Malaysian Applied Biology (2023) 52(5): 129-136
https://doi.org/10.55230/mabjournal.v52i5.icfic03

Research

Native and Modified Sago (*Metroxylon sagu*) Starches as an Ingredient in The Formulation of Low Glycaemic Food Product

Mohd Alhafiizh Zailani[1,2], Hanisah Kamilah[1,3], Awang Ahmad Sallehin Awang Husaini[4], Awang Zulfikar Rizal Awang Seruji[5] and Shahrul Razid Sarbini[1,3]*

1. Department of Crop Science, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Kampus Bintulu Sarawak, Malaysia
2. Centre for Pre-University Studies, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
3. Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
4. Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia
5. CRAUN Research Sdn. Bhd., Jalan Sultan Tengah, Petra Jaya, Kuching, Sarawak, Malaysia
*Corresponding author: shahrulrazid@upm.edu.my

ABSTRACT

Native sago starch has a high content of resistant starch (RS) which is associated with low glycaemic and beneficial to individuals with obesity and diabetes. Additionally, the RS is linked to the prebiotic properties exhibited by starch. This study aimed to evaluate the predicted glycaemic index (pGI) and probiotic growth rates of food formulated with native or modified starches in the formulation of a breakfast drink. The sago starch was modified *via* microwave heat treatment (MHT) with different treatment duration or *via* pre-treatment followed by MHT. The formulation of food was performed by replacing a portion of wheat starch at percentages of 25, 50, or 75%. The pGI was determined by measuring the amount of glucose produced during *in vitro* digestion. Meanwhile, the probiotic growth rates were conducted by monitoring the optical density of *Lactobacillus casei* and *Bifidobacterium lactis* for 24 hr. Comparatively, food formulated with 50 and 75% starch showed lower pGI than other formulations. This was correlated with the increase of RS in food products. Meanwhile, the probiotic growth rates increase for a few of the formulations mostly with a higher pGI or low RS content which is contributed by the accessibility for fermentation to occur. In conclusion, the findings suggest the substitution of 50% wheat flour with native or modified sago starches is sufficient to increase RS content and lower the pGI of formulated food. In the future, investigation of RS components contributing to probiotic growth is needed to enable the exploration of new prebiotics with low glycaemic.

Key words: Functional food, glycaemic index, prebiotic, probiotic, retrograded starch

Article History
Accepted: 8 November 2023
First version online: 15 December 2023

Cite This Article:
Zailani, M.A., Kamilah, H., Awang Husaini, A.A.S., Awang Seruji, A.Z.R. & Sarbini, S.R. 2023. Native and modified sago (*Metroxylon sagu*) starches as an ingredient in the formulation of low glycaemic food product. Malaysian Applied Biology, 52(5): 129-136. https://doi.org/10.55230/mabjournal.v52i5.icfic03

INTRODUCTION

The interest in functional food has increased among consumers focusing on improving or maintaining their health. Hence, this increases the studies on various food components which include carbohydrates and proteins. Starch is one of the most studied food ingredients as it can provide a low glycaemic index value for food. This is contributed by the presence of resistant starch components in the starch itself (Zailani *et al*., 2023). Food that contains low glycaemic index components is beneficial for diabetes patients and individuals with obesity (Bello-Pérez *et al*., 2021). Many studies have focused on increasing the resistant starch content in starch *via* various treatments such as chemical, enzymatic, and physical treatments (Zailani *et al*., 2023). Examples of modified starches displaying low glycaemic index were banana, maize, and cassava starches (Bello-Pérez *et al*., 2021; Eyinla, *et al*., 2021; Paramasivam *et al*., 2021).

As starch has been used in the formulation of food products, for example in fish crackers and tempura mix, the supplement of resistant starch can create an opportunity to enhance the functional food properties between different food products. Examples of foods that are studied by adding

resistant starch include cookies, pasta, muffins, bread, and breakfast cereals (Arp, Correa, & Ferrero, 2021; Himat *et al*., 2021). This process is conducted by replacing a percentage of the actual ingredient such as wheat flour or by changing it to 100% starch. For instance, studies were conducted by replacing wheat flour with purple yam and millet flour (Liu *et al*., 2019; Sharma & Gujral, 2019). This results in the preparation of food with lower digestibility.

Starch with low digestibility was associated with the prebiotic properties of starch (Zaman & Sarbini, 2015). By definition, a prebiotic is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson *et al*., 2017). As a result, gut microbiota will be modulated, hence, benefiting the host. Gut microbiota is essential in the alteration of host metabolism in response to food intake (Farag *et al*., 2020). For example, caffeine is converted to purine by the gut microflora through a demethylation process. In addition, these microflorae assist in the production of gamma-aminobutyric acid which is a by-product of the fermentation of food (Farag *et al*., 2020). This compound is reported to exhibit multiple biological properties that benefit the host, for example, it assists in the prevention of type 1 diabetes, neural disease, and immunological disorders (Diez-Gutiérrez *et al*., 2020). Additionally, butyric acid was reported to be produced from the fermentation of resistant starch by gut microbiota such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus helveticus* (Raigond *et al*., 2015; Geng & Zhao, 2015). The produced butyric acid has been reported to possess anticancer and anti-inflammatory properties (Foglietta *et al*., 2014; Chen & Vitetta, 2020).

Sago starch is obtained by wet extraction of the pith of a sago palm, *Metroxylon sagu*. It was reported to contain highly resistant starch which gave it low digestibility (Arshad *et al*., 2018). There is a lack of investigation of sago starch formulated in food products. Moreover, the information on food formulated with sago starch digestibility and prebiotic properties is limited. Therefore, the current study determined the functional food properties of formulation of food products incorporated with native and modified sago starches.

MATERIALS AND METHODS

Materials

Food-grade sago starch was contributed by CRAUN Research Sdn. Bhd. (Sarawak, Malaysia). Chemicals used in this study were purchased from HmbG chemicals (analytical grade), while inulin was from Orafti®. Resistant Starch Assay Kit was acquired from Megazyme (Ireland) while MRS agar and broth from Difco™. The bacteria used in this study were obtained from the Biology Laboratory at the Centre for Pre-University Studies (Universiti Malaysia Sarawak). The general-purpose wheat flour was purchased from local stores.

Microwave heat treatment of sago starch

Native sago starch was treated using two different methods that were microwave heat treatment with different treatment durations and pre-treated microwave heat treatment. Microwave heat treatment with different treatment duration was performed as described by Zailani *et al*. (2021). The moisture content of native sago starch was adjusted to 30% and stored overnight at 4 °C. The starch was then microwave-heat treated at four different time durations which were 5 (M5), 10 (M10), 15 (M15), and 20 (M20) min. The starch was then dried at 50 °C overnight before being cooled and stored until further analysis.

Meanwhile, the pre-treated-microwave heat treatment of native starch was conducted by pre-treating the starch *via* two pre-treatments which were distilled water washing and soaking in cold distilled water. In distilled water washed starch, the native starch was washed with distilled water (1.00:1.75). It was then kept overnight at 4 °C before microwave heat treatment for 15 min. The treated starch was oven-dried at 50 °C overnight, cooled, and stored to yield washed-microwave heated starch (W15). On the other hand, the cold-soaked-microwave heated sago starch (S15) was prepared by suspending native starch in distilled water (1:5) with constant stirring (1 hr). The suspension was then stored (4 °C, overnight) and followed by microwave heat treatment for 15 min and filtration. The modified starch obtained was oven-dried (50 °C) for two days (Zailani *et al*., 2022).

Formulation of food products

The sago starch-based food products were formulated by replacing a percentage of wheat flour with starch. The percentage of native and modified sago starch used was 25, 50, and 75%. The ingredients were homogeneously mixed before being platted to an approximate thickness of 1 mm. The food was then baked in a preheated oven for 12 min at a temperature of 180 °C. For a control, the food was formulated with the same ingredient except it uses 100% flour with no addition of starch. The food product was let to cool to ambient temperature and stored in a zip-lock bag until further analysis.

Determination of resistant starch content

The Megazyme Resistant Starch Assay Kit (K-RSTAR) was used in the determination of resistant

starch content. In brief, the food products were ground by pestle and mortar and digested using a mixture of amylase and amyloglucosidase as described in the assay kit protocol (Megazyme, 2019). The obtained solid supernatant was then washed with ethanol solution and added with potassium hydroxide followed by stirring. Sodium acetate buffer was added to the mixture followed by amyloglucosidase solution and incubated for 30 min at 50 °C. The mixture was added with glucose oxidase/peroxidase (GOPOD) solution which was used as an indicator to determine the concentration of glucose available in the mixture. The D-glucose and sodium acetate buffer were the standard and blank used. A UV-Vis spectrophotometer (V-730, Jasco) was used to measure the absorbance of the samples, blank, and standard at 510 nm.

In vitro digestion of food products

The food products were digested following methods by Lux *et al*. (2012) and Mandalari *et al*. (2008). The *in vitro* digestion was completed in oral, gastric, and duodenal phases. Briefly, sodium chloride solution (0.15 M, pH 7.0) was mixed with the samples. In the oral phase, the mixture was added with α-amylase and incubated for 5 min at 37 °C. This was followed by adjusting the pH to 2.5 and mixing with pepsin and gastric lipase with incubation in a shaking incubator for 2 hr at 37 °C. Meanwhile, in the duodenal phase, the digest from the gastric phase was pH adjusted to 6.5. This was followed by the addition of calcium chloride, bile salts, α-chymotrypsin, Bis-Tris (pH 6.5), trypsin, and pancreatic lipase. Incubation of the mixture was conducted for 1 hr in a shaking incubator at 37 °C. Blank was prepared the same as the digestion of samples excluding samples. Meanwhile, the positive control used in this study was inulin (Mandalari *et al*., 2008; Lux *et al*., 2012).

Predicted glycaemic index determination of food products

The sample was suspended in HCl-KCl buffer (pH 1.5) before added with pepsin in HCl-KCl buffer (pH 1.5) and incubated for 1 hr at 40 °C in a shaking water bath. The mixture pH was adjusted to 6.0 followed by the addition of maleate buffer (pH 6.0), pancreatic α-amylase (93.75 CU/mL), and amyloglucosidase (9.375 U/mL). The mixture was then incubated with shaking at 37°C for 3 hr. The mixture was sampled every 30 min and added to an ethanol solution (95%). The absorbance at 510 nm was measured upon mixing the samples with GOPOD reagent and incubated at 50 °C for 20 min (Tsai & Lai, 2021). The hydrolysis percentage, hydrolysis constant, hydrolysis index, and the predicted glycaemic index for each sample were calculated as described by Tsai and Lai (2021).

Determination of bacterial growth rates

Three bacteria were used in the growth rate determination which were *Lactobacillus casei* (*L. casei*)*, Bifidobacterium lactis* (*B. lactis*)*,* and *Escherichia coli* (*E. coli*). *L. casei* and *B. lactis* were chosen in this study as these bacteria are probiotics that are normally consumed and present in the human gut. *E. coli* was chosen as it is an enteric bacterium that is also present in the gut. The bacteria were cultured in a sterilized MRS broth for 24 – 48 hr followed by streaking onto MRS agar. A single colony was taken and added into MRS broth followed by incubation (37 °C, 24 – 48 hr). The optical density was adjusted to 1.0 at a wavelength of 620 nm using a UV-Vis spectrophotometer (Okolie *et al*., 2019).

The food samples, inulin (positive control), and glucose were prepared to a concentration of 0.1% by mixing them with sterilized MRS broth. The samples, inulin, and glucose were pipetted into a 96-well plate which was then followed by the addition of bacterium culture. The plate was sealed and incubated for 24 hr at 37 °C. The optical density of each plate was measured at a wavelength of 620 nm every 30 min. A graph was plotted using the optical densities and used in the determination of bacterial growth rates as described in Okolie *et al*. (2019).

Statistical analysis

The data obtained was analyzed using One Way ANOVA and followed by Tukey's test (p<0.05). Pearson's correlation study was also conducted with the same alpha value and conducted using Statistical Package for Social Sciences (IBM® SPSS® Version 20).

RESULTS AND DISCUSSION

Digestibility of formulated foods

The formulation of food products by substituting common ingredients such as wheat flour with modified starch has been conducted previously to enhance the functional food properties of the products (Laguna *et al*., 2011). The current study uses native and modified sago starches in the formulation of a breakfast drink. A breakfast drink is a simple food product that is consumed in the morning for energy. Additionally, these products contain dietary fibers which benefit the consumer. Based on the result (Figure 1), it can be observed that the percentage of resistant starch content in the food product increases as the percentage of starch used to replace wheat flour increases. Several food products displayed the same percentage of resistant starch content as the control (100% wheat flour)

which were the food formulated by replacing 25% of wheat flour except for M20 formulations. These observations were tallied with the correlation studies which indicate the negative correlation between the amount of wheat flour used with the percentage of resistant starch content (r=-0.703, p<0.001). The flour:starch replacement ratio of 1:1 (50% starch) was sufficient to increase the RS content of the food product which was significantly better than the 100% flour formulation. However, the pre-treated microwave heat treated starch formulated food showed a lower resistant starch content which may be due to the influence of pre-treatments that enabled starch granule damage (Zailani *et al*., 2022). Hence, reducing the percentage of resistant starch in food products. Additionally, the amount of resistant starch content was almost similar to the dietary fiber content in commercially available breakfast drinks which is between 8 – 10% mostly for the microwave heat-treated starch at different treatment durations. In addition, the increase in RS content may help in decreasing the production of lipids through increasing lipid oxidation benefitting obese individuals (Raigond *et al*., 2015).

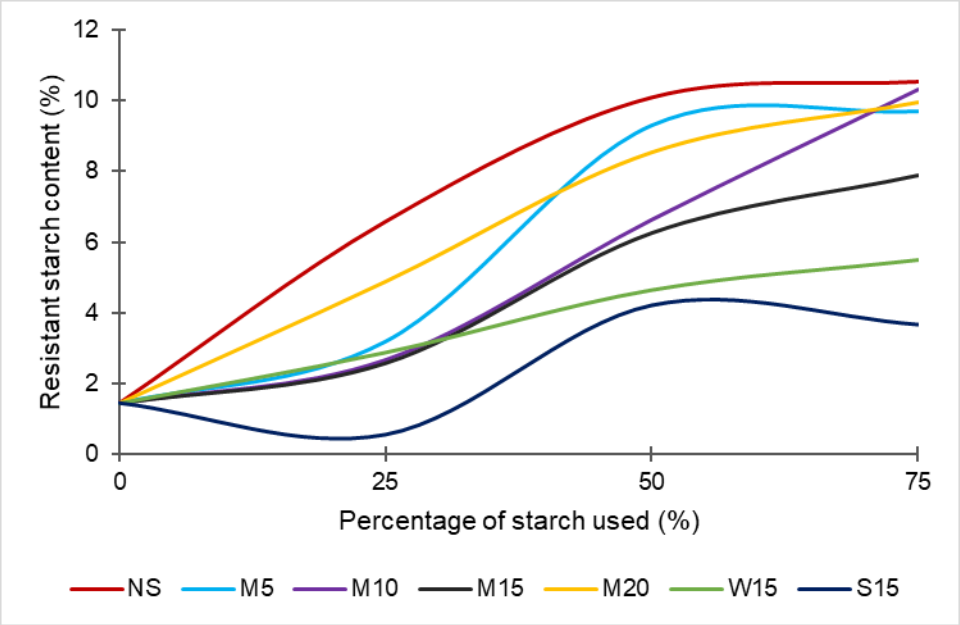

Fig. 1. The resistant starch content of food formulated with native and modified starches at different flour substitution percentages. Note: NS – native sago starch, M5 – microwave heated sago starch (5 min), M10 – microwave heated sago starch (10 min), M15 – microwave heated sago starch (15 min), M20 – microwave heated sago starch (20 min), W15 – washed-microwave heated sago starch (15 min) and S15 – cold-soaked-microwave heated sago starch (15 min)

Predicted Glycaemic Index of Formulated Foods

The predicted glycaemic index (pGI) of formulated food was within a range of 56 – 120 with four formulations having values that exceeded 100% (Figure 2). This was displayed by M10 (25%), M15 (25%), M20 (25%), and S15 (75%) formulations. Meanwhile, the two formulations had the lowest pGI which were M10 (50%) and M10 (75%) with the values of 56.3 and 56.7, respectively. Based on Figure 2, it was observed that the pGI was lower when the amount of flour substituted was between 50 to 75%. A moderate positive correlation was observed between the percentage of flour and pGI (r=0.4551, p=0.002) indicating that the increase in the percentage of starch helps in the decrease of the pGI value of the formulated food. Based on Venn *et al*., (2014), the glycaemic index is classified into three high groups (≥70), moderate (between 55 - 70), and low (≤55) glycaemic index. Hence, most of the food formulated with starch had pGI values in moderate groups which may be beneficial to the consumer. The pGI values showed a negative correlation with the RS content (r=-0.618, p<0.001) which implied the increase in RS content can help in lowering the GI value of food products. This finding was similar to previously published literature where the increase in the RS content had millet flour substituted food product yielded a lower glycaemic index (Sharma & Gujral, 2019; Kaimal *et al*., 2021). This was also observed in a retrograded starch known as Novelose 300 which lowers the glycaemic response when it is used in the formulation of food (Raigond *et al*., 2015).

Bacterial growth rates of formulated foods

The bacterial growth rates of formulated foods have differed from each bacterium (Figure 3). The *L. casei* growth rates were within a range of 0.196 to 0.320 h^{-1}, while *B. lactis* growth rates were between 0.201 to 0.298 hr^{-1}. Meanwhile, for the enteric bacteria, *E. coli*, the growth rates were between 0.050 to 0.098 hr^{-1}. For the *L. casei*, formulation W15 (25%) has the highest growth rate value while W15 (75%) was the lowest. Compared to the control food (0.261 hr^{-1}), W15 (25%) was significantly

higher with an increase of around 22%. Meanwhile, for the growth rates of *B. lactis*, the highest value was displayed by M10 (25%) while the lowest value was W15 (50%). The M10 (25%), M15 (75%) (0.284 hr^{-1}), and W15 (25) (0.273 hr^{-1}) which had the highest growth rates were significantly higher than the control food (0.234 hr^{-1}). The increment in *B. lactis* growth rates was between 16 to 28%. On the other hand, the growth rates of *E. coli* decrease when compared with the control food (0.074 hr^{-1}) except for M5 (25%), M10 (25%), M15 (25, 50%), M20 (25, 50%), S15 (25%). The increase in these probiotics' growth rates while the decrease in the enteric bacterium growth rates suggests the potential of these food products to modulate the gut microbiota.

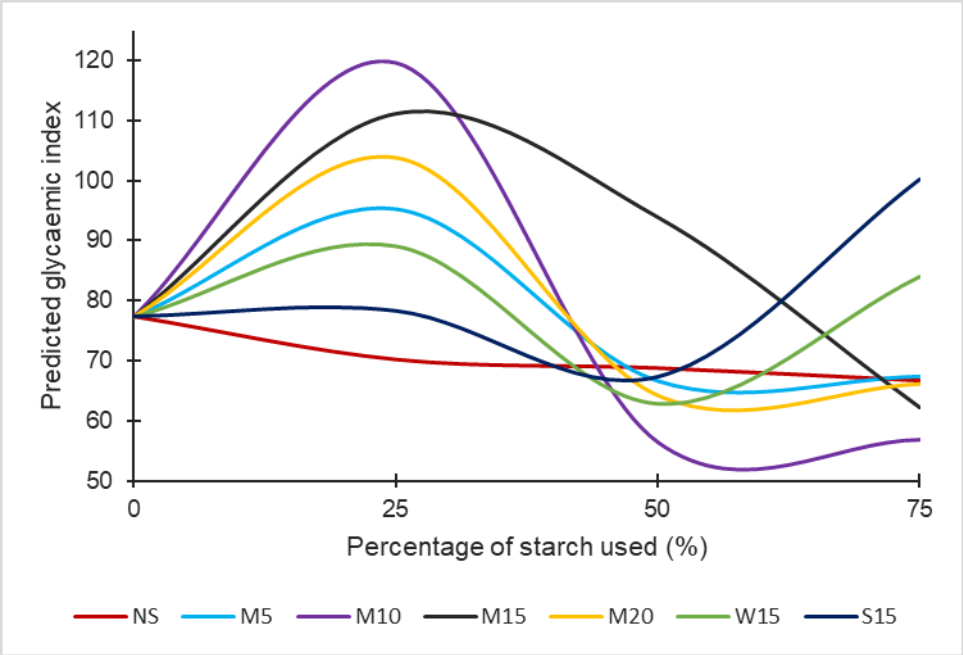

Fig. 2. The predicted glycaemic index of food formulated with native and modified starches at different flour substitution percentages (0, 25, 50, or 75%).
Note: NS – native sago starch, M5 – microwave heated sago starch (5 min), M10 – microwave heated sago starch (10 min), M15 – microwave heated sago starch (15 min), M20 – microwave heated sago starch (20 min), W15 – washed-microwave heated sago starch (15 min) and S15 – cold-soaked-microwave heated sago starch (15 min)

Based on the correlation studies, there were correlations observed between the percentage of starch used in the formulation with the bacterial growth rates. The correlations were observed as moderate negative correlations for the *L. casei* (r=-0.415, p=0.005) and *E. coli* (r=-0.628, p<0.001) growth rates. This indicates that the growth rates of these bacteria were affected by the starch percentage. Additionally, there was a moderate negative correlation was observed between the resistant starch content with the growth rate of *E. coli* (r=-0.585, p<0.001). This suggests that the presence of resistant starch in the food product by adding native or modified sago starch can slow the growth rates of enteric bacteria, *E. coli*. Furthermore, the growth rate of *E. coli* was also correlated with the pGI of the food (r=0.704, p<0.001) indicating the role of resistant starch in the formulated food. By increasing the RS content in the starch, delaying the growth of this bacterium is possible. Besides that, resistant starch type 3 (RS3) was reported to have a high rate of fermentation by the intestinal microbiota as observed for the *L. casei* growth rate (Sharp & Macfarlane, 2000). However, most of the formulated food did not display better growth rates for *L. casei* and *B. lactis*. Hence, this finding may be influenced by the experimental conditions which limit the fermentation by the enzymatic activities of these bacteria. In this study, the growth rates were determined in aerobic conditions, while the lactobacilli and bifidobacterial were predominant in the human colon normally in anaerobic conditions (Mu *et al*., 2018; Matejčeková *et al*., 2019). These differences in experimental conditions may influence the bacterial enzymatic activities thus affecting the outcome (González *et al*., 2004; Ashraf & Smith, 2015). Resistant starch was the only dietary fiber reported to produce a higher butyrate concentration when fermented by the gut microbiota (Raigond *et al*., 2015). This substance was reported can prevent and inhibit colonic carcinogenesis, epithelial defense barrier, and more (Canani *et al*., 2011). Moreover, the use of RS in food formulation benefits in providing balanced energy for hours through slow fermentation by gut microbiota (Raigond *et al*., 2015).

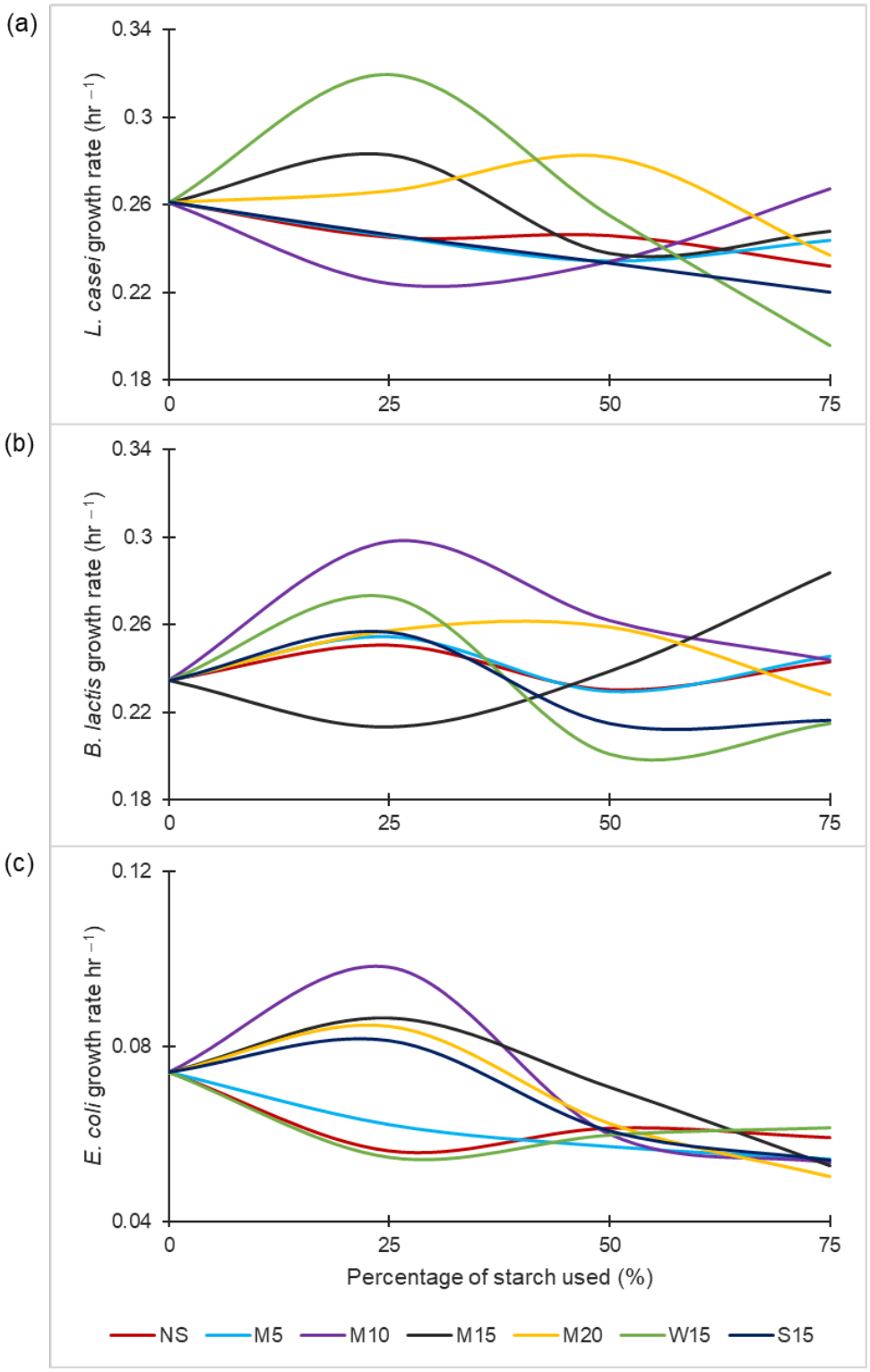

Fig. 3. The growth rates of (a) *Lactobacillus casei*, (b) *Bifidobacterium lactis*, and (c) *Escherichia coli* of formulated food products. Note: NS – native sago starch, M5 – microwave heated sago starch (5 min), M10 – microwave heated sago starch (10 min), M15 – microwave heated sago starch (15 min), M20 – microwave heated sago starch (20 min), W15 – washed-microwave heated sago starch (15 min) and S15 – cold-soaked-microwave heated sago starch (15 min)

CONCLUSION

The formulation of the food product by replacing wheat flour portion with starch was performed to produce lower glycemic food. The formulation uses native, and microwave heat-treated modified starch in the formulation. Formulations with 50 or 75% starch showed an increase in the RS percentage than the control (100% wheat flour) formulation. Furthermore, the increase of the starch concentration also contributed to a lower predicted glycaemic index of food formulated with starch except for pre-treated microwave heat-treated starch formulations. However, the pre-treated microwave heat-treated starch

formulation showed a better probiotic growth rate, especially for the W15 (25%). In conclusion, the incorporation of 50% starch in food was adequate to achieve a higher resistant starch content with a lower glycaemic index than using 100% wheat flour. Future research should focus on the components in the resistant starch that influence the growth rates of probiotics.

ACKNOWLEDGEMENTS
The authors would like to express their gratitude to Sarawak Research and Development Council (RDCRG/CAT/2019/26) for financing this project *via* a Catalyst Grant.

ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Agama-Acevedo, E., Pacheco-Vargas, G., Gutierrez-Meraz, F., Tovar, J. & Bello-Perez, L.A. 2019. Dietary fiber content, texture, and in vitro starch digestibility of different white bread crusts. Journal of Cereal Science, 89: 102824. https://doi.org/10.1016/j.jcs.2019.102824

Arp, C.G., Correa, M. J. & Ferrero, C. 2021. Resistant starches: A smart alternative for the development of functional bread and other starch-based foods. Food Hydrocolloids, 121: 106949. https://doi.org/10.1016/j.foodhyd.2021.106949

Arshad, N., Zaman, S., Rawi, M. & Sarbini, S.R. 2018. Resistant starch evaluation and in vitro fermentation of lemantak (native sago starch), for prebiotic assessment. International Food Research Journal, 25(3): 951-957.

Ashraf, R. & Smith, S.C. 2015. Selective enumeration of dairy based strains of probiotic and lactic acid bacteria. International Food Research Journal, 22(6): 2576-2586.

Bello-Pérez, L.A., Flores-Silva, P.C., Sifuentes-Nieves, I. & Agama-Acevedo, E. 2021. Controlling starch digestibility and glycaemic response in maize-based foods. Journal of Cereal Science, 99: 103222. https://doi.org/10.1016/j.jcs.2021.103222

Canani, R.B., Costanzo, M.D., Leone, L., Pedata, M., Meli, R. & Calignano, A. 2011. Potential beneficial effects of butyrate in intestinal and extraintestinal disease. World Journal of Gastroenterology, 17(12): 1519-1528. https://doi.org/10.3748/wjg.v17.i12.1519

Chen, J. & Vitetta, L. 2020. The role of butyrate in attenuating pathobiont-induced hyperinflammation. Immune Network, 20(2): e15. https://doi.org/10.4110/in.2020.20.e15

Diez-Gutiérrez, L., Vicente, L.S., Barrón, L.J., Villarán, M. d. & Chávarri, M. 2020. Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. Journal of Functional Foods, 64: 103669. https://doi.org/10.1016/j.jff.2019.103669

Eyinla, T.E., Sanusi, R.A. & Maziya-Dixon, B. 2021. Effect of processing and variety on starch digestibility and glycemic index of popular foods made from cassava (*Manihot esculenta*). Food Chemistry, 356: 129664. https://doi.org/10.1016/j.foodchem.2021.129664

Farag, M.A., Abdelwareth, A., Sallam, I.E., Shorbagi, M.e., Jehmlich, N., Fritz-Wallace, K., Schäpe, S.S., Rolle-Kampczyk, U., Ehrlich, A., Wessjohann, L.A. & von Bergen, M. 2020. Metabolomics reveals impact of seven functional foods on metabolic pathways in a gut microbiota model. Journal of Advanced Research, 23: 47-59. https://doi.org/10.1016/j.jare.2020.01.001

Foglietta, F., Serpe, L., Canaparo, R., Vivenza, N., Riccio, G., Imbalzano, E., Gasco, P. & Zara, G.P. 2014. Modulation of butyrate anticancer activity by solid lipid nanoparticle delivery: An in vitro investigation on human breast cancer and leukemia cell lines. Journal of Pharmacy & Pharmaceutical, 17(2): 231-247. https://doi.org/10.18433/J3XP4R

Geng, Q. & Zhao, X.H. 2015. Influences of exogenous probiotics and tea polyphenols on the production of three acids during the simulated colonic fermentation of maize resistant starch. Journal of Food Science and Technology, 52(9): 5874-5881. https://doi.org/10.1007/s13197-014-1662-6

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K. & Reid, G. 2017. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Review: Gastroenterology & Hepatology, 14: 491-502. https://doi.org/10.1038/nrgastro.2017.75

González, R., Blancas, A., Santillana, R., Azaola, A. & Wacher, C. 2004. Growth and final product formation by *Bifidobacterium infantis* in aerated fermentations. Applied Microbial and Cell Physiology, 65: 606-610. https://doi.org/10.1007/s00253-004-1603-9

Himat, A.S., Gautam, S., Garcia, J.P., Vidrio-Sahagún, A.X., Liu, Z., Bressler, D. & Vasanthan, T. 2021.

Starch-based novel ingredients for low glycemic food formulation. Bioactive Carbohydrate and Dietary Fibre, 26: 100275. https://doi.org/10.1016/j.bcdf.2021.100275
Kaimal, A.M., Mujumdar, A.S. & Thorat, B.N. 2021. Resistant starch from millets: Recent developments and applications in food industries. Trends in Food Science & Technology, 111: 563-580. https://doi.org/10.1016/j.tifs.2021.02.074
Laguna, L., Salvador, A., Sanz, T. & Fiszman, S.M. 2011. Performance of a resistant starch rich ingredient in the baking and eating quality of short-dough biscuits. LWT - Food Science and Technology, 44: 737-746. https://doi.org/10.1016/j.lwt.2010.05.034
Liu, X., Lu, K., Yu, J., Copeland, L., Wang, S. & Wang, S. 2019. Effect of purple yam flour substitution for wheat flour on in vitro starch digestibility of wheat bread. Food Chemistry, 284: 118-124. https://doi.org/10.1016/j.foodchem.2019.01.025
Lux, S., Scharlau, D., Schlörmann, W., Birringer, M. & Glei, M. 2012. In vitro fermented nuts exhibit chemopreventive effects in HT29 colon cancer cells. British Journal of Nutrition, 108(7): 1177-1186. https://doi.org/10.1017/S0007114511006647
Mandalari, G., Faulks, R.M., Rich, G.T., Lo Turco, V., Picout, D.R., Lo Curto, R.B., Bisignano, G., Dugo, P., Dugo, G., Waldron, K.W., Ellis, P.R. & Wickham, M.S. 2008. Release of protein, lipid, and vitamin E from almond seeds during digestion. Journal of Agricultural and Food Chemistry, 56(9): 3409-3416. https://doi.org/10.1021/jf073393v
Matejčeková, Z., Vlková, E., Liptáková, D. & Valík, Ľ. 2019. Preliminary screening of growth and viability of 10 strains of *Bifidobacterium* spp.: Effect of media composition. Fermentation, 5(2): 38. https://doi.org/10.3390/fermentation5020038
Megazyme. 2019. Megazyme. Retrieved from www.megazyme.com: https://www.megazyme.com/documents/Assay_Protocol/K-RSTAR_DATA.pdf
Mu, Q., Tavella, V.J. & Luo, X.M. 2018. Role of *Lactobacillus reuteri* in human health and diseases. Frontier in Microbiology, 9: 757. https://doi.org/10.3389/fmicb.2018.00757
Odenigbo, M.A., Asumugha, U.V., Ubbor, S. & Ngadi, M. 2013. In vitro starch digestibility of plantain and cooking-banana at ripe and unripe stages. International Food Research Journal, 20(6): 3027-3031.
Okolie, C.L., Mason, B., Mohan, A., Pitts, N. & Udenigwe, C.C. 2019. The comparative influence of novel extraction technologies on in vitro prebiotic-inducing chemical properties of fucoidan extracts from *Ascophyllum nodosum*. Food Hydrocolloids, 90: 462-471. https://doi.org/10.1016/j.foodhyd.2018.12.053
Paramasivam, S.K., Saravanan, A., Narayanan, S., Shiva, K.N., Ravi, I., Mayilvaganan, M., Pushpa, R. & Uma, S. 2021. Exploring differences in the physicochemical, functional, structural, and pasting properties of banana starches from dessert, cooking, and plantain cultivars (*Musa* spp.). International Journal of Biological Macromolecules, 191: 1056-1067. https://doi.org/10.1016/j.ijbiomac.2021.09.172
Raigond, P., Ezekiel, R., & Raigond, B. 2015. Resistant starch in food: a review. Journal of The Science of Food and Agriculture, 95: 1968-1978. https://doi.org/10.1002/jsfa.6966
Sharma, B., & Gujral, H.S. 2019. Modulation in quality attributes of dough and starch digestibility of unleavened flat bread on replacing wheat flour with different minor millet flours. International Journal of Biological Macromolecules, 141: 117-124. https://doi.org/10.1016/j.ijbiomac.2019.08.252
Sharp, R. & Macfarlane, G.T. 2000. Chemostat enrichments of human feces with resistant starch are selective for adherent butyrate-producing *Clostridia* at high dilution rates. Applied and Environment Microbiology, 66(10): 4212-4221. https://doi.org/10.1128/AEM.66.10.4212-4221.2000
Tsai, P.C. & Lai, L.S. 2021. In vitro starch digestibility, rheological, and physicochemical properties of water caltrop starch modified with cycled heat-moisture treatment. Food, 10: 1687. https://doi.org/10.3390/foods10081687
Venn, B.J., Kataoka, M. & Mann, J. 2014. The use of different foods in determining the glycemic index of starchy and non-starchy test foods. Nutrition Journal, 13: 50. https://doi.org/10.1186/1475-2891-13-50
Zailani, M.A., Kamilah, H., Husaini, A. & Sarbini, S.R. 2021. Physicochemical properties of microwave heated sago (*Metroxylon sagu*) starch. CyTA - Journal of Food, 19(1): 596-605. https://doi.org/10.1080/19476337.2021.1934550
Zailani, M.A., Kamilah, H., Husaini, A., Awang Seruji, A.Z.R. & Sarbini, S.R. 2023. Starch modifications via physical treatments and the potential in improving resistant starch content. Starch. 2200146. https://doi.org/10.1002/star.202200146
Zailani, M.A., Kamilah, H., Husaini, A., Awang Seruji, A.Z.R. & Sarbini, S.R. 2022. Functional and digestibility properties of sago (*Metroxylon sagu*) starch modified by microwave heat treatment. Food Hydrocolloids, 122: 107042. https://doi.org/10.1016/j.foodhyd.2021.107042
Zaman, S.A. & Sarbini, S.R. 2015. The potential of resistant starch as prebiotic. Critical Reviews in Biotechnology, 1-7. https://doi.org/10.3109/07388551.2014.993590