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Short Communication

The Digestibility and Bacterial Growth Rates of Microwave Treated Sago (*Metroxylon sagu*) Starch

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ABSTRACT

Sago starch is rich in resistant starch (RS) but less utilised than other commercial starches. Hence, modification is essential to give an add-on value to the starch. Thus, the objective was to determine the influence of microwave heat treatment (MHT) on the digestibility and probiotic growth rates of sago starch. In this study, the starch was treated by MHT for durations of up to 20 min. The digestibility and bacterial growth rates increase as the treatment duration increases to 15 min. It implies the potential of the MHT in increasing the digestibility of the sago starch and improving its prebiotic property based on probiotic growth rates.

Keywords: Functional food, glycaemic, prebiotic, resistant starch, starch modification

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INTRODUCTION

Sago starch is acquired from the wet milling of the *Metroxylon sagu* plant, a native of Southeast Asia (Zhu, 2019). Nowadays, this plant has been cultivated in various countries, including Malaysia (Achudan et al., 2020). A single pith of the sago palm can yield up to 82% of sago starch (Zhu, 2019). Nevertheless, this starch is underutilised

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compared to potato and maise starches, which led to its modifications (Singh & Nath, 2012; Sondari, 2018). The modification is essential to improve the starch's physicochemical and functional food properties, adding value to the starch and increasing its utilisation.

Physical treatment is known for its simple and cost-effective elements among modification techniques. Among them, microwave heat treatment has been used widely for its heating efficiency and time-saving features. Microwave starch treatment has been performed on Bambara groundnut, corn, and potato starches (Oyeyinka et al., 2019; Wang et al., 2019). The microwave irradiation caused changes in the granules' surface of starch (Oyeyinka et al., 2019). Changes on the granule surface will impact starch's physicochemical, functionality, and digestibility due to the formation of pinholes, fractures, pores, or a destroyed granule structure.

Regarding digestibility, starch is categorised into rapidly digestible, slowly digestible, and resistant starch. Sago starch contains a high resistant starch content of up to 62% (Arshad et al., 2018). The slowly digestible and resistant starches are favoured in food formulations, contributing to their low glycaemic properties, which are valuable for diabetic patients (Chung et al., 2008). Moreover, resistant starch is linked to the prebiotic properties of starch. A prebiotic is a substance that can promote the growth of good gut bacteria and suppress pathogenic bacteria (Zaman & Sarbini, 2015). Prebiotics is digested by good gut bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. producing by-products, short-chain fatty acids useful to the host's health. The resistant starch has mutual characteristics with the prebiotic, which can withstand digestion by the human digestive system and cannot be absorbed by the small intestine (Zaman & Sarbini, 2015). This statement was supported by an *in vivo* study in which resistant starch from maize flour was used; it displayed an increase in *Lactobacillus* bacteria while decreasing *Escherichia coli* (Khan et al., 2022). In another study, the resistant starch obtained from potato starch exhibited an increase in *Lactiplantibacillus plantarum* subsp. *plantarum* and butyric acid content (Wang et al., 2022). Meanwhile, previous studies on sago starch treated by enzymatic debranching followed by autoclaving, cooling and annealing have shown the highest *Lactobacillus* sp. count compared to control, fructo-oligosaccharide (Loo et al., 2010).

However, heating starch as a modification treatment reduces the resistant starch content (Chen et al., 2017). Lowering the resistant starch content may also lower the prebiotic properties of sago starch. Therefore, this study evaluated the effect of microwave heat treatment on digestibility and bacterial growth rates of sago starch.

MATERIALS AND METHODS

Materials

Food-grade native sago starch in the current study was obtained from CRAUN Research Sdn. Bhd., Kuching, Malaysia. The resistant starch assay kit was acquired from Megazyme,

Ireland, which is AOAC Method 2002.02 and AACC Method 32-40.01 complied. Chemicals used in the current study were purchased from a local supplier and were in analytical reagent grade. *Lactobacillus casei*, *Bifidobacterium lactis*, and *Escherichia coli* were acquired from the biology laboratory, Centre for Pre-University Studies, Universiti Malaysia Sarawak.

Modification of Sago Starch

The modification of native sago starch started with adjusting its moisture content by adding distilled water to 30%. The starch was sealed and maintained at 4 °C overnight. It was followed by the microwave heat treatment with a 20% power setting for 5, 10, 15, or 20 minutes using a microwave oven (EMO-2505, 900 W, ELBA). The treated starch was then oven-dried (Heraeus T12 oven, Thermo Scientific) at 50 °C overnight. The modified starches were labelled M5 (Microwave heat treatment for 5 minutes), M10 (Microwave heat treatment for 10 minutes), M15 (Microwave heat treatment for 15 minutes), and M20 (Microwave heat treatment for 20 minutes) and kept in a sealed airtight bag at ambient temperature away from direct sunlight and heat until further use (Zailani et al., 2021).

Resistant Starch Content

Determining resistant starch content in uncooked and cooked modified starches was performed using Megazyme Resistant Starch Assay Kit (K-RSTAR). The starch sample was used as it is for the uncooked sample. Meanwhile, for the cooked sample, the modified starch was cooked by boiling and cooled before digestion. The samples were digested using a mixture of amylase and amyloglucosidase in a shaking incubator (16 h, 37 °C, 200 strokes/min). Ethanol solution was added to wash and remove non-resistant starch, followed by centrifugation. The pellet obtained was mixed with potassium hydroxide in an ice bath and stirred continuously. Sodium acetate buffer (pH 3.8, 1.2 M) was added to the mixture, followed by amyloglucosidase solution (3,300 U/mL), with heating at 50 °C for 30 min. Glucose oxidase/peroxidase reagent was mixed with the sample mixture, and their absorbance was recorded at 510 nm using a UV-Vis spectrometer.

Bacterial Growth Rates

Meanwhile, Okolie et al. (2019) used a method to determine the bacterial growth rates of *Lactobacillus casei*, *Bifidobacterium lactis*, and *Escherichia coli*. The bacteria were cultured in a De Man, Rogosa and Sharpe (MRS) broth for 24–48 h in an incubator at 37 °C. The culture was streaked onto a sterilised MRS agar and incubated overnight. A single bacteria colony was transferred into MRS broth and incubated for 24–48 h. Prior

to use, the optical density of the bacteria was adjusted to 1.0 at a wavelength of 620 nm using a UV-Vis spectrometer. This study was performed on resistant starch fractions of uncooked and cooked samples. The sample preparation followed the digestion process as the determination of resistant starch. The sample was mixed into a sterilised MRS broth to a concentration of 0.1 and 0.5% w/v. Positive and negative controls were inulin and glucose, respectively, and prepared at 0.1 and 0.5% w/v of concentration. Aliquots of 200 µL of samples and controls were placed into a 96-well plate separately. 7.5 µL of bacterium culture was added and sealed with parafilm in each well. Incubation of the plate was conducted at 37 °C for 24 h. The optical density at 620 nm was measured every 30 min using a microplate reader. Graphs were plotted to determine the bacterial growth rates as described by Okolie et al. (2019).

Statistical Analysis

One-way ANOVA was used to analyse the data, which was then analysed by Tukey's test (Zailani et al., 2021). Meanwhile, a correlation study was performed using Pearson's Correlation. The significant level was set at 0.05. Statistical Package for Social Sciences (IBM® SPSS® Statistics Version 20) was used for the statistical analyses.

RESULTS AND DISCUSSION

The starch treated by microwave heating with different treatment duration displayed a decrease in the resistant starch content (Figure 1). A strong negative correlation ($r = -0.748$, $p = 0.033$) between the resistant starch content and treatment duration was observed for the uncooked samples. The decline in the resistant starch content may be caused by the formation of pinholes, cracks, and rough granule surfaces (Oyeyinka et al., 2019). These disruptions in the starch granule structure permitted the digestion enzymes to access the internal structure of the granules resulting in the digestion of the starch (Li et al., 2020). Among the modified starches, M5 had the highest resistant starch content. The short treatment duration might have had a low impact on the granule structure and retaining parts resistant to digestion. Meanwhile, the resistant starch contents were drastically lower for the cooked samples compared to the uncooked counterparts. No significant difference was observed between the

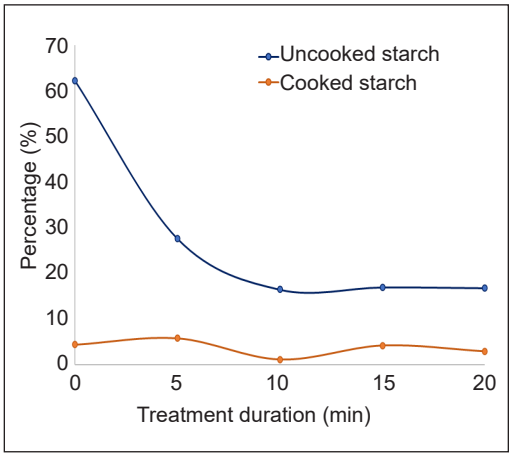
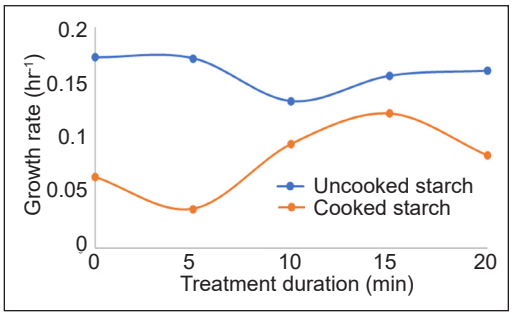


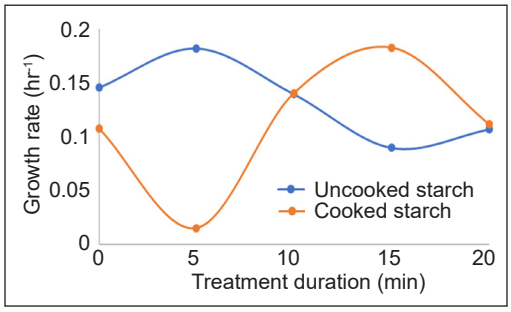
Figure 1. The resistant starch content of sago starches against microwave treatment duration

cooked sample content of resistant starch. Cooking the starch destroyed the granule's structure by undergoing gelatinisation. As the sago starch has a gelatinisation temperature of around 79 °C, this process was possible during the preparation of cooked starch samples (Ying et al., 2020). The gelatinisation process caused the leaching of amylose and low molecular weight amylopectin chains (Li et al., 2020; Fan et al., 2019). It causes the components to be susceptible to enzymatic digestion, lowering the resistant starch content.

Meanwhile, the bacterial growth rates of starches at different treatment duration were lower than the positive standard used, inulin. However, the resistant starch samples showed an interesting pattern of growth rates as the treatment duration increased. The resistant starch of cooked samples with a treatment duration of 15 minutes showed the highest bacterial growth rates among the cooked samples. The growth rates of uncooked resistant starch samples showed a decreasing pattern against treatment duration, as seen in correlation for *B. lactis* (r = -0.841, p = 0.001) and *E. coli* (r = -0.814, p = 0.001) (Figure 2). It is possibly linked to the formation of double helices between amylose molecules, which were produced by the breakage of α-(1,6)- and α-(1,4)-glycosidic bonding and with amylopectin molecules (Yang et al., 2017). These double helices form during the cooling and storage of the sample, where the retrogradation process occurs. The double helix structure may limit the bacteria's fermentation of the starch components. Meanwhile, moderate positive correlations between cooked resistant starch samples and treatment durations were observed for *L. casei* (r = 0.600, p = 0.039) and *B. lactis* (r = 0.583, p = 0.047) growth rates. The cooked RS of M10 (*L. casei*) and M15 (*L. casei* and *B. lactis*) exhibited higher growth rates than the native starch. Probably



(a)



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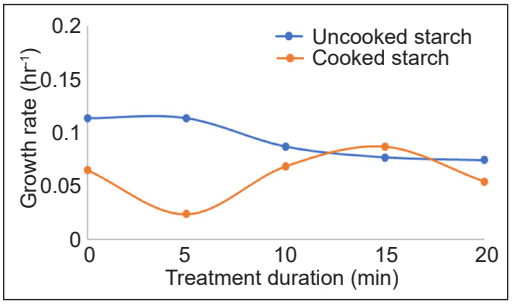

(c)

Figure 2. (a) *Lactobacillus* casei, (b) *Bifidobacterium lactis* and (c) *Escherichia coli* growth rates for uncooked and cooked resistant starch samples of microwave-treated sago starch

cooking the sample reduces the mixture's complexity and enables bacteria's fermentation of the components. Meanwhile, M5 cooked resistant starch had its bacterial growth rates decreased, which may be associated with the short treatment duration, which reduces the chances of reducing the entanglement of its complex mixture. Slight changes were seen in uncooked resistant starch of M10 (*L. casei*), M15 (*E. coli*), and M20 (*E. coli)* bacterial growth rates compared to the native starch.

CONCLUSION

In conclusion, the microwave heat treatment with different treatment duration on sago starch changes its digestibility by digestive enzymes and fermentation by tested bacteria. The treatment causes a decrease in the resistant starch fractions by changing the starch granules' structure. An increase in the treatment duration influences the amount of resistant starch in each sample, linked to a higher number of pinholes and pores on the surface of the granules. Additionally, the treatment also improves the growth rates of *L. casei* and *B. lactis* for cooked-resistant starch samples. It suggests that the microwave heat treatment can enhance the digestibility and prebiotic property of the sago starch, especially for the cooked samples.

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