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Research Article

Physicochemical Properties of Glycan within Swiftlet's Nest (*Aerodramus fuciphagus*) as Potential Prebiotic

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Abstract

Swiftlet's nest from *Aerodramus fuciphagus* species has been classified as one of the nutritious and medicinal delicacy in curing many illnesses. The swiftlet's nest compound was found to be rich in glycan in the form of mucin glycoprotein. Glycan can be involve in shaping gut microbiota, most of which cannot be digested by human digestive system whereas can be used by gut microbiota. In this study, physicochemical properties in terms of degree of polymerization (DP) and molecular weight of the swiftlet's nest glycan were evaluated in order to determine its potential as prebiotic, the food for gut microbiota. Prebiotic test was conducted by adding the prebiotic into 'de Man Rogosa Sharpe' (MRS) broth and inoculated with probiotic bacteria (*Lactobacillus acidophilus, Lactobacillus bulgaricus and Streptococcus thermophilus*). Swiftlet's glycan contained DP value of seven in average. The molecular weight of the glycan was in the range of 66.6 kDa to 21.2 kDa which showed a various size of glycan compound. Prebiotic tests exhibited positively high growth of probiotic bacteria supplemented with glycans in the form of hydrolysate (glycopeptide chains). This study demonstrated that glycans are potential as prebiotic that can be beneficial togut microbiota.

Keywords: *Aerodramus fuciphagus; Lactobacillus acidophilus*; Swiftlet's Nest

Introduction

Influx of glycan into the intestine (mostly from diet and host mucosal secretions) is one of major factor shaping the composition and physiology of gut microbiota. Symbiotic microorganisms that reside in the human intestine are adept at foraging glycans and polysaccharides, including those in dietary plants (starch, hemicellulose and pectin), animal-derived cartilage and tissue (glycosaminoglycans and N-linked glycans), and host mucus (O-linked glycans). Humans are generally consuming abundance of animal- and plant-derived dietary glycan. However, human genome is lack of encoded enzyme to digest most type of glycan, which leave a potential to the glycan to be a prebiotic compound [1].

Prebiotics may function to selectively stimulate growth of beneficial bacteria; either the species established in the colon or externally administered probiotic bacteria. Many possible benefits can be obtained from the consumption of prebiotics such as gut health maintenance, colitis and cancer prevention, immuno-stimulation, reduction of cardiovascular disease, prevention of obesity and constipation [2]. Potential prebiotic oligosaccharides can be classified according to their chemical constituents and degree of polymerization [3].

Alcalase enzyme is an endo-protease which has very broad substrate specificity, where it can hydrolyse most peptide bonds within a protein molecule. The use of alcalase enzyme in preparation of prebiotic compound is to remove the protein component from glycoprotein complex in a way that similar to the digestive system, leaving some amino acids or short peptides remained attach to the glycan due to its glycosidic bond. Instead, chemical removal of protein component may remove all protein components, leaving a purified chain of glycan free from protein. Whereas, the glycosidic

bond between the amino acid and glycan was included in the indigestible bond by enzyme digestion system [4].

The mucin of swiftlet's nest (*Aerodramus fuciphagus*) was considered as a natural source of a glycan-rich material [5]. Some of the glycan had been characterized and was classified as O-linked and N-linked oligosaccharide chains which attached to protein chain forming a complex of mucin glycoprotein [6,7]. As the nest consists of mucin types of glycoprotein, it can serve as a lubricant for digestion and protective agent from diseases [8]. This study was conducted to determine the physicochemical properties of the glycan as a potential prebiotic material.

Materials and Methods

Materials

Swiftlet's nest source was obtained from Mobile Harvesters Malaysia Sdn. Bhd, collected from swiftlet's house in Pahang. Chemicals and enzyme used in this study were purchased from Sigma-Aldrich, USA. Alcalase enzyme used is a protease from *Bacillus licheniformis*. The strains of probiotic bacteria, *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus* were given by a graduate student from UniversitiKebangsaan Malaysia.

Carbohydrate analysis and calculation of degree of polymerization

The official methods of the Association of Official Analytical Chemistry [9] were used to determine the protein, moisture, fat and ash contents of the nest. Carbohydrate content was determined by deducting the total mass of compound with the mass of protein, moisture, fat and ash. Reducing sugar was determined by dinitrosalicylic acid (DNS) reagent method based on Saqib and Whitney [10]. Dinitrosalicylic acid (DNS) reagent was prepared by mixing 1 g DNS and 30 g sodium potassium tartarate and dissolved in 80 ml of 0.5 M sodium hydroxide (w/v) using some heat. The reagent was mark-up to 100 ml using distilled water. An amount of 1 ml sample (1 mg/ml; w/v) was added to 4 ml DNS reagent. The sample-reagent mixture was boiled for 5 minutes and cooled down to room temperature prior reading the absorbance at wavelength 540 nm using a spectrophotometer (Model UV-160A, Shimadzu, Kyoto, Japan).

Calculation of the glycan's degree of polymerization (DP) was based on the following equation [11]:

Degree of polymerization (DP) = (Amount of total sugar)/(Amount of reducing sugar)

Alkaline hydrolysis (β-Elimination)

Protein removal from swiftlet's nest glycoprotein complex was done using alkaline hydrolysis as described by Fukuda [12]. 200µg of glycoprotein was incubated in 0.075M sodium hydroxide and 1M sodium borohydride for 17h at 45oC in a screw-cap vial. The reaction was interrupted by addition of 3-4 drops of glacial acetic acid. After neutralization with 3-4 drops of acetic acid the samples are dried on Speed Vac and reconstituted in 100µl of water.

Enzymatic hydrolysis

Enzyme alcalase was added to substrate with ratios 1% of enzyme to substrate and the hydrolysis was carried out for 3 hours. The hydrolysates were heated in boiling water for 5 mins to inactivate the enzyme, and then centrifuged at 4°C and 4,000rpm for 10 mins. The supernatant was filtered using Whatman No.1 and the filtrate was freeze-dried before stored for further analysis.

Measurement of molecular weight

Measurement of molecular weight for swiftlet's glycan and hydrolysate were done following the method described by Schägger [13] using 12% of electrophoresis gel concentration. Carbohydrate staining was done using Pierce Glycoprotein Staining Kit and the staining method was based on periodic acid Schiff staining reagent as described by [14].

Determination of prebiotic activity

The response as prebiotic was evaluated using a culture media with the same composition of MRS broth but replacing the carbohydrate source with the boiled and hydrolyzed nest samples. This bacterial growth was also evaluated in the prepared MRS broth without carbohydrate source, which was used as control. The carbohydrate concentration in all the assays was 5 g/L. The activated probiotics was inoculated with 1% (v/v) and incubated at 37˚C for 24 h, when the growth of mixed strains was in the stationary phase. The optical density (OD) value of the cultures at 622 nm and colony forming unit (CFU) on MRS agar were measured to evaluate the growth of probiotics after 24 h of fermentation [15].

Statistical analysis

All data collected was analyzed using analysis of variance (ANOVA) and Duncan's multiple range tests O'Mahony [16]. Significant

differences in means between samples were determined at 5% confidence level ($P < 0.05$). The analysis was done using SPSS software version 23.

Results

The degree of polymerization (DP) value obtained for the carbohydrate analyses was 7 in average. The DP value obtained is within the range of the glycan length based on the structure reported by Wieruszeski., *et al*. [6] and Yagi., *et al*. [7], which showed a DP value from 7 to 21 (14 in average). The molecular weight of swiftlet nest's glycan was in a range of 21.2 kDa to 66.6 kDa. The molecular weight of swiftlet nest's hydrolysate (contain traces of peptides linked with the glycan chain) was in a range of 31.4 kDa to 66.9 kDa, as shown in Figure 1. Xian., *et al*. [17] have reported the molecular weight of digested swiftlet's nest through in vitro digestion system was reduced to 70 kDa, 40 kDa and below. The results were proof that the glycan can resist human digestion system and have a potential to be carried into colonic environment, where the colon microbiota resides.

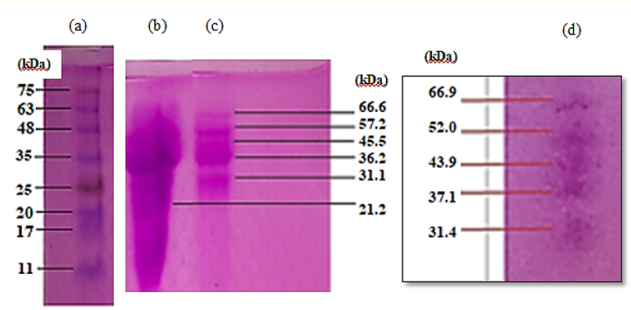
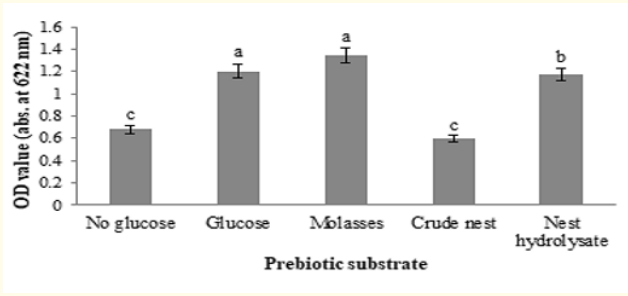
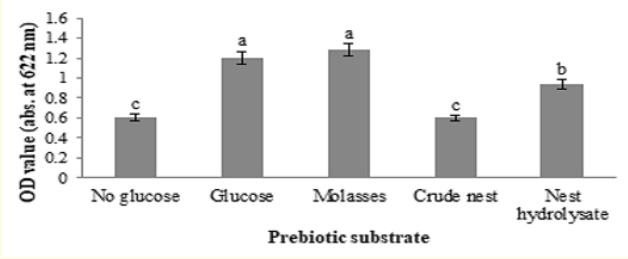


Figure 1: Molecular weight of swiftlet's nest glycan and hydrolysate on electrophoresis gel.
(a) Protein standard with specific molecular weight;
(b) Swiftlet's nest glycan replicate 1; (c) Swiftlet's nest glycan replicate 2; (d) Swiftlet's nest hydrolysate with alcalase.

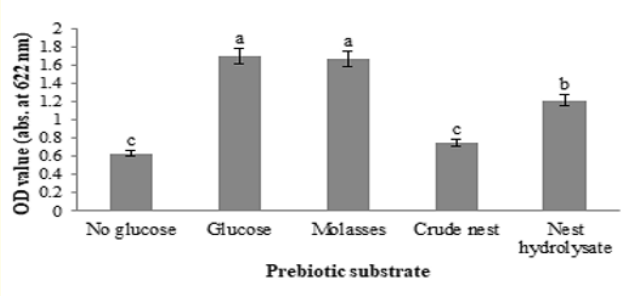
The prebiotic test showed that the probiotic bacteria (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus*) can grow well and significantly the highest growth in the MRS media containing glucose and molasses compared to other mixtures media. The probiotic bacteria have a significant growth in MRS media provided with swiftlet's nest hydrolysate compared to the negative control (no glucose) and swiftlet's crude nest, as presented in Figures 2 and 3. The growth number through OD values and CFU counts showed that different species of bacteria can give a different degree of prebiotic utilization, as shown in the significant growth of Lactobacillus acidophilus much higher than *Lactobacillus bulgaricus* and *Streptococcus thermophilus* much higher than both Lactobacillus species in the MRS media provided with the nest hydrolysate.



(a) Growth of *Lactobacillus acidophilus**



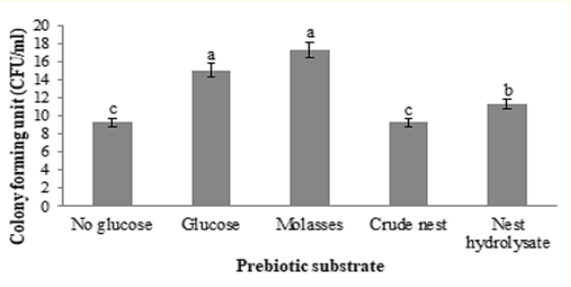
(b) Growth of *Lactobacillus bulgaricus**



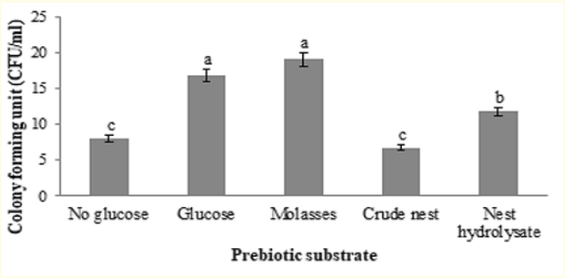
(c) Growth of *Streptococcus thermophilus**

*a-c Different letters indicate significant differences ($P<0.05$).

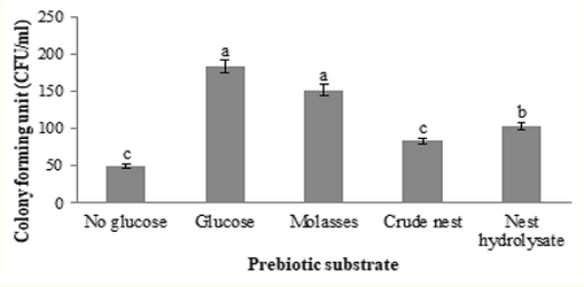
Figure 2: Optical density (OD) value from the growth of probiotic bacteria in MRS broth after 24 hours of fermentation process.



(a) Growth of *Lactobacillus acidophilus**



(b) Growth of *Lactobacillus bulgaricus**



(c) Growth of *Streptococcus thermophilus**

*a-c Different letters indicate significant differences (P<0.05).

Figure 3: Colony forming number (CFU x 107 CFU/ml) on MRS agar from the growth of probiotic bacteria in MRS broth after 24 hours of fermentation process.

Discussion

Prebiotic materials such as inulin, fructoologisaccharide and galactooligosaccharide can have different range of DP, where inulin having the DP value in the range of 2 to 60 fructose-unit, whereas fructooligosaccharide having DP value 2 to 9 fructose-unit in chains [18,19]. The prebiotic materials can also have a variety of monosaccharide-unit within their oligosaccharide chain [20]. The swiftlet's nest glycan had been characterized to have a variety monosaccharide within the chain, which include galactose, mannose, fucose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid [21]. This variety of monosaccharide unit can be beneficial to the selectivity of bacterial species in order to influence the growth of beneficial bacteria within gut bacterial community [22].

Another characteristic of prebiotic materials is that the prebiotic have to be indigestible through the digestive system and persist in intact form until reaching the colon environment. Hence, the prebiotic will be available to stimulate the growth of beneficial bacteria and further improve the host health and well-being [23]. The beneficial bacteria in the colon can be both of the bacterial species which normally resides in the colon and either orally consumed from food and beverage products such as yoghurt and vitagen. The glycan from swiftlet's nest has shown its ability to resist human digestive system through in-vitro digestibility test [17]. The intact molecular weights that escape the digestion were almost similar to the molecular weight of extracted glycan in this study. This result could suggest the intact glycan chain can resist the digestion process while protein compound in the complex glycoprotein was degraded through the process. Therefore, the chances for the glycan to be prebiotic are high.

The preparation of swiftlets nest hydrolysate was to imitate the normal consumption, where the digested nest should be available in the form short-chain of glycopeptide. The positive growth of probiotic bacteria using the hydrolysate exhibited the ability of bacterial species to degrade this type of prebiotic and improve their growth. The inability of the probiotic bacteria to degrade the crude nest is probably due to the intact form of the glycoprotein complex, which showed the importance of digestion, conversion of glycoprotein into shorter chain of glycopeptide while reducing the molecular weight of its compound. In a study by Li., *et al*. [15], inulin with lower DP value showed a higher and active degree of utilization than higher DP. The study was supporting the findings obtained in this study.

Conclusion

This study has shown the swiftlet's nest glycan with a degree of polymerization (DP) value of 7 in average has the characteristics to be a prebiotic. The nest in the form of glycopeptide exhibited the ability to improve the growth of probiotic bacteria (*Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Further study can be carried out to determine the activity and effect of swiftlet's nest glycopeptide to the bacterial community within colon environment. The swiftlet's nest glycopeptide also can be used as prebiotic ingredients in the innovation of new food product development for the benefit of food and swiftlet industries.

Acknowledgements

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Conflict of interest

There is no conflict of interest in this study.

Bibliography

1. Koropatkin NM., *et al*. "How glycan metabolism shapes the human gut microbiota". *Nature Review Microbiology* 10 (2012): 323.

2. Patel S and Goyal A. "The current trends and future perspectives of prebiotics research: A review". *Biotechnology* 2 (2012): 115-125.

3. Anadón A., *et al*. "Prebiotics and probiotics: an assessment of their safety and health benefits". Probiotics, prebiotics, and synbiotics". Bioactive foods in health promotion. Elsevier, San Diego, USA (2016): 3-23.

4. Capuano E. "The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect". *Critical reviews in food science and nutrition* 57 (2017): 3543-3564.

5. Ma FC and Liu DC. "Review sketch of the edible bird's nest and its important bioactivities". *Food Research International* 48 (2012): 559-567.

6. Wieruszeski JM., *et al*. "Structure of the monosialyl oligosaccharides derived from salivary gland mucin glycoproteins of the Chinese swiftlet (genus Collocalia)". *Journal of Biological Chemistry* 262 (1987): 6650-6657.

7. Yagi H., *et al*. "The expression of sialylated high-antennary N-glycans in edible bird's nest". *Carbohydrate Research* 343 (2008): 1373-1377.

8. Derrien M., *et al*. "Mucin-bacterial interactions in the human oral cavity and digestive tract". *Gut microbes* 1 (2010): 254-268.

9. Association of Official Analytical Chemistry - AOAC. "Official methods of analysis of the Association of Official Analytical Chemists". 15th Ed. Arlington (1990): 1105-1106.

10. Saqib AAN and Whitney PJ. "Differential behaviour of the dinitrosalicylic acid (DNS) reagent towards mono-and di-saccharide sugars". *Biomass Bioenergy* 35 (2011): 4748-4750.

11. Hayisama-Ae W., *et al*. "A potential synbiotic beverage from fermented red seaweed (Gracilariafisheri) using Lactobacillus plantarum DW12". *International Food Research Journal* 21 (2014): 1789-1796.

12. Fukuda M. "β-Elimination for Release of O-GalNAc-Linked Oligosaccharides from Glycoproteins and Glycopeptides". *Current protocols in molecular biology* 31 (1995): 17-15.

13. Schägger H. "Tricine-sds-page". *Nature Protocols* 1.1 (2006): 16-22.

14. Trivedi SM., *et al*. "Modified technique for periodic acid-Schiff staining of glycoproteins on agarose-film electrophoretograms". *Clinical Chemistry* 29 (1983): 836-839.

15. Li W., *et al*. "Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from Jerusalem artichoke". *Carbohydrate Polymers* 121 (2015): 315-319.

16. O'Mahony Michael. "Sensory evaluation of food: statistical methods and procedures". *Routledge* (2017).

17. Xian XM., *et al*. "Study on degradation of protein of the edible birds' nest (Aerodramus) in vitro". *Journal of Chinese Medicinal Materials* 33 (2010): 1760-1763.

18. Luo D., *et al*. "Effects of inulin with different degree of polymerization on gelatinization and retrogradation of wheat starch". *Food Chemistry* 229 (2017): 35-43.

19. Zhu L., *et al*. "Inulin with different degrees of polymerization modulates composition of intestinal microbiota in mice". *FEMS Microbiology Letter* 364 (2017).

20. Lie A and Pedersen LH. "Analysis of human milk oligosaccharides using high-performance anion-exchange chromatography with pulsed amperometric detection". 11th Danish Conference on Biotechnology and Molecular Biology: Denmark (2016).

21. Kathan RH and Weeks DI. "Structure studies of Collocalia mucoid. I. Carbohydrate and amino acid composition". *Archives Biochemistry Biophysics* 134.2 (1969): 572-576.

22. Tailford LE., *et al*. "Mucin glycan foraging in the human gut microbiome". *Frontiers in Genetics* 6 (2015): 1-18.

23. Al-Sheraji SH., *et al*. "Prebiotics as functional foods: A review". *Journal of Functional Foods* 5 (2013): 1542-1553.

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