Utilization Of *Cilembu* Sweet Potato (*Ipomoea batatas* (L.) Lam.) As Prebiotic Functional Beverage

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Abstract *Cilembu* sweet potato is a native cultivar of sweet potatoes in Indonesia. After baking, *Cilembu* sweet potato was known to produce a sugar liquid similar to glucose syrup which can functioned as a prebiotic content, useful in supporting gut's healthy microflora. In this research *Cilembu* were baked at three different baking temperatures (150ºC, 175ºC, 200ºC) with three different baking time (60 minutes, 90 minutes, 120 minutes) to induce the formation of the sugar syrup and were formulated as a novel prebiotic functional beverage. All formulation were analyzed for its prebiotic activity score and reducing sugar content. Baking induced change in the composition of *Cilembu* sweet potatoes which increased the inulin content (from 5.5% to 8.7%) and total reducing sugar from (1.7 to 3,1%) (w/w) of the baked *Cilembu* sweet potatoes. The prebiotic activity score of the baked sweet potatoes were ranging from (0.587 to 0.866) with prebiotic index of (0.89 to 0.91). The *Cilembu* sweet potato functional beverage made with 35 g sweet potato and 35:65 milk to water ratio resulted in a functional beverage with nutritional composition of 1.35% protein, 0.09% fat, 0.59% ash, and 16.76% carbohydrate with moderate likeness and contained a prebiotic activity score of 1.334 ±0.037 and prebiotic index of 0.945.

Keywords: *Cilembu* sweet potato, functional beverages, prebiotic score
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Introduction

Up to 1000 different species of bacteria live in the colon due to its desirable environment such as slow transit time, nutrient availability, and favorable pH. The amount of harmful bacteria must be limited by maintaining the probiotic bacteria [1]. Probiotics may provide important strategy for the prevention and treatment of gastrointestinal infections including prevention of pathogen proliferation and function, stimulating host's immune function, and suppressing the severity and duration of disease in treatment.

Sweet potato contains dietary fiber which cannot be digested by human's digestion system. Sweet potatoes is known to be rich in prebiotic compound, specifically fructo-oligosaccharides, inulin, and raffinose, which supports the growth of beneficial probiotic bacteria in human's intestine [1]–[3]. Especially *Cilembu* variety is known for their distinct sweetness and their ability to be liquified upon heating [4], [5] *Cilembu* . There are compositional change in the carbohydrate content of sweet cassava upon heating [6] thus the re-formation of the carbohydrate might enhance their prebiotic activity.

In this research, sweet potatoes will be utilized as a material to create a functional beverage containing sweet potatoes and skim milk. The sweet potatoes were first baked in different time and temperature combination to obtain the sweet potatoes with the highest prebiotic activity. The prebiotic activity of the *Cilembu* sweet potatoes is shown as prebiotic score and prebiotic index. The prebiotic score and prebiotic index will describe the relative ability of a given prebiotic to produce specific effects invitro in terms of measurement of microbial populations and growth rates against gut microflora. Prebiotic index reflects the ability of a given substrate to support the growth of an organism on it compares with the growth of the microorganism on non-prebiotic substrate such as glucose as control [7]. The final product prebiotic score and index will be compared with other similar beverages made with inulin and yam.

Materials and methods

Materials

The materials used in this research were *Cilembu* sweet potato (*Ipomoea batatas* L.) obtained from farmer in Sumedang-West Java with approximate dimension of 5 cm diameter and 15-20 cm length and age of 2-3 weeks after harvest, skim milk “Diamond” brand, and mineral water with brand “AQUA”. Materials used for analysis was *Lactobacillus plantarum* culture and *Escherichia coli* culture from IPB. Reagents used were crystal violet, iodine, ethyl alcohol, safranin, nutrient agar, nutrient broth, MRS agar, MRS broth, glucose, buffer phosphate, hexane, potassium sulfate, selenium, concentrated sulfuric acid, hydrogen peroxide 35%, sodium hydroxide solution 35%, boric acid solution 4%, standardized hydrogen chloride (0.2 N), distilled water, and mixed indicator.

Methods

Cilembu sweet potato was roasted with different the baking temperature and baking time to produce *Cilembu* sweet potato with the highest prebiotic activity score. The *Cilembu* sweet potato was baked on 150ºC, 175ºC, and 200ºC, with baking time was set for 60 minutes, 90 minutes, and 120 minutes [4]. The best combination of time and roasted temperatures was done by analyzing the reducing sugar content, prebiotic score and prebiotic index of the baked sweet potatoes. Sample with the highest prebiotic activity score and prebiotic index was then analyzed for its oligosaccharides compounds and concentration using High Performance Liquid Chromatography (HPLC). The chosen treatment was then formulated into a prebiotic beverage by combination with skim milk.

The analysis that was conducted in this research includes chemical, physical, and sensory evaluation. Chemical analysis that was done is proximate analysis [8], [9], total reducing sugar [6], inulin content [10], prebiotic score and prebiotic index [7], [11]. The sensory analysis consisted of hedonic tests which cover color, taste, aroma, texture (viscosity), and overall quality[12]. The collected data were processed using IBM SPSS 22.0 using Analysis of Variance (ANOVA).

Prebiotic activity assay [11], [13]

Prebiotic activity assay was determined based on the change in cell biomass from 0 hour to 24 hour of growth of probiotic strain on certain concentration of prebiotic compared to the same concentration of glucose relative to the change in cell biomass of a mixture of enteric strains grown under the same conditions. Quantitative data was obtained using equation by Huebner et al. (2007):

$$\text{Prebiotic activity score} = \frac{((xp24 - xp0) - (xg24 - xg0))}{(yp24 - yp0)\ (yg24 - yg0)} \quad (1)$$

x : probiotic (log OD),
y : enteric bacteria (log OD);
p : on prebiotic,
g : on glucose.
24 : after 24-hour incubation,
0 : after 0-hour incubation.

Prebiotic index [7], [11]

Prebiotic index (Ipreb) was calculated as the ratio of probiotic growth in the prebiotic containing solution compared to probiotic growth in a control carbohydrate. A value higher than 1 means that the carbohydrate has a positive effect on probiotic growth.

$$\text{Prebiotic index} = \frac{CFU\ of\ Probiotic\ in\ Prebiotic\ Solution}{CFU\ of\ Probiotic\ in\ Control\ Solution} \quad (2)$$

Prebiotic Solution : *Cilembu* sweet potatoes / Functional beverages / test subject
Control Solution : Glucose

Inulin content [10]

Prebiotic analysis was done to determine the prebiotic compound qualitatively and quantitatively, which is represented as inulin content. The standard solvent was made by weighing approximately 100 mg standard maltitol, dextrose, galactose, fructose, lactose, and sucrose and placing into volumetric flask 100 ml, dissolved with aquabidest homogeneously. This 1000 ppm liquor was diluted into 5, 25, and 50 ppm by adding internal standard concentration of 50 ppm. After filtration using 0.45 µm, it was inserted to a vial and injected to HPAEC. Sample was prepared by extraction. Sample was homogenized and weighed to 100 ml beaker glass containing 1 g fructan, added with ±40 ml hot water and KOH 0.05N or HCl 0.05N until the pH was in range of 6.5-8. The solution was placed in 100 ml volumetric flask, heated at 85ºC and poured to beaker glass and diluted up to 1% fructan concentration. An amount of 50 grams was taken for direct analysis (A) added by 15 g buffer acetate until the pH was 4.5 and added by 35 g amyloglucosidase (51U/mg). Incubation was done for 30 minutes at 60º±2ºC and weighed (B). An amount of 10 grams was added by inulinase solution (56 g enzyme/100 g fructan, incubated in water bath at 60ºC for 30 minutes. After cooling and weighing (C), the solution A, B, and C were diluted, added with internal standard (glucoheptose) 20 ppm. Samples were filtered and injected into HPAEC.

Reducing Sugar Content [14], [15]

Reducing sugar content can be calculated using the Luff Schoorl method. Luff Schoorl reagent was made by dissolving 143.78 grams of natrium carbonate (NaCO50 grams of citric acid, and 25 grams of $CuSO_4$ by adding distilled water in 1-liter volumetric flask. Sample was prepared by blending and filtering until homogenous. An amount of 10 mL of sample was put into Erlenmeyer flask with boiling chips and added by 15 mL distilled water and 25 mL Luff Schoorl reagent. The Erlenmeyer was then refluxed for 10 minutes and cooled down. An amount of 10 mL potassium iodide 20% (KI) and 25 mL sulfuric acid 25% (H_2SO_4) were added to the Erlenmeyer. Titration was done using natrium thiosulfate ($Na_2S_2O_3$) 0.1N until the color of the solution changed into light brown. Few drops of starch indicator 0.5% were added so that there was blue color on the solution. Titration continued until the solution becomes milky white in color. Blank was made with the same steps but replaced the sample with distilled water.

Results and discussion

Determination of the baking temperature and baking time for Cilembu sweet potato

Cilembu sweet potato used in this research was baked to produce desirable sweet taste and mushy texture. During baking, carbohydrates break down into simpler sugars (Chan et al , 2014; Lai et al., 2013; Woolfe, 1992). Thus, it is expected for the baked *Cilembu* sweet potato to have higher content of oligosaccharides which might act as a source of the prebiotic compound. best baking temperature and baking time is determined by the highest prebiotic activity score and highest total reducing sugar content among the treatments. Prebiotic content of baked *Cilembu* sweet potato is measured for its activity regarding its ability to support the growth of probiotic bacteria relative to other organisms and another non-prebiotic substrate.

Higher baking time and longer roasting period is found to increase the present of reducing sugars. Sample baked in high temperature produces higher sugar probably due to the action of starch hydrolysis. Starch is degraded by alpha-amylase enzyme and beta-amylase present in fresh sweet potato into disaccharides and monosaccharides including sucrose, glucose, and fructose. The starch content of baked sweet potato decreases while the reducing sugars increase, compared to fresh sweet potato [6], [16]..

Table 1. Prebiotic score and % reducing sugar

Treatment	% Reducing sugar	Prebiotic score	Prebiotic Index
150ºC, 60 min	1,700 ± 0,10[a]	0,575± 0,06[a]	0,89
150ºC, 90 min	2,933 ± 0,21[d]	0,733 ± 0,12[c]	0,90
150ºC, 120 min	2,233 ± 0,06[c]	0,703 ± 0,07[c]	0,86
175ºC, 60 min	1,800 ± 0,10[b]	0,800 ± 0,12[c]	0,92
175ºC, 90 min	2,233 ± 0,25[c]	0,712 ± 0,13[c]	0,86
175ºC, 120 min	2,767 ± 0,06[c]	0,653 ± 0,11[b]	0,91
200ºC, 60 min	2,667 ± 0,23[c]	0,803 ± 0,03[d]	0,91
200ºC, 90 min	3,100 ± 0,36[c]	0,866 ± 0,10[e]	0,93
200ºC, 120 min	2,300 ± 0,52[c]	0,717 ± 0,13[c]	0,79

The reducing sugar content of *Cilembu* sweet potato done in this research is much higher than the several other research on the sugar content of the sweet potatoes have sugar content ranging from 0.17-0.89%, depending on the cultivar[1], [8]. The difference result might be caused by different cultivar, plantation technique and environment, as well as sample preparation. Baked sweet potatoes had a significantly higher number of total sugars which included fructose, sucrose, maltose, and glucose. Sucrose was the major sugar in fresh sweet potato, higher than fructose and glucose, while maltose was almost not detected. However, during baking, maltose is formed and shown to become the major component of sugar in baked *Cilembu* sweet potato[6]. Baking temperature had significantly impacted sucrose degradation. The caramelization of sucrose not only changed color but also converted sucrose to form oligomers and polymers.

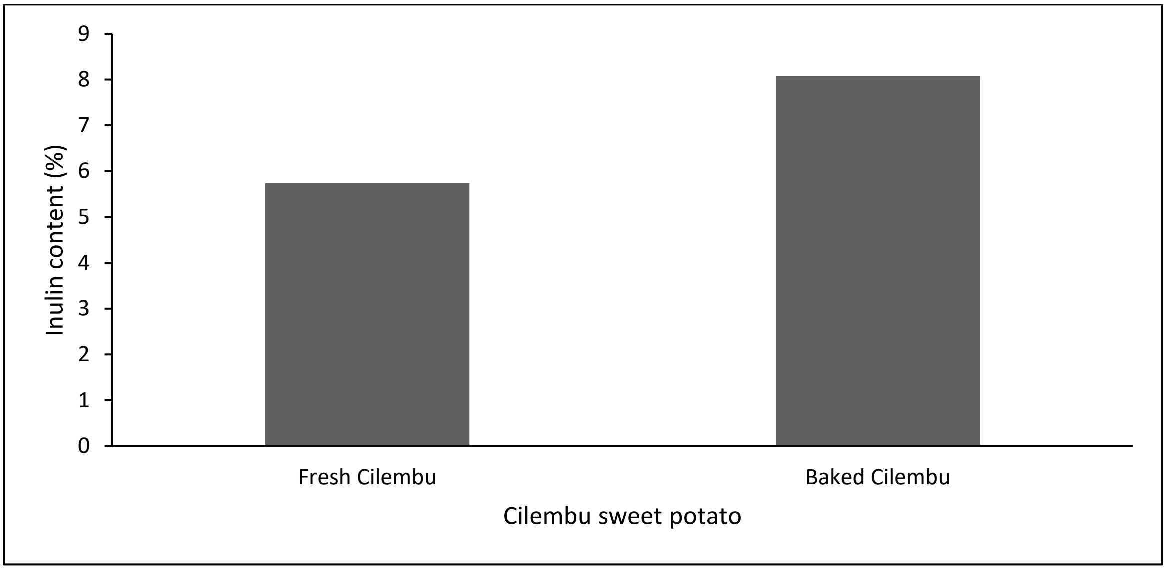

Figure 1. Inulin content of fresh *Cilembu* compared to baked *Cilembu*

After baking, it is shown that both baking time and period of baking also affected the prebiotic score of the baked *Cilembu* sweet potato. The rise in the temperature and the longer the *Cilembu* sweet potatoes is baked increases the prebiotic score, the same way it increases the reducing sugar content. The prebiotic score was shown to be highly correlated with the prebiotic index ll samples, in which the increase of temperature and time of roasting was shown to increase in prebiotic index. The *Cilembu* sweet potatoes that are baked for a longer period (90-120min in all temperature) showed an increase in the prebiotic index. However, prolong heating (200ºC for 120 min) shown to reduce the prebiotic index. From previous research, it is shown that after baking, there is a significant increase in the amount of raffinose and maltose from raw potatoes [16], [17], [18]. Raffinose is an oligosaccharide known for its ability to increase the growth of the gut microbiome [19]. From the HPLC analysis, it can also be seen that there is a significant increase in the inulin content of baked *Cilembu* sweet potatoes compared to the fresh ones. Baked *Cilembu* sweet potato contains inulin in the amount of 8.08%. The previous study stated that inulin compound of sweet potato is 0.33±0.04% [10]. This might happen due to various factors including the different cultivar, different techniques and environment, and others. In addition, the inulin of baked *Cilembu* sweet potato is higher than to the raw one which is 5.74% (Figure 1).

The increase in the oligosaccharide content of the baked sweet potatoes explains the possible rise of the prebiotic score and index of them. The highest prebiotic score was found in the hour baking time with 200ºC roasting temperature 0,866± 0,10. This score is comparably higher than several other research for fermented breadfruit (0.45), sargassum (0.58) and lower than the prebiotic score of longan flour (1.44)[11], [13], [20]. The prebiotic activity score of all treatments is 0.729±0.037 in average means that baked. *Cilembu* sweet potato could support the growth of probiotic bacteria better than other (enteric) bacteria. In conclusion, it can be noted that baked *Cilembu* sweet potato contains high prebiotic compounds.

Effect of different Cilembu sweet potato concentration and skim milk concentration toward physicochemical characteristics of the functional beverage

Cilembu sweet potato which is baked at 200°C and 90 minutes was then turn into a functional beverage with skim milk addition. Skim milk was added to increase the protein content of the beverages with yoghurt as a standard. Based on statistical analysis there is no significant difference of pH value among the products. Difference in sweet potato concentration and milk to water ratio also do not give significant difference among the products. The pH of all products is 6.79±0.01, which is close to neutral. This may indicate that the product is in normal condition and safe to consume. Although the normal pH of sweet potato is 5.30-5.60 the pH of the product could be higher due to the addition of water and milk. The normal pH of water is 7.0, while normal pH of milk is 6.6-6.7.

Table 2. Physical and sensory characteristic of the *Cilembu* sweet potato beverages

Parameter	Value
Protein	1.35 (% wb)
Fat	0.09 (% wb)
Ash	0.59 (% wb)
Carbohydrate	16.76 (% wb)
pH value	6.79±0.01
Hue Color	97.48±1.67
Viscosity	89-375 cps
Color	5.06-5.2
Aroma	4.71-4.95
Texture	4.42-4.63
Overall acceptance	4.65-4.88

Cilembu sweet potato functional beverage contains protein of 0.88%. The protein mostly comes from the addition of skim milk ("Diamond" brand) of which the protein content is approximately 4%. The protein content of sweet potato beverage is relatively low, ranging from 1-14.2% (db). The proper formula was measured for its prebiotic activity score.

The statistical analysis) shows there is no interaction between two factors (α=0.05). The factor of different sweet potato concentration and different milk to water ratio do not affect the hue value of all samples. Hue values of all samples are 97.48±1.67, representing the yellow color. The yellow color is contributed by sweet potato due to its β-carotene pigment, which is influenced by the addition of milk and water that give white and transparent color respectively [1], [8]. Since the difference of sweet potato concentration used in each treatment is only 5% (w/v), it might cause no significant difference in term of color.

The viscosity of *Cilembu* sweet potato functional beverage was measured using viscometer. The result is presented in centipoise (cP) unit as shown Table 3. According to statistical analysis increasing sweet potato concentration and ratio of milk to water will also increase the viscosity of *Cilembu* sweet potato functional beverage. There are several factors that affect the viscosity of a product are concentration of solute and presence of suspended matter. There is usually positive nonlinear relationship between the concentration of the solute and the viscosity at constant temperature. In this case, the solute concentration is conditioned by the milk to water ratio. On the other hand, suspended matter may increase the viscosity depending on its concentration. It may also affect the type of viscosity flow. In this research, the suspended matter is contributed by the sweet potato with the presence of the solid matter will increase the viscosity of the beverage.

Hedonic test or affective test is a method that offers people choices of some products and sees if there is any clear preference from the majority of the panellists. In this research the functional drink sensory acceptance is assessed based on the aroma, flavor, and color acceptance. Study shows that color can affect the flavor intensity, sweetness and salinity, susceptibility, preference and even judgment of safety of products. Thus, color is commonly used to evaluate food quality. As described, panelists' preference toward the color of all products lies on value above 5, which means slightly like. The addition ofsweet potato concentration and higher milk to water ratio generate a significantly darker color. This result correlates to the color objective measurement in chapter that the lower concentration of sweet potato gives a lighter color. Aroma is one of the most important factors related to food palatability because it directly affects the flavor perception in the mouth, even before the taste does. *Cilembu* sweet potato functional beverage was evaluated for its aroma acceptance.

In term of aroma, the aroma acceptance of all samples is 4.81±0.08 in average, which means that the panelists slightly like the products' aroma. Different sweet potato concentration and different milk to water ratio affect the aroma intensity significantly. However, the sweet potato aroma does not affect the preference of the panelists. The key feature that contributed to the acceptance of the functional drink is the taste of the product. Taste comes through the interaction between water-soluble molecules with receptors on the tongue and oral cavity receptors that will transmit that impulse to the brain. Higher concentration of sweet potato gives by a sweeter taste derived from the amount of sweet potato paste added and shown to increase the overall likeness of the product.

Sample of 1 ml *Cilembu* sweet potato functional beverage was taken and analyzed and compared with functional beverages made with pure inulin and yam, to compare their prebiotic effect in food matrix.

Table 3. Prebiotic score and prebiotic index of the *Cilembu* sweet potato beverages

Analysis	*Cilembu* Beverages	Yam Based Beverages	Inulin Based Beverages
Prebiotic Score	1.334 ±0.037.	0.635 ±0.015	1.520 ±0.042
Prebiotic Index	0.945	0.729	1.290

The result shows that *Cilembu* sweet potato functional beverage has prebiotic activity score of 1.334 ±0.037. and prebiotic index 0.945, close to 1. This score is higher than the average prebiotic activity score and prebiotic index of baked *Cilembu* sweet potato in table1. Pure inulin-based beverages shown to have the highest prebiotic score and index. Inulin can be fermented by probiotic bacteria resulting in the production of short-chain fatty acids that lower the pH in the colon thus inhibit the growth of pathogenic bacteria [10][21]. The ability of prebiotic source food to enhance the growth of probiotic bacteria can be seen from Figure 2.

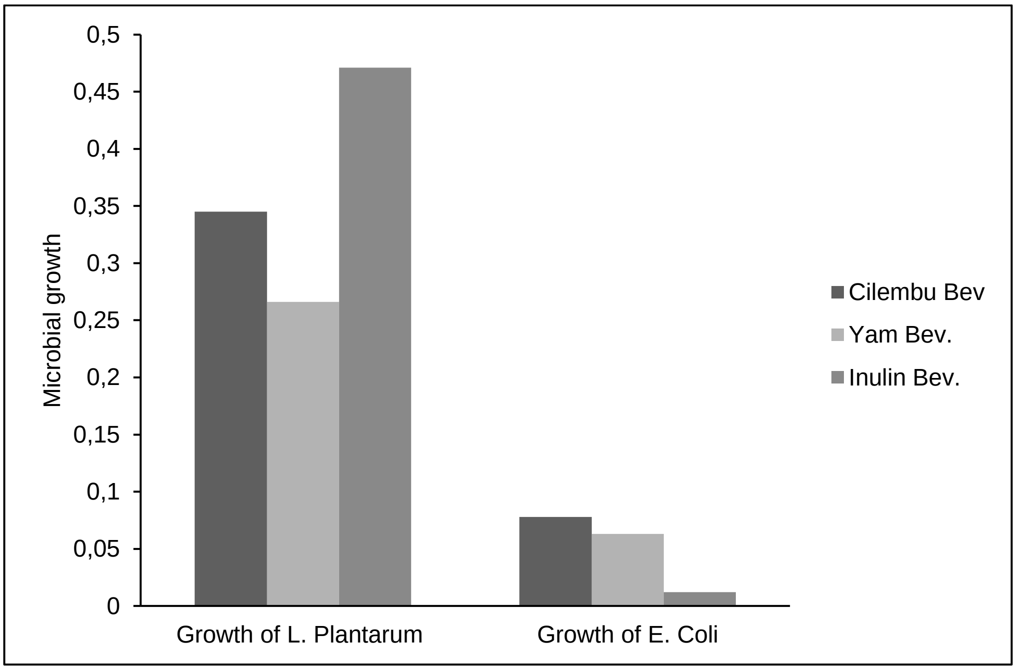

Figure 2. Microbial growth of *L. plantarum* and *E. coli* (OD_{600}) after 24 hours

From Figure 2, the different growth of probiotic microorganism (*L. plantarum*) and pathogenic microorganism (*E. coli*) after 24h in the functional beverages can be observe. It can be seen that all three beverages are able to induce the growth of probiotic microorganism significantly better than the pathogenic bacteria. This result is in accordance with the prebiotic index score of the three beverages. *Lactobacillus* strains had the ability to ferment, not only sugars but also the oligosaccharides. In previous research, similarly the presence of fructo-oligosaccharide and inulin caused an increase in the numbers of all probiotic bacteria, indicating a possible synergetic effect[21]. *Cilembu* based beverages is shown to able to support the growth of probiotic bacteria compare to the pathogenic bacteria.

Conclusion

Baking sweet *Cilembu* shown to increase the prebiotic activity by converting the carbohydrate into reducing sugar newly formed oligosaccharides such as inulin that can acts as a prebiotic source. The baked Cilembu sweet potatoes shown to have a significant increase in their inulin content and total reducing sugar compared to the fresh *Cilembu* sweet potatoes, indicating the breakdown of long chain carbohydrate into smaller oligosaccharides. *Cilembu* sweet potato with baking condition at 200ºC for 90 minutes contains inulin about 8.08%, as representative of prebiotic compounds, have a prebiotic activity score of 0,866 ± 0,10, a prebiotic index of 0.93 and a total reducing sugars of 3,100 ± 0,36.The *Cilembu* sweet potato functional beverage made with 35 g sweet potato and 35:65 milk to water ratio resulted in a functional beverage with nutritional composition of 1.35% protein, 0.09% fat, 0.59% ash, and 16.76% carbohydrate with moderate likeness and have a prebiotic activity score of this 1.334 ±0.037 and prebiotic index of 0.945.

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