Isolation and Characterization of Inulin from Sweet Potato (*Ipomoea batatas* L. Cilembu) with Maggot Frass Fertilization as Prebiotic in Synbiotic Yoghurt

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Abstract. Cilembu sweet potato (*Ipomoea batatas* L. Cilembu) has a sweet potato with high inulin content. However, sweetness and inulin content decrease if sweet potatoes are planted outside the optimal environment. Thus, this study aimed to test the effect of maggot frass fertilizer on the inulin content of Cilembu sweet potato and evaluate its bioactivity as a prebiotic. The research methods included inulin isolation from Cilembu sweet potato, inulin identification, prebiotic activity test, yoghurt starter production, synbiotic yoghurt fermentation, and yoghurt analysis. Inulin was isolated by precipitation using a mixture of ethanol and water to obtain inulin powder yields of different fertilization doses: 0 (P1), 5 (P2), and 10 (P3) kg/5 m^2, of 10.01, 21.07, and 22.97%, respectively. The results of in vitro prebiotic activity tests have shown an increase in the growth of *Lactobacillus bugaricus and Streptococcus thermophilus*. Inulin powder from sweet potato increased the viability of lactic acid bacteria in synbiotic yoghurt, with the highest increase at P3 dose with 5% inulin concentration (2.5×10^9 cfu/mL), highlighting its potential as a probiotic. In conclusion, maggot frass fertilizer increased the inulin content in Cilembu sweet potato and prebiotic activity in yoghurt.

1 Introduction

The development of functional food products has increased over time. This is also influenced by increasing public awareness of a healthy lifestyle by consuming foods that are beneficial to health. Yoghurt is one of the most well-known functional food products and a healthy food source of nutritional value. Yoghurt is a dairy product made through lactic acid fermentation by lactic acid bacteria species, namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus [1]. Yoghurt consumption from year to year

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continues to increase; as assessed from food consumption figures, the consumption rate of fermented milk, including yoghurt, for Indonesians per day is 155 g/person [2]. Thus, the consumption rate of yoghurt in Indonesia can be used as a potential for developing new products. The quality of yoghurt can be improved by combining the benefits of probiotic bacterial starter cultures with prebiotics; the combination of the two is called synbiotics [3]. Synbiotics can synergistically affect the function of probiotics because they provide suitable substrates.

Synbiotic yogurt consists of beneficial bacteria in the form of probiotic microorganisms and indigestible carbohydrates known as prebiotics. Prebiotics stimulate the growth of bacteria that benefit the body [4]. Inulin is commonly used as a prebiotic. It is one of the most widely used prebiotics worldwide, for example, in the food industry as a sugar substitute, stabilizing agent, texturizing agent, and in functional food development.

The world's source of inulin is from the extraction of Jerusalem artichoke (*Helianthus tuberosus* L) and chicory (*Cichorium intybus* L). However, these plants are also cultivated in temperate regions [5]. Therefore, their distribution is limited and difficult to find in Indonesia. *Dahlia* sp., *Dioscorea esculenta*, and *Pachyrhizus erosus*, which can produce inulin and grow in Indonesia. However, their cultivation is difficult with limited results [6]. Therefore, alternative sources of inulin, such as Cilembu sweet potatoes, are required in Indonesia. According to previous research, Cilembu sweet potatoes have the highest inulin content compared with other types of sweet potatoes [7]. Cilembu sweet potato had the highest inulin content (6.47 %) compared with several other tubers: purple sweet potato, white sweet potato, *gadung, uwi, ganyong,* and arrowroot. Ubi Cilembu has a high total sugar content, especially fructose, compared to other sweet potato varieties, making it the primary source of inulin [8].

Sweet potato Cilembu is a superior local commodity from Cilembu Village, West Java, Indonesia. It is known for its sweet honey-like flavor, chewy meat structure, good taste, and soft texture [9]. The sweetness of Cilembu sweet potatoes is influenced by fertilization factors. Black soldier fly (BSF) maggot frass fertilizer is an organic fertilizer that supports plant growth. This fertilizer contains macro- and micronutrients and beneficial microorganisms such as nitrogen-fixing bacteria and phosphate solvents. This study aimed to isolate and characterize inulin obtained from Cilembu sweet potato treated with maggot frass fertilizer and compare it with the functional groups of the standard inulin. In addition, tests were conducted to determine the effect of inulin addition at several concentrations on the quality of synbiotic yogurt and the role of inulin as a prebiotic on bacterial growth in synbiotic yogurt.

2 Materials and method

2.1 Materials

Cilembu sweet potato seedlings were obtained from Kebun Merdesa (Bogor, Indonesia). Maggot frass fertilizer from PT. Biomagg Sinergi Internasional, Depok, Indonesia. *Lactobacillus bulgaricus* (Lb) and *Streptococcus thermophilus* (St) were obtained from the IPB Culture Collection, IPB University. Standard inulin (chicory), standard glucose, fructose, starch, de Man Rogosa Sharpe Agar (MRSA), de Man Rogosa Sharpe Broth (MRSB), 3,5-dinitrosalicylic acid (DNS) reagent, 30% ethanol, petroleum ether, Whatman filter paper No. 40, concentrated sulfuric acid (H_2SO_4), potassium sulfate (K_2SO_4), 40% sodium hydroxide (NaOH), boric acid (H_3BO_3), and 0.1 N standard sulfuric acid (H_2SO_4) were bought from Sigma, Germany.

2.2 Isolation, purity measurement and characterization of inulin from Cilembu sweet potato

The inulin isolation method described by Andrinto *et al.* (2022) [10]. Cilembu sweet potato powder received maggot frass fertilizer with P1, P2, and P3 concentrations of 0, 5, and 10 kg/5 m^2, respectively. The materials used were previously analyzed for proximate and reducing sugars. Water was added to the powder in a ratio of 1:2 (w/v). NaOH was then added to the solution until the pH level was 7, and the solution was heated to 80 °C for 30 min. The solution was filtered after cooling, and the filtrate was collected. The filtrate was added to 30% ethanol up to 40% of the filtrate volume. The solution was kept for 24 h until a precipitate was formed. The separation was performed by gradually reducing the filtrate until a precipitate was obtained. The precipitate thus obtained was inulin. Inulin was re-isolated by adding water to the wet inulin. The precipitate was then dried and pulverized. The inulin content was determined by calculating the difference in reducing sugars before and after hydrolysis, then multiplying by a constant of 0.995 according to Saengkanuk *et al.* [14]. The amount of reducing sugar was measured using the DNS method. The functional groups of the isolated inulin were characterized using the osazone method and an FTIR spectrophotometer.

2.3 Preparation of synbiotic yogurt

Yoghurt was prepared as previously described by Indriantianti *et al.* (2015) [11]. In the preparation of the yoghurt starter, 50 mL of fresh milk was pasteurized at 80 °C for 15 min while stirring. The milk was left at 37 °C and inoculated with bacteria (2.5 mL). Milk was then incubated at 37 °C for 24 h. Yoghurt fermentation was carried out with milk, with every 50 mL added with a variation in inulin concentration (0, 1, 3, and 5%). The milk was pasteurized at 80 °C for 15 min, then cooled to 37 °C, and 5 % (v/v) yoghurt seeds consisting of 2.5% *Lactobacillus Bulgaricus* and 2.5% *Streptococcus thermophilus* were added. The fermentation was performed at 37 °C for 7 h.

2.4 Quality testing of synbiotic yogurt

Yogurt quality testing was then performed according to Indriantianti *et al.* (2015) [11]. The synbiotic yoghurt quality parameters analyzed included lactic acid bacteria (LAB) viability, pH (acidity), protein, fat, moisture, and ash content. LAB viability was assessed by counting the number of colonies formed using the Standard Plate Count (SPC) method and expressed as colony-forming units per mL (cfu/mL) [15]. The pH was measured using a pH meter. Protein content was determined using the Kjeldahl method, while fat content was analyzed using the Soxhlet extraction method. Water and ash contents were measured using the gravimetric method.

2.5 Prebiotic activity assay

Prebiotic activity was evaluated using suspensions of *Lactobacillus bulgaricus* (Lb) and *Streptococcus thermophilus* (St). 0.2 mL bacterial suspension was added to a Petri dish, followed by 0.25 mL of inulin solution at concentrations of P1, P2, and P3. Subsequently, 4.75 mL the molten MRSA medium was added to the Petri dish. The mixture was homogenized and allowed to solidify at room temperature. Once solidified, plates were incubated in an inverted position at 37 °C for 24 h. The number of colonies formed was determined using the Standard Plate Count (SPC) method and expressed in colony-forming units per milliliter (cfu/mL).

2.6 Data analysis

The data analysis in this study was a qualitative analysis of FTIR readings of standard inulin and inulin isolated from Cilembu sweet potatoes. Quantitative data included yogurt quality using ANOVA and Duncan's test at the 95% level. Software used in data analysis was Microsoft Excel and SPSS 24.

3 Result and discussion

3.1 Inulin isolation from Cilembu sweet potato

The isolated inulin from Cilembu sweet potato was obtained in the form of a white powder (Figure 1), with inulin yields of 6.00, 12.64, and 13.78 grams from an initial dry weight of 60 grams, resulting in yields of 10.01%, 21.07%, and 22.97%, respectively. Based on the extraction results in Table 1, treatment P3 produced the highest yield, whereas *Dioscorea esculenta* (lesser yam) showed the lowest yield. Several factors, including genetic variation, harvesting age, growing environment, and tuber chemical composition, influence inulin yield. A higher starch content generally results in greater yield. Additionally, the filtration process significantly affects the yield; a greater amount of soluble starch separated from the tuber residue can lead to a higher yield. Smaller starch granules tend to be lost during extraction, which may reduce the final yield [8]. Cilembu sweet potato contains starch granules that settle quickly and tend to adhere to the bottom, contributing to a higher yield owing to its high carbohydrate content.

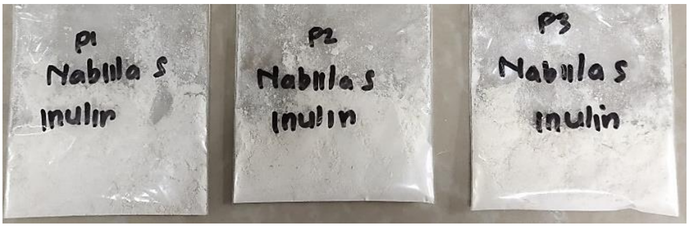


Fig. 1. Inulin Isolation Results from Cilembu Sweet Potato. The doses of maggot frass fertilizer were variated, P1 is 0 kg/5 m^2, P2 is 5 kg/5 m^2, and 10 kg/5 m^2

3.2 Measurement of Purity of Isolation Results

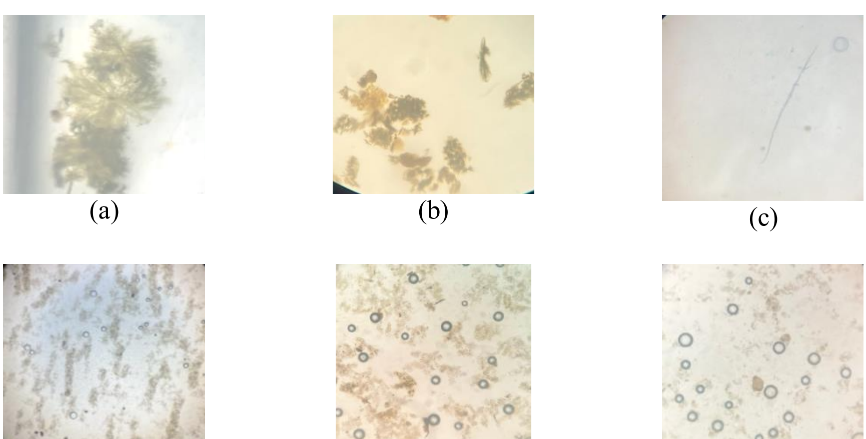
The results of the reducing sugar measurements before and after hydrolysis and the calculation of inulin purity are presented. The purities of inulin isolated from Cilembu sweet potato were 38.93%, 37.24%, and 39.30%, respectively, whereas the actual inulin contents obtained were 3.89%, 7.84%, and 9.02%, respectively. Inulin content was calculated by multiplying the inulin yield percentage by the inulin purity percentage. The data on inulin yield, purity, and actual content for each treatment are shown in Table 1. The study showed that the highest inulin mass was produced from Cilembu sweet potatoes. Environmental factors can influence inulin content; high soil moisture tends to reduce inulin concentrations, and conversely, lower soil moisture can lead to higher inulin levels.

Table 1. Yield, purity, and actual content levels of inulin from Cilembu sweet potatoes with different treatments and several inulin-producing tubers.

Material	Yield (%)	Purity	Actual content
P1	10.01	38.93	3.89
P2	21.07	37.24	7.84
P3	22.97	39.30	9.02
Gembili[9]	2.32	67.66	1.56
Dahlia[9]	3.22	78.21	2.51
Chicory[6]	14	95	13.3
Jerusalem Artichoke[6]	21	18.75	3.93
Yacon[7]	4.86	44	2.15

3.3 Characterization of Functional Groups by Osazone Test and FTIR Spectroscopy

The osazone test was the first characterization performed on the isolated inulin, as shown in Figure 2. An osazone test was conducted to observe the crystal morphology of the isolated inulin, which was then compared with the crystal forms of standard inulin (chicory), standard glucose, standard fructose, and starch.



(a) (b) (c)

(d) (e) (f)

Fig. 2. Osazon test results (a) cassava starch, (b) fructose, (c) glucose, (d) chicory inulin standard, (e) inulin P1, (f) inulin P2, (g) inulin P3, (a-g) magnification 100 times, (h) chicory inulin standard, (i) yacon inulin ((h-i) magnification 40 times [10].

The results of osazon testing are shown in Figure 2, which shows the shape of glucose crystals, such as needle-like elongated crystals and needle-like fructose, that gather in association with simple carbohydrate compounds with osazon. The isolated inulin had a crystal structure similar to that of standard sigma inulin after mixing with the osazon reagent. It can be seen from the cotton-ball-shaped inulin crystals of all the tested inulins. This aligns with previous research showing that starch crystals look like a large cotton ball structure, while inulin is shaped like a smaller cotton ball [10].

Subsequent characterization was conducted using Fourier-transform infrared (FTIR) spectroscopy. The FTIR spectra of chicory inulin and isolated inulin are shown in Fig. 3. FTIR spectroscopy is commonly used to characterize carbohydrates based on their functional groups [10]. The interpretation of FTIR spectra requires a correlation table, which indicates the types of functional groups corresponding to specific wavelength ranges and can be applied to identify various chemical compounds. The FTIR spectrum of isolated inulin was compared to that of standard inulin (chicory).

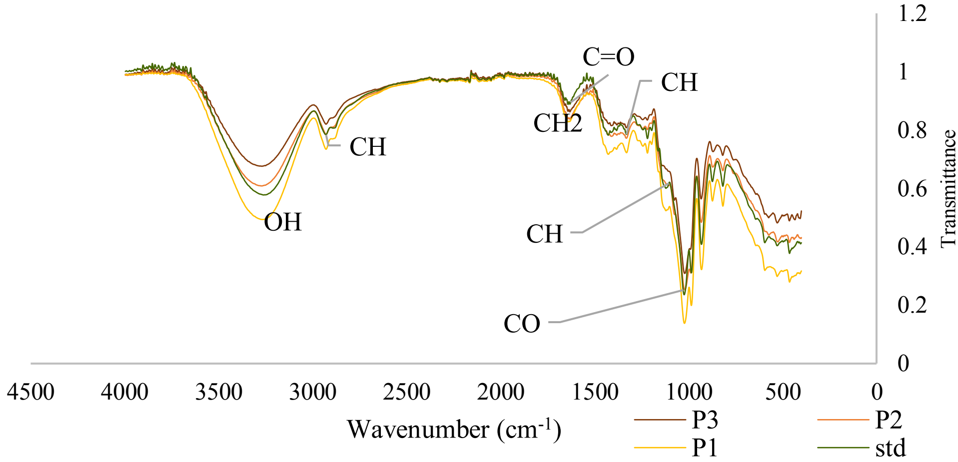


Fig. 3. FTIR spectrum of chicory inulin, inulin isolated from Cilembu sweet potato P1, inulin isolated from Cilembu sweet potato P2, and inulin isolated from Cilembu sweet potato P3

The FTIR spectrum of chicory inulin was compared with the wave correlation table, which shows the functional groups present in the sample (Table 2). Characterization of the isolated inulin using FTIR was compared with that of chicory inulin as a control. FTIR spectra of standard inulin compared with inulin sourced from P1, P2, and P3 are shown in Table 2. The functional groups of inulin P1, P2, and P3 were similar to the standard inulin functional groups, namely, OH, CH, CH2, C=O, and C-O groups. This is in line with research conducted by Andrianto *et al.* [10]. This indicates that the functional groups identified based on the absorption bands at wave numbers in chicory inulin are almost the same as those of isolated inulin; thus, the FTIR characterization of P1, P2, and P3 from Cilembu sweet potato is similar to that of chicory inulin based on the number value and shape of the spectrum.

Table 2. FTIR wave number values of literature, standard inulin and isolated inulin

Wavenumber value (cm^{-1})					
Chicory Inulin (standard)	P1	P2	P3	FTIR Literature[7]	Functional group
3255	3257	3312	3296	3400-3200	OH
2929	2925	2916	2929	29 40-2915	C-H
1627	1623	1650	1648	1750-1600	C=O
1455	1453	1459	1467	1485-1445	CH_2
1330	1342	1322	1320	1350-1320	C-H
1105	1113	1142	1109	1225-950	C-H

3.4 Quality characteristics of synbiotic yoghurt

Analysis of the quality of synbiotic yoghurt included the viability of lactic acid bacteria (LAB). Viability is the number of cells that are still alive in a population. A high viability of LAB cells indicates better yogurt quality [12]. Adding inulin can improve the quality of yoghurt, as indicated by the increase in LAB viability shown in Table 3.

Table 3. Quality analysis result of synbiotic yoghurt

Treatment	**LAB viability (cfu/mL)**	**pH**	**Protein content (%)**	**Fat content (%)**	**Water content (%)**	**Ash content (%)**
$P_1$0%	1,58 x10^7	4,53	4,34^c	3,94	83,78^g	0,18^a
$P_1$1%	1,73x10^7	4,48	4,44^d	3,91	82,73^d	0,65^b
$P_1$3%	2,03x10^8	4,4	4,10^a	4,15	82,73^c	0,68^c
$P_1$5%	2,81x10^8	4,35	4,51^e	4,35	82,00^b	0,85c
$P_2$0%	1,73 x10^7	4,49	4,34^c	3,87	82,97^f	0,15^a

$P_2$1%	1,50 x10^8	4,41	4,48^d	4,20	82,89^d	0,69^c
$P_2$3%	2,95x10^8	4,4	4,92^g	4,28	82,54^c	0,70^b
$P_2$5%	2,50x10^8	4,38	4,80^f	4,39	81,60^b	0,87^{cd}
$P_3$0%	1,92 x10^7	4,47	4,34^c	3,85	83,31^{fg}	0,18^a
$P_3$1%	1,09 x10^8	4,47	4,84^f	4,30	82,67^e	0,68^b
$P_3$3%	2,89x10^8	4,3	4,29^c	4,34	81,64^c	0,90^{cd}
$P_3$5%	2,02x10^9	3,9	4,25^b	4,36	81,30^a	0,92^d

Notes: Numbers followed by the same letter in the same column indicate no significant differences based on Duncan's test ($\alpha = 0.05$).

The viability of lactic acid bacteria was highest in yoghurt supplemented with 5% inulin and P3 and lowest in yoghurt supplemented with 0% inulin. The addition of inulin resulted in significant growth of LAB. All synbiotic yoghurts produced met the required number of bacteria Codex Stan 243-2003. This aligns with previous research showing that the highest viability of lactic acid bacteria was found in yogurt with 7% inulin (1.0×10^8 cfu/mL), and the least with 0%, namely 1.58×10^7 cfu/mL [11].

Yoghurt formula with lactic acid bacteria cultures of *S. thermophilus* and *L. bulgaricus* obtained pH values ranging from 4.0 to 4.5. The quality requirement for good yogurt is a pH level of 3.90 - 4.50 [12]. The addition of inulin decreased the pH to 3.9, which remained within the limits set by the Indonesian National Standard (SNI). A decrease in pH was observed in yogurt with the addition of 5% inulin sourced from P3. This was in line with previous research showing that increasing the amount of starch can reduce the pH of synbiotic yogurt [13]. A decrease in pH is influenced by the activity and growth of lactic acid bacteria (LAB), which produce pectinolytic and cellulolytic enzymes. These enzymes break down the starch cell walls, resulting in a concentration gradient: a higher concentration within the LAB due to enzyme release and a lower concentration in the starch as its structure degrades. Consequently, starch enters the bacterial cells, where it and lactose are hydrolyzed and converted into lactic acid.

The protein content of synbiotic yoghurt did not exhibit a consistent trend of increase or decrease with the addition of inulin. Previous research has shown that prebiotic addition has no effect on the protein content of synbiotic yogurt [14]. In contrast, the highest fat content (4.39 %) was observed in the sample with 5% inulin derived from the P3 source, whereas the lowest fat content (3.85 %) was recorded in the sample without inulin (0%). This is because inulin extracted from P3 Cilembu sweet potato has a higher intrinsic fat content than that extracted from P1, as shown in a previous study. The addition of inulin significantly increased the fat content. The initial fat content of the raw materials plays a crucial role in determining the final fat content of yoghurt. These results are consistent with those of previous studies indicating that prebiotic supplementation affects the fat content of synbiotic yoghurt. For instance, the addition of three different prebiotic sources—mocaf, commercial inulin, and suweg flour—demonstrated that suweg flour produced the highest fat content (approximately

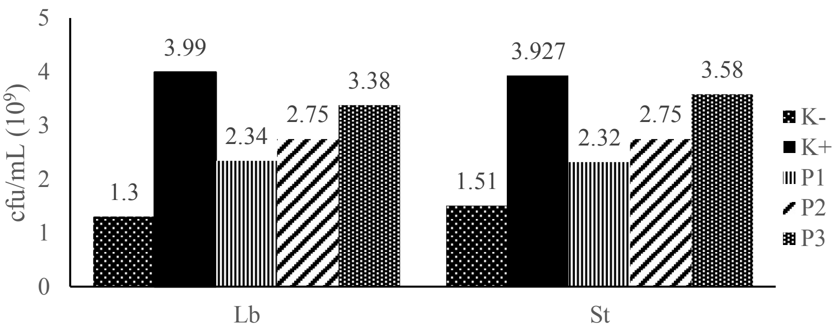
5.13%) and the highest protein content at a 4% concentration [3]. A higher fat content contributes to improved flavor and a smoother texture in yoghurt.

The highest water content in the synbiotic yogurt was 0% inulin addition (83.37%), and the lowest was 5% inulin addition in P3 (81.30%). The addition of inulin significantly reduced the water content. The decrease in water content was in line with the increase in viscosity owing to the addition of prebiotics. Previous research has shown that the addition of prebiotics from purple sweet potato extract to yogurt causes an increase in total solids, increased viscosity, and decreased water content. A higher concentration of prebiotics makes yogurt thicker because of the increase in total solids. During fermentation, insoluble compounds are converted into a more soluble form, which decreases water content [12]. This is also in line with previous research related to the analysis of the lowest water content, namely in synbiotic yogurt with 4% commercial inulin compared with lower concentrations (2% and 3%) . The moisture content was lower than the standard results in textural instability, but did not affect other properties [15].

The ash content shows the mineral content in yogurt, which is good for the body and for LAB to survive in the intestine. In food products, ash content is influenced by the mineral content of the starting material. As shown in Table 3, the highest ash content was found in yogurt with 5% inulin (0.93%), and the lowest in 0% inulin (0.19%). The addition of inulin resulted in a significant increase in the ash content. The value obtained met the ash content of yoghurt required according to a maximum SNI of 1% [2]. Previous research showed that the addition of 2% commercial inulin produced the lowest ash content (0.86%), whereas the addition of 4% *suweg* flour produced the highest ash content (1.02%) [3]. Synbiotic yogurt with the addition of inulin tends to increase the ash content because of yogurt due to its increased mineral content [12].

3.5 Prebiotic activity assay result

The results of the prebiotic activity test of inulin isolated from Cilembu sweet potato were compared with those of the positive control (chicory inulin) and negative control (without inulin), as shown in Figure 4. The prebiotic activity test showed that adding inulin powder from Cilembu sweet potato (P1, P2, and especially P3) increased the number of L. bulgaricus and S. *thermophilus* colonies compared to media without inulin. P3 inulin, in addition to 5% inulin, resulted in the highest colony growth compared to P1, P2, and the control.



Prebiotic activity tests showed that adding inulin from Cilembu sweet potato (P1, P2, and especially P3) increased the number of Lb and St colonies compared to media without inulin. P3 inulin, in addition to 5% inulin, resulted in the highest colony growth compared to the others. The results of the prebiotic activity tests are shown in Figure 4. This is also in line with previous research showing that inulin from Jombang plants can increase the number of colonies of *L. bulgaricus* and *Bifidobacterium bifidum* [11]. It was observed that higher inulin supplementation in synbiotic yoghurt led to an increased population of *L. bulgaricus* and *S. thermophilus* compared to the control sample, in line with the increasing inulin concentration. This increase in probiotic counts is attributed to the role of inulin as a prebiotic compound [4].

4. Conclusions

Inulin from Cilembu sweet potato, with the highest amount of fertilization, can increase prebiotic activity by increasing the number of Lactobacillus bulgaricus and Streptococcus thermophilus. Inulin from Cilembu sweet potato is of good quality when preparing synbiotic yogurt. The results of this study have the potential to be produced on an industrial scale as a synbiotic yogurt drink that is beneficial to health.

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