The Effect Of Substrate Concentration In Fermentation Of Sago Starch Short Chain Oligosaccharides By Lactobacillus Spp.

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Abstract: Experiments were conducted to study the potential of sago starch short chain oligosaccharides to enhance the growth of *L. acidophilus, L. bulgaricus* and *L. casei*. The sago starch was hydrolysed by 1% (v/w) α-amylase (Termamyl 120L) at 65°C for 0-48 h. Hydrolysis resulted in sago starch short chain oligosaccharides (SCO) with DP1 up to DP5. The fermentations of sago starch SCO at various concentrations; 1%, 2%, 4% and 6% (v/v) by *Lactobacillus* spp. were studied. Results showed that the growth of all *Lactobacillus* sp. were positively correlated with SCO concentration up to 4% (v/v) but no further improvement on the growth with inclusion of 6% (v/v) SCO in the media. *L. casei* showed the highest growth compared to *L. acidophilus* and *L. bulgaricus* in all fermentation conditions. Comparison studies of *Lactobacillus* sp. growth on commercial prebiotics; fructooligosaccharide and inulin showed that growth in sago starch SCO was as good as in commercial prebiotics. Conclusively, sago starch SCO had an effect on *Lactobacillus sp.* growth and thus its potential in serving as prebiotics is worth to be explored.

Index Terms: Sago starch; Short chain oligosaccharides; Prebiotic, *Lactobacillus* spp.

1 Introduction

Presently, the use of foods that promote a state of wellbeing, better health and reduction of the risk of diseases have become popular as the consumer is becoming more and more health conscious. In this sense, there has been a lot of attention paid to specific types of dietary carbohydrates, namely the non-digestible oligosaccharides (NDOs). There is currently great deal of interest in the use of prebiotic oligosaccharides as functional food ingredients to manipulate the composition of colonic microflora in order to improve host health [1]. Prebiotic is defined as non digestible but fermentable food ingredients that confers a health benefit on the host associated with modulation of microbiota in the colon [2]. Sago (Metroxylon sagu) has been an economically important crop of Sarawak. The focus of the development is in Mukah division. To date, a total of 15,006 hectares of peat land in Mukah had been designated and progressively developed [3]. Sago palm, which grows in swamp areas inhabitable for most other crops is also the world's highest starch producer, however the utilization of this material is still limited as compared to other countries due to the limited data of basic knowledge on its physico-chemical and functional properties. The potential of sago starch oligosaccharides as an alternative source of prebiotics may lead to the development of a new food ingredient, thus promoting the economic growth of Sarawak. In this present work, the suitability of sago starch short chain oligosaccharides (SCO) in promoting the growth of probiotics namely Lactobacillus acidophilus, L. bulgaricus and L. casei was studied.

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2 Material and methods

2.1 Materials

Sago starch (*Metroxylan sagu*) was obtained from Nitsei Sago Industries Sdn. Bhd., Penang, Malaysia. Commercial FOS (Raftilose P85, ORAFTI, Tienen, Belgium) and inulin (Raftiline, ORAFTI, Tienen, Belgium) were kindly supplied by DPO (M) Sdn. Bhd. (Kuala Lumpur, Malaysia). Thermostable α-amylase (Termamyl 120L, Novo Nordisk, Bagsvaerd, Denmark) was purchased from Science Technics Sdn Bhd (Kuala Lumpur, Malaysia). Maltooligosaccharide standards were purchased from Sigma Chemicals Co. (St. Loius, USA). *Lactobacillus casei* FTCC 0442, *Lactobacillus bulgaricus* FTCC 0411 and *Lactobacillus acidophilus* FTCC 0291 were purchased from Food Technology Research Centre, MARDI, Serdang, Malaysia All microbiological culture media were obtained from Oxoid Ltd. (Hampshire, England). All other chemicals were analytical grade and obtained either from Merck, Darmstadt Co. or Sigma Chemical Co.

2.2 Sago starch oligosaccharides preparation

About 2 g of raw starch was mixed with 100 ml of 0.05 M citrate-phosphate buffer solution, pH 6.2 and heated at 60°C for 2 hours in an oven. Five ml of 50 ppm $CaCl_2$ was added into pretreated 2.0 % (w/v) starch solution. Enzymatic hydrolysis was initiated by addition of 1 % (v/w of starch) Termamyl 120L into the reaction mixture and incubated at temperature 65 °C with constant shaking (Clifton, UK) for 48 hours. Aliquots of the reaction mixtures were removed periodically and centrifuged at 2500 rpm for 10 minutes (Sigma Sartorius, Germany). The enzymatic reaction was stopped by adjusting the pH of supernatant was adjusted to pH 3.7 - 3.9 with 0.01 N HCl and further incubated in a water bath at 95 °C for 20 minutes. The solution was kept at −18 °C until it is needed for analysis with HPLC and applied as substrate in fermentation media..

2.3 Determination of sago oligosaccharide profiles by HPLC

Determination was performed using Waters HPLC system (Waters, Millford, USA) and signals were detected by Refractive Index Detector (Waters, Millford, USA). The

concentration of oligosaccharide samples were referred to maltooligosaccharide standards (Sigma Co., USA) of degree of polymerization (DP) 1 to 5.

2.4 Inoculum and seed medium preparation

A single colony of individual *Lactobacillus* spp. from MRS plate agar was transferred into 15 ml MRS broth in test tubes. The broths were then placed into an anaerobic jar (Oxoid Ltd., Hampshire, England) which contained Anerogen envelopes (Oxoid Ltd., Hampshire, England) to create anaerobic environment and incubated at 37 °C for 24 hours. Total viable count of *L. acidophilus*, *L. bulgaricus* and *L. casei* were counted and populations in the range of 10^8 – 10^9 CFU/ml were obtained.

2.5 Fermentation medium preparation

The fermentation medium was prepared according to deMann *et al*. [4] except that the glucose was replaced by sago short chain oligosaccharides, FOS or inulin. The media was then autoclaved at 121°C for 15 minutes. About 10 mg of oligosaccharide samples were added into 1 L of the fermentation medium. About 3 ml precultured medium which contained 10^6 CFU/ml of individual species of Lactobacilli was transferred into 20 ml fermentation medium in duplicate 30 ml screw cap vials. Unagitated fermentation was carried out inside an anaerobic jar (Oxoid Ltd., Hampshire, England) containing Anerogen envelopes (Oxoid Ltd., Hampshire, England) at 37 °C for 24 hours. Samples were analysed for bacterial growth based on total viable count method. Plates were incubated in anaerobic condition as above at 37 °C for 48 hours. All data was subjected to statistical analysis.

3 Results and Discussion

3.1 Determination of sago starch SCO profile by HPLC

Figure 3.1 shows hydrolysis profiles of sago starch with 1% (v/w) α–amylase Termamyl 120L at 65 °C for 48 hours. Termamyl 120L yielded products of SCO of DP5 (maltopentaose), DP3 (maltotriose), DP2 (maltose) and DP1(glucose). The DP4 (maltotetraose) was also produced by Termamyl 120L but in a very low and insignificant and it's became the reason why it did not appears in the Figure. Possible explanation of lower yields of DP4 could be due to the immediate hydrolysis of DP4 to DP2 + DP2 and/or to DP1 + DP3 [5]. It can be observed that the rate of maltopentaose (DP5) that has been produced was at a greater rate at early stage of hydrolysis especially at the first 10 hours of hydrolysis as compared to other components of oligosaccharides. Govindasamy *et. al* [6] had also mentioned that DP5 is the predominant compound with DP1, DP2 and DP3 produced in much lesser amounts after which the degradation of starch occurred. Gorinstein *et. al* [7] had mentioned sago starch hydrolysed with Termamyl alone gave a final product composed mainly of DP1 and DP2 but low in DP3, DP5, DP7 and DP9 which could explain the reducing yield of DP5 towards the end of hydrolysis process.

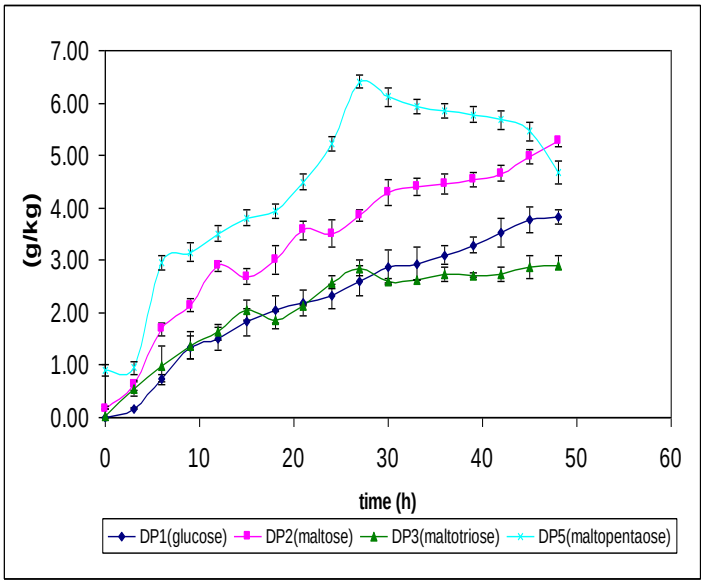

Figure 3.1 Sago starch SCO profile by enzymatic hydrolysis with 1% (v/w) Termamyl 120L at 65°C

3.2 Total viable count of *Lactobacillus* spp. on different substrate fermentation.

To study the effects of sago starch SCO on the growth of *L. acidophilus*, *L. bulgaricus* and *L. casei* in vitro, the bacteria were cultured in carbohydrate-free MRS medium supplemented with sago starch SCO at final concentrations of 1%, 2%, 4%, or 6% (v/v) obtained from previous section as shown in Figure 3.1 at 37 °C for 24 hours. Carbohydrate-free MRS medium was used as the control.

Table 3.1 Total viable count of *L. acidophilus* in different sago starch SCO concentration and other fermentation substrates

Substrate concentration (% v/v)	Time of hydrolysis (h)		
	3	27	48
1.0	7.50 ± 0.23^{b}	7.38 ± 0.62^{b}	7.69 ± 0.04^{b}
2.0	8.06 ± 0.08^{cd}	7.70 ± 0.23^{bc}	7.78 ± 0.23^{b}
4.0	8.46 ± 0.06^{d}	8.23 ± 0.07^{cd}	8.11 ± 0.13^{bcd}
6.0	8.10 ± 0.09^{cd}	7.81 ± 0.23^{bcd}	7.83 ± 0.06^{bc}
Blank	5.20 ± 0.12^{a}	5.20 ± 0.12^{a}	5.20 ± 0.12^{a}
FOS	8.51 ± 0.36^{d}	8.51 ± 0.36^{d}	8.51 ± 0.36^{d}
Inulin	7.99 ± 0.18^{c}	7.99 ± 0.18^{bcd}	7.99 ± 0.18^{bc}
Glucose	8.27 ± 0.09^{cd}	8.27 ± 0.09^{cd}	8.27 ± 0.09^{cd}

Values are mean ± SD (n=2). Means within a column with different superscripts differ significantly ($P < 0.05$)

Table 3.2 Total viable count of *L. bulgaricus* in different sago starch SCO concentration and other fermentation substrates.

Substrate concentration (% v/v)	Time of hydrolysis (h)		
	3	27	48
1.0	7.66 ± 0.08^{b}	7.31 ± 0.23^{b}	7.79 ± 0.14^{bc}
2.0	8.15 ± 0.16^{c}	8.26 ± 0.16^{d}	8.21 ± 0.16^{cd}
4.0	8.43 ± 0.15^{cd}	8.75 ± 0.30^{d}	8.38 ± 0.18^{de}
6.0	7.54 ± 0.18^{b}	7.13 ± 0.25^{b}	8.13 ± 0.26^{bcd}
Blank	5.20 ± 0.22^{a}	5.20 ± 0.22^{a}	5.20 ± 0.22^{a}
FOS	8.72 ± 0.23^{d}	8.72 ± 0.23^{d}	8.72 ± 0.23^{e}
Inulin	7.70 ± 0.14^{b}	7.70 ± 0.14^{c}	7.70 ± 0.14^{b}
Glucose	8.35 ± 0.16^{cd}	8.35 ± 0.16^{d}	8.35 ± 0.16^{de}

Values are mean ± SD (n=2). Means within a column with different superscripts differ significantly ($P < 0.05$)

Table 3.3 Total viable count of *L. casei* in different sago starch SCO concentration and other fermentation substrates.

Substrate concentration (% v/v)	Time of hydrolysis (h)		
	3	27	48
1.0	7.96 ± 0.33[b]	7.02 ± 0.04[b]	7.74 ± 0.31[b]
2.0	8.09 ± 0.15[b]	8.05 ± 0.10[b]	8.14 ± 0.18[b]
4.0	10.28 ± 0.20[d]	10.42 ± 0.19[e]	10.27 ± 0.18[d]
6.0	8.67 ± 0.37[c]	9.81 ± 0.10[d]	10.10 ± 0.31[d]
Blank	6.16 ± 0.18[a]	6.16 ± 0.18[a]	6.16 ± 0.18[a]
FOS	9.06 ± 0.21[c]	9.06 ± 0.21[c]	9.06 ± 0.21[c]
Inulin	8.98 ± 0.27[c]	8.98 ± 0.27[c]	8.98 ± 0.27[c]
Glucose	9.09 ± 0.23[c]	9.09 ± 0.23[c]	9.09 ± 0.23[c]

Values are mean ± SD (n=2). Means within a column with different superscripts differ significantly ($P < 0.05$)

From the Table 3.1, 3.2, 3.3, the results indicated that all lactobacilli strains could utilize sago starch SCO. Compared with the control medium, there are great significant differences on all lactobacilli strains growth in the medium containing sago starch SCO. Generally, as the substrate concentrations increased, total viable count of the bacteria increased as well. Gibson and Roberfroid [8] had reported that growth of several *Lactobacillus spp*. had been stimulated in the presence of fermentable carbohydrates. The result shows that among the different concentration of sago starch SCO studied, 4% (v/v) substrate concentration was the best growth substrate for *L. acidophilus*, *L. bulgaricus* and *L. casei* as exhibited by highest count. Bacteria counts reduced when substrate concentration increased from 4% (v/v) to 6% (v/v), suggesting that increasing of substrate concentration did not improve the fermentability of *Lactobacillus spp*. Although the *Lactobacillus* spp. growth in 6 % (v/v) sago starch SCO showed slightly weaker effect on bacteria growth as compared with other concentrations, its effect was still higher than the control medium. According to Wang *et al.* [9], the key criterion for a prebiotic is the demonstration of the selective stimulation of growth of one particular or a limited number of potentially beneficial bacteria in the complex gut microbiota after the consumption of a particular food. Data should demonstrate that the number of bacteria in that particular population increased significantly, whereas others did not change or even decreased. It is found from this study that addition of 4% (w/v) sago SCO increased the numbers of lactobacilli which as good as to the medium containing Inulin, FOS or glucose which indicates that the studied sago starch SCO has the ability to serve as prebiotics as good as the commercial prebiotics. *L. casei* has been reported as the most oxygen tolerant of *Lactobacillus* sp. studied, made them become the most adaptive species within the species of Lactobacillus [10], [11]. Therefore, the growth of *L. casei* is better than *L. acidophilus* and *L. bulgaricus*.

4 Conclusion

In conclusion, the fermentation studies described in this paper suggest that substrate concentration of 4 % (v/v) is the best concentration for fermentation of *Lactobacillus* sp. to be optimum and further additional of concentration will not improve the fermentation process. The fermentability of sago starch SCO was found to be comparable from those commercial prebiotics of FOS and Inulin. Sago starch SCO had an effect on *Lactobacillus sp.* growth and thus its potential in serving as prebiotics is worth to be explored.

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