan); scanning electron microscope (SEM; JEOL JSM-5410LV, USA); Hettich EBA20 centrifuge (Germany); hotplate stirrer (STUART CB162, UK); vortex mixer (GEMMY-VM-300 Taiwan); microwave oven (EMM20K22B, China); thermostatic water tank (Bluepard DK-8AD, China); and autoclave (HYSC AC-100, Korea).

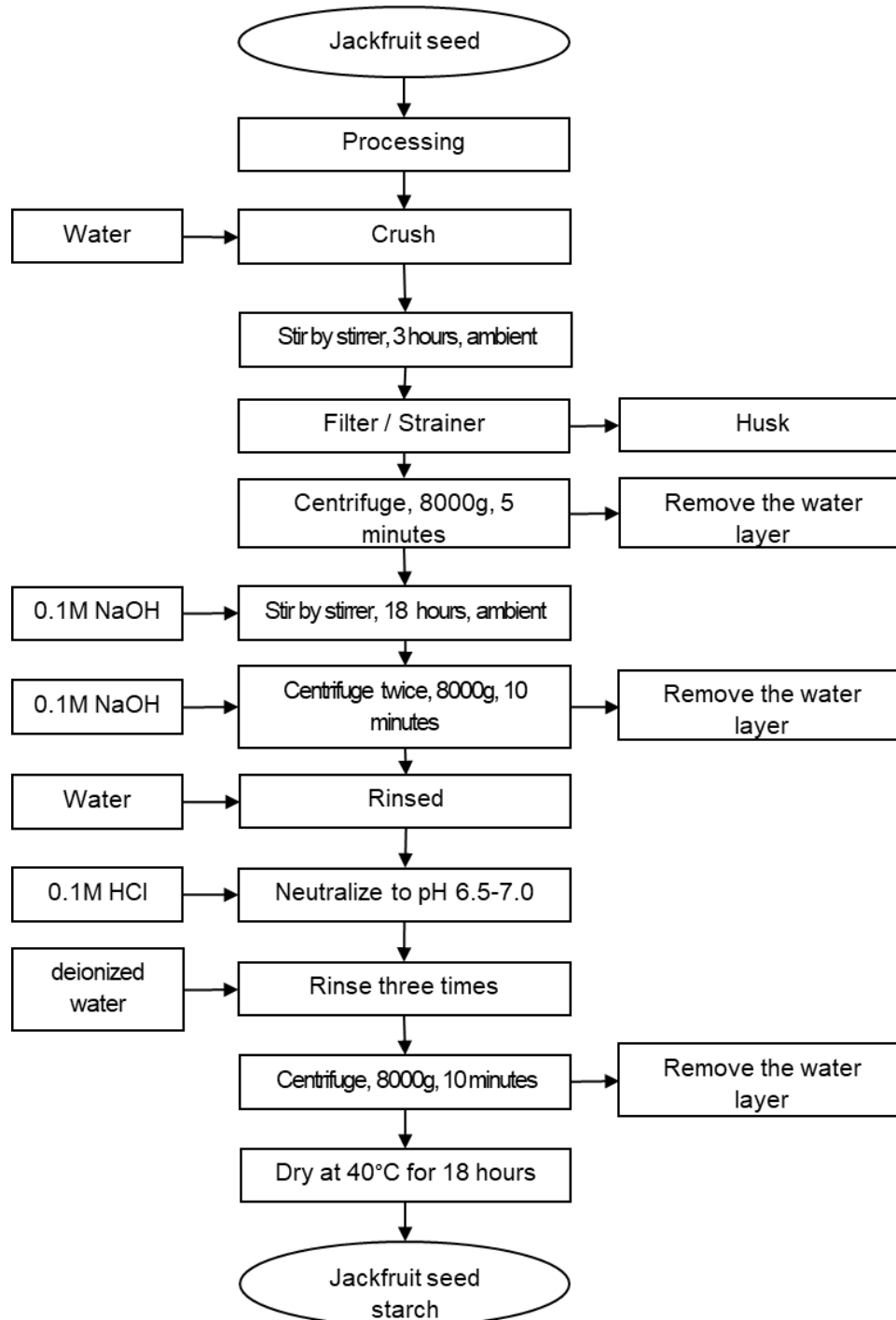
### Laboratory methods

#### Process of collecting jackfruit seed starch

Jackfruit seed starch was collected by chemistry method following previous study of Wong *et al.* (2021), as shown in Figure 1. After peeling the outer hard shell, jackfruit seeds were ground well and diluted with distilled water (1:2), and continually stirred (500 rpm) for 3 h at room temperature. Then, the residue was removed using a sieve or filter cloth.

The collected milk suspension was centrifuged at 8,000 g for 5 min, and the supernatant was skimmed. Sodium hydroxide 0.1 M was added to the bottom slurry to dissolve the remaining proteins. Suspension was kept at room temperature for 18 h with continual stirring. Then, it was centrifuged at 8,000 g in 10 min (25°C), and rinsed twice with sodium hydroxide 0.1 M. The supernatant was skimmed, and the remaining brown layer was also removed. After that, the sample was washed thoroughly with water, and neutralised with hydrochloric acid 0.1 M until the pH was

approximately 6.5 to 7.0. The starch sample was washed three more times with distilled water to remove any remaining salt, and re-centrifuged at 8,000 g for 10 min at room temperature. The moisture of starch suspension was adjusted to 70% before it was dried by drier at 40°C in 18 h. Dry starch sample was ground by powder grinder. Efficiency of starch recovery from jackfruit seeds was calculated by the last starch volume divided by the jackfruit seeds volume used for extraction (Wong *et al.*, 2021).



**Figure 1.** Process flow of jackfruit seed starch extraction.

### Thermal processing methods

The production of jackfruit seed starch samples involved mixing with water (to a moisture content between 50 - 53%) to form a cohesive mass, then

shaping small cakes of consistent weight and size. The implementation of thermal processing techniques (Table 1) was modified based on prior research studies.

**Table 1.** Heat processing processes.

Method	Condition			Reference
	Temperature	Time	Result	
Boiling	100°C	5, 10, 15, 20, 25, 30, and 35 min	Make cool to define RS.	Thuy and Van Tai (2022)
Steaming	100°C	5, 10, 15, 20, 25, 30, and 35 min	Make cool to define RS.	Thuy and Van Tai (2022)
Deep-frying	160°C	3, 5, 7, and 9 min	From yellowish to brown-yellow colour. Make cool to define RS.	Yadav (2011)
Steam sterilisation	121°C	10, 15, and 20 min	- Dry sample retained its original colour, white, nice. - Well done sample was wet, soft, and plastic.	Yadav (2011)
Baking	150°C	10-15-20, and 25 min	Well done sample was from light yellow to dark yellow colour. Make cool to define RS.	Yadav (2011)
Microwaving	105°C, 800 W	20, 30, and 40 s	The sample was consistently well done, plastic, and had typical smell of well-done cake.	Marconi <i>et al.</i> (2000); Liu <i>et al.</i> (2021)

### Determination of resistant starch content

The RS content was analysed by AOAC 2002.02 method. Briefly, 0.1 gram of starch was poured into the test tube having sodium acetate buffer solution 0.1 M (pH 4.5), added with porcine pancreatic  $\alpha$ -amylase and amyloglucosidase from *Aspergillus niger*, mixed thoroughly with vortex mixer, and hydrolysed in bain-marie tank shaking at 37°C in 16 h. The reaction was ceased by adding ethanol or alcohol, and the RS was recovered by centrifugation. The liquid portions were accumulated to scale digested starch (DS). The residue was drained in air, dissolved with KOH 2 M in ice water environment, and magnetically stirred, then added with acetate buffer solution pH 3.8 in order to adjust pH to about 4.5. The dispersed starch residual was the RS content hydrolysed using amyloglucosidase in bain-marie tank shaking at 37°C in 30 min, and then the solution was further diluted into 100 mL. Glucose absorption was determined by glucose oxidase-peroxidase (GOPOD) reagent at 510 nm, and compared with standard sample to determine the RS content. The calculated RS content was glucose content  $\times$  0.9. Total starch content of the sample was

obtained by the addition of DS and RS (McCleary *et al.*, 2002).

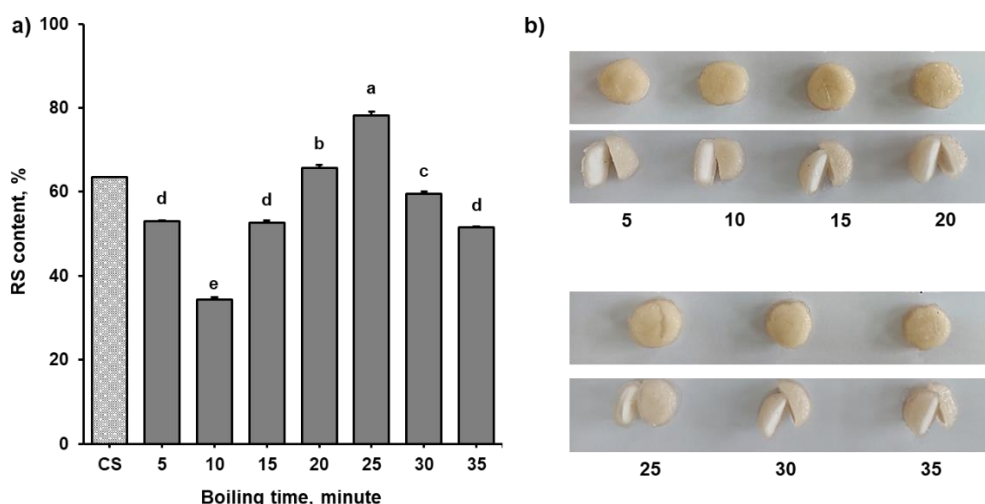
### Statistical analysis

The experiments were conducted with three replicates, and statistically processed using Microsoft Excel 2016 and Statgraphic Centurion 19.1.2 software. Results were expressed as mean  $\pm$  standard deviation of three replicates. Significance level was when  $p \leq 0.05$ .

## Results and discussion

### Boiling

Boiling can increase or decrease RS depending on the types of food. Herein, the starch samples were boiled in the same mode but analysed at different periods of time. The obtained results are shown in Figure 2. Over the first 5 min, the RS content decreased from 63.48 to 52.97%. In the next 5 min, it sharply decreased to 34.35%. Then, RS steadily increased over boiling period, and in the final 25 min, it reached the highest of 78.22%, an increase of 14.74% from the original RS. When boiling to 30<sup>th</sup>



**Figure 2.** Effects of boiling on RS in jackfruit seed starch: (a) RS contents in jackfruit starch samples over boiling times, and (b) images of jackfruit seed starch samples over boiling times (min). Different lowercase letters indicate significant differences within the same column at 95% confidence level.

and 35<sup>th</sup> min, RS steadily decreased to 51.50%. When jackfruit seed starch sample was boiled in a long period of time, it led to starch gelatinisation and degradation, then amylose chains would rearrange, and RS increased. If the boiling continues, the arrangement of amylose chains is inhibited and loosens, or the bond steadily weakens, so RS would decrease. According to Wang *et al.* (2010), while the RS volume of some boiled beans decreased, that of green bean increased. The decrease in RS content was caused by catabolism of amylose inhibitors generated during boiling; the increase in RS content was caused by the reverse degradation of starch generated after boiling and gelatinisation.

According to another research on RS in rice, especially rice having low and medium amylose content, RS decreased when boiled for there was no volume of linear ingredients higher than other starches (Sagum and Arcot, 2000). This finding was completely consistent with RS of jackfruit seed starch observed in the present work for its amylose content was up to 35.37%.

### Steaming

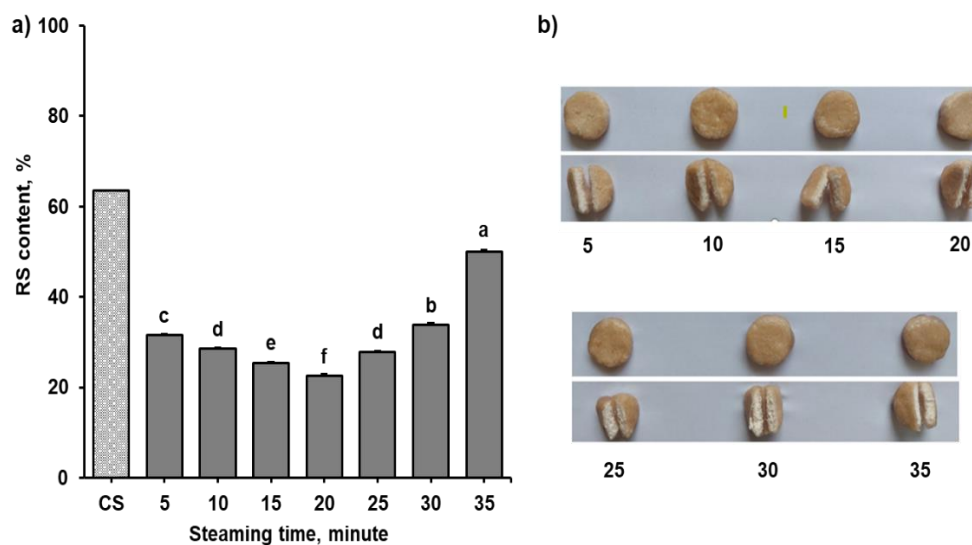
Steaming is a commonly used food processing method, and in several studies on RS content, it was observed that RS mostly increased after steaming (de Almeida Costa *et al.*, 2006; Dhital *et al.*, 2010). However, in the present work, the RS gradually decreased throughout the steaming period. Subsequently, with prolonged steaming, the RS

content increased, reaching 49.98% after 35 min, although still lower than the initial RS content, as depicted in Figure 3. According to Ek *et al.* (2012) and Kolarič *et al.* (2020), when starchy products are heated through steaming or boiling, starch granules absorb water, swell, and gelatinise, while amylose is degraded and leached out of the solution. Higher levels of gelatinisation require longer durations; this may explain the decrease in RS during steaming (Thuy and Van Tai, 2022).

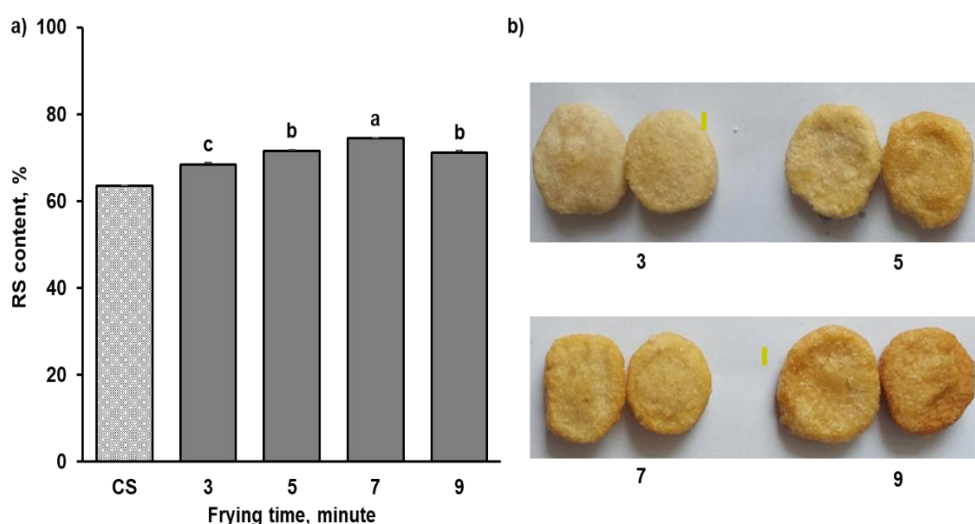
However, according to another study by Dhital *et al.* (2010) on wheat, steaming increased both moisture content and RS. Another study on three types of beans: black, red, and lima beans, which initially had RS levels ranging from 1.7 to 2.2%, showed a significant increase in RS after steaming at normal pressure for 90 min; black beans increased from 2.2 to 18.9%, red beans from 1.7 to 21.2%, and lima beans from 2.0 to 30.7%. This indicated that steaming is considered a preparatory step contributing to the formation and enhancement of RS compared to the initial levels. Tovar and Melito (1996) also indicated that raw beans contained significantly less RS, approximately 9 to 15 times lower, compared to starch processed from previously heat-treated seeds.

### Frying

Frying often increases RS content of foods (Goñi *et al.*, 1997; Pinthus *et al.*, 1998). In the present work, as shown in Figure 4, RS content in almost all



**Figure 3.** Effect of steaming on RS in jackfruit seed starch: (a) RS contents in jackfruit seed starch samples over steaming times, and (b) images of jackfruit seed starch samples over steaming times (min). Different lowercase letters indicate significant differences within the same column at 95% confidence level.



**Figure 4.** Effect of frying on RS in jackfruit seed starch: (a) RS contents in jackfruit seed starch samples over frying times, and (b) images of jackfruit seed starch samples over frying times (min). Different lowercase letters indicate significant differences within the same column at 95% confidence level.

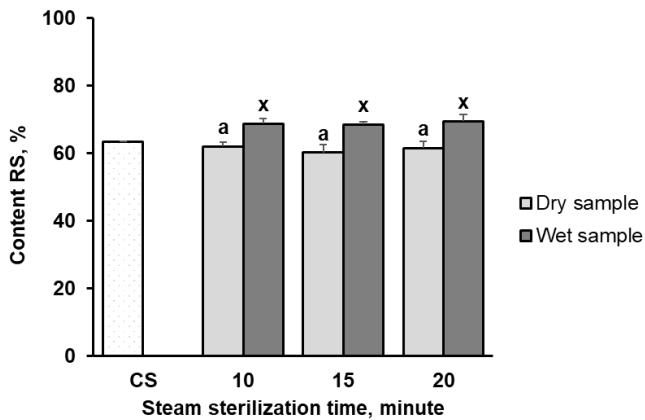
samples increased, from 63.48% up to 74.37%, and from 7.19 - 14.46% after frying. Goñi *et al.* (1997) opined that after frying, there was a linear increase in oil amount, and decrease in moisture in the sample; the lower the moisture was, the more RS content was, and in conclusion, RS was formed from heat degradation of starch resulting in the variation of structure when the water content was low.

In Dhital *et al.* (2010) study on wheat flour samples, RS increased from 7 to 12%. But according to research by Pinthus *et al.* (1998) done on maize, potato, and rice samples, all showed that RS only

slightly increased from 4.3 to 5.4%. This was explained by Yadav (2011) that there was more fat to form lipid-amylose complexes during deep-frying; when starch sample had high moisture, the amylose chain crystallisation process was not inhibited, and more persistent than the one with low moisture content, thus resulting in increase in RS function. However, prolonged frying can lead to visually unappealing samples, and the degradation of lipid-amylose chain bonds, causing a tendency for RS to decrease.

### Steam sterilisation

The results of the steam sterilisation process on two samples with different moisture contents are presented in Figure 5.



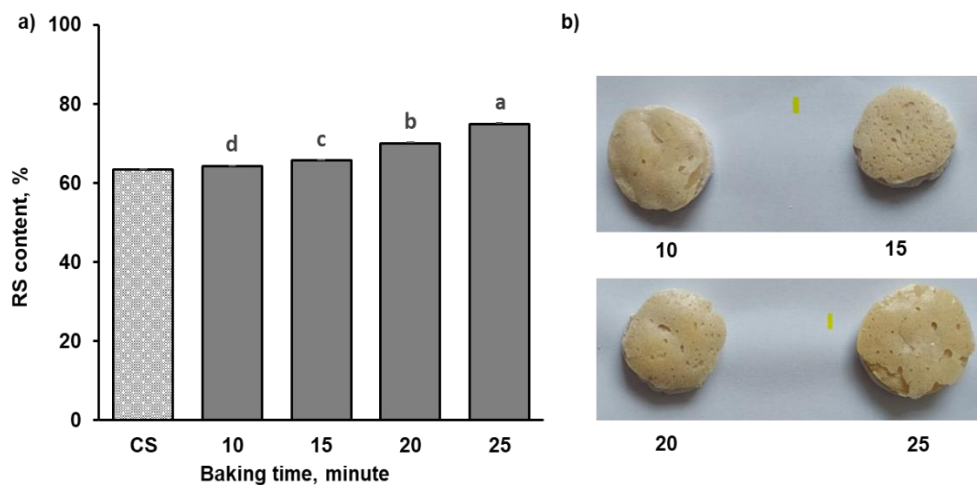
**Figure 5.** Effect of steam sterilisation on RS in jackfruit seed starch from two samples with different moisture contents. Different lowercase letters indicate significant differences within the same column at 95% confidence level.

For dry starch sample (11% moisture), the RS content decreased by  $2 \pm 3\%$ , whereas for wet starch sample added with water (50 - 53% moisture) to knead into dough, RS considerably increased; the most increase was for steaming for 20 min (69.50%). This aligned perfectly with the explanation of Ashwar *et al.* (2016), where there were two steps in RS production process by method of steam sterilisation - reverse degradation. For wet sample, starch was hydrated. When steamed, the gelatinisation took

place, and the amylose chains convoluted randomly. During heat lowering to ambient temperature, linear plastic amylose chains crystallise, and form a tight mass; the twists are stabilised thanks to hydrogen bond, and able to resist hydrolysis of amylase enzyme, which leads to RS content increasing. Therefore, for the dry sample, due to its low moisture content, gelatinisation could not occur, and under the influence of high temperature, some starch was degraded, leading to a decrease in RS content.

### Baking

When cake sample was baked, its RS content increased; at initial 10 min of baking, RS content increased by about 1.2%, to 25<sup>th</sup> min of baking, RS increased to 15.3% as shown in Figure 6. This was explained by Yadav (2011) for high amylose content in jackfruit seed starch, with high moisture of the baked cake sample, plus by the temperature condition/prolonged baking time which caused spreading and crystallisation of starch chains, resulting in production of much resistant starch from the starch. Yadav (2011) also opined that the traditional baking method destroyed  $\alpha$ -D-1-6 glucosidic bond of amylopectin which led to short chains formation. In a study by Kale *et al.* (2002), extending the baking time of whole wheat bread from 15 to 35 min increased the RS content from 17.98 to 45.6%. Åkerberg *et al.* (1998) baked mixtures of barley and wheat flour with varying amylose levels at 200°C for 45 min, resulting in baked products with high RS content, with 44% being amylose.



**Figure 6.** Effect of baking on RS in jackfruit seed starch: (a) RS contents in jackfruit seed starch samples over baking times, and (b) images of jackfruit seed starch samples over baking times (min). Different lowercase letters indicate significant differences within the same column at 95% confidence level.

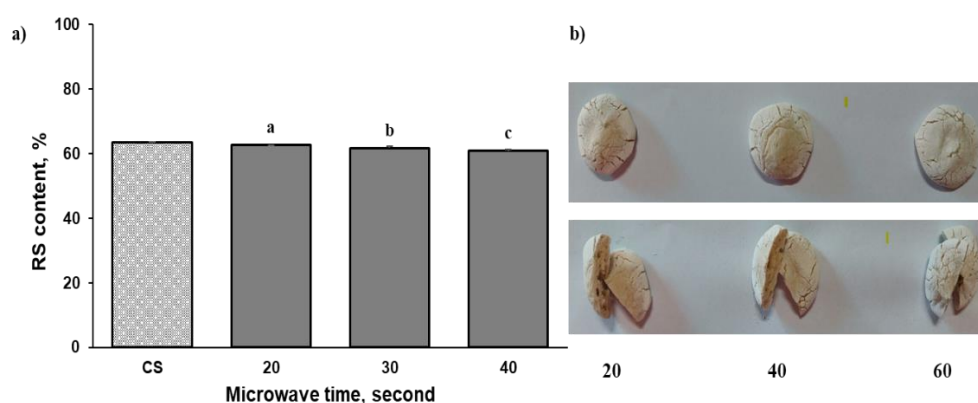


### Microwaving

Microwaving increases the digestibility of starch from roots and some kinds of bean, which means that RS would decrease; however, it depends on processing conditions, change in starch structure, and can lead to increase of RS2 content (Marconi *et al.*, 2000).

The results presented in Figure 7 indicate that there is not substantial variation in RS content with short microwave cooking times, corresponding to RS content values of 62.54, 61.48, and 60.83%. This aligned with Yang *et al.* (2016) study on RS in

microwaved potatoes. According to the research by Hódsági *et al.* (2012) on effect of microwaving on maize and wheat starch, commercial resistant starches of Hi-maize™ 260 and Fibersym™ RW, the samples processed by microwave for 30 and 150 s had negligibly varied results in the digestibility of starch RS concerning microwave energy. Therefore, for the microwaved jackfruit seed starch sample, RS content steadily decreased over cooking time. However, this depends on many other factors such as moisture of starch, additives, *etc.*, and this warrants further research for clarification.



**Figure 7.** Effect of microwave cooking on RS in jackfruit seed starch: (a) RS contents in jackfruit seed starch samples over microwave cooking times, and (b) images of jackfruit seed starch samples over microwave cooking times (s). Different lowercase letters indicate significant differences within the same column at 95% confidence level.

### Conclusion

The effect of common processing methods including boiling, steaming, frying, steam sterilisation, baking, and microwaving on RS contents in jackfruit seed starch samples was assessed according to processing time. In which, boiling, frying and baking - the common heating methods that would have effect in increasing sensory value of products - were the best options in order to significantly increase RS content in jackfruit seed starch *via* processing process. The RS content reached 78% after boiling for 25 min, 75% after baking for 25 min, and 74% after frying for 7 min. These findings could serve as background data for further research and development of jackfruit seed starch-supplemented food and processing methods in order to obtain both sensory and health values. In addition, the effect of heating methods on RS content

in jackfruit seed starch in the presence of other ingredients in food recipes also needs further research.

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