



## Research article

# Potential of *Musa sapientum* Linn. for digestive function promotion by supporting *Lactobacillus* sp.



Patthanakorn Jaiturong<sup>a,b</sup>, Nachtharinee Laosirisathian<sup>b</sup>, Busaban Sirithunyalug<sup>b</sup>, Sukum Eitssayeam<sup>c</sup>, Sasithorn Sirilun<sup>b,d,e</sup>, Wantida Chaiyana<sup>b,e,f,\*</sup>, Jakkapan Sirithunyalug<sup>b,f,\*\*</sup>

<sup>a</sup> Graduate School, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>b</sup> Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>c</sup> Department of Physics and Materials, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>d</sup> Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceutical, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>e</sup> Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand

<sup>f</sup> Research Center of Pharmaceutical Nanotechnology, Chiang Mai University, Chiang Mai, 50200, Thailand

## ARTICLE INFO

## Keywords:

Food science  
Food technology  
Microbiology  
*Musa sapientum*  
*Lactobacillus*  
Resistant starch  
Food supplements  
Functional food  
Digestive function

## ABSTRACT

*Lactobacillus* is a beneficial bacteria that could inhibit pathogenic potential of other microorganisms. This is the first study to develop a potential tablet from *Musa sapientum* Linn. (locally known as Kluai Namwa) using the direct compression method to support *Lactobacillus* sp. We compared the amount of resistant starch and prebiotic properties of the dry powder from unpeeled raw fruit, peeled raw fruit, and starch from *M. sapientum*. These dry powders were formulated into tablets using the direct compression method and evaluated for their prebiotic index compared to their native powder. Resistant starch, which possessed the highest prebiotic index, generated a tablet that possessed remarkable in vitro prebiotic properties. All tablets met the requirement of the United States Pharmacopeia. Therefore, resistant starch tablets from *M. sapientum* are suggested for use as a health promotion product.

## 1. Introduction

*Lactobacillus* is a large species of facultative anaerobic gram-positive rod-shaped bacteria that is a member of the normal human microbiota, belonging to the lactic acid bacteria group (LAB). *Lactobacillus* have been recognized as a normal flora that inhibit pathogenic bacteria by producing antimicrobial substances, such as bacteriocins, hydrogen peroxide, and lactic acid. The activity of *Lactobacillus* sp. relies on the production of bacteriocins and low molecular weight compounds that limit the pathogenic potential of other microorganisms (Martinez et al., 2014; Wang et al., 2014).

*Lactobacillus* is generally found in various parts of the human body, e.g., the digestive tract, urinary system, and genital system. Additionally, *Lactobacillus* can be found in some fermented foods such as cheeses, yogurt, and wine, as well as dietary or food supplements that contain live bacteria (Mcfarlane and Cummings, 1999; Martinez et al., 2014; Goldstein et al., 2015). Since *Lactobacillus* has been reported as an effective treatment to prevent both infectious and antibiotic-associated diarrhea

(Vanderhoof et al., 1999), many people take food supplements containing *Lactobacillus* to treat general digestion problems. *Lactobacillus* was reported to be beneficial for irritable bowel syndrome (IBS), chronic idiopathic constipation (CIC) (Ford et al., 2014), breastfed infants with colic (Johnson et al., 2015; Sung et al., 2018), inflammatory bowel disease (IBD) (Le and Yang, 2018), the growth inhibition of *H. pylori* in the gut (Liyuan et al., 2018), stomach pain, constipation, colon inflammation, and control of cancer progression through postponing the metastasis process (Heydaria et al., 2019; Yuea et al., 2020). However, products from *Lactobacillus* have a short shelf life, requiring special storage condition and high technology in the production process. Therefore, consumption of food supplements that promote the *Lactobacillus* growth would be an alternative attractive study.

Resistant starch has been used as a major component in food supplements used to promote beneficial bacteria growth (Sajilata et al., 2006; Fuentes-Zaragoza et al., 2010). It is not hydrolyzed after consumption (about 120 min) and not absorbed in the stomach and small intestine either (Englyst et al., 1992; Asp et al., 1996). Thus, it is an

\* Corresponding author.

\*\* Corresponding author.

E-mail addresses: [wantida.chaiyana@gmail.com](mailto:wantida.chaiyana@gmail.com) (W. Chaiyana), [jakkapan.s@cmu.ac.th](mailto:jakkapan.s@cmu.ac.th) (J. Sirithunyalug).

<https://doi.org/10.1016/j.heliyon.2020.e05247>

Received 2 September 2020; Received in revised form 18 September 2020; Accepted 8 October 2020

2405-8440/© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

excellent substrate to ferment in the digestive tract via *Lactobacillus*, leading to the formation of short-chain fatty acids (SCFAs) (Bird et al., 2000; Topping and Clifton, 2001). Especially resistant starch type 2 (RS2) are a nature one in a certain granular form found in some plants including green bananas and raw potatoes (Asp et al., 1996; Sajilata et al., 2006). *Musa sapientum* Linn. (a banana locally known as Kluai Namwa) is an herbaceous perennial plant belonging to the family Musaceae, which has been cultivated throughout Thailand. Moreover, *M. sapientum* is easily obtainable, cheap, and commonly consumed. The unripe or green *M. sapientum* is a good source of excellent RS2 (Vatanasuchart et al., 2012). However, during the ripening process, enzymes change the banana flour to simple sugar. Therefore, RS2 is resistant to enzymes when they have not been cooked (Gao et al., 2016).

*M. sapientum* powder is consumed but has not been produced into a tablet form before. Therefore, this study aimed to develop a tablet form of unpeeled raw *M. sapientum* fruit powder (URB), peeled raw *M. sapientum* fruit powder (PRB), and *M. sapientum* starch (BS) to enhance the compliance of consumers. Additionally, a tablet form is portable and convenient for consumers to have an exact amount of resistant starch.

## 2. Materials and methods

### 2.1. Plant materials

The unpeeled raw *M. sapientum* fruit powder (URB), peeled raw *M. sapientum* fruit powder (PRB), and *M. sapientum* starch (BS) were prepared from raw *M. sapientum* (Kluai Namwa) fruits aged 90–120 days. They were collected in the Mae Wang District, Chiang Mai Province, Thailand from January 2017 to March 2017. The voucher specimen of *M. sapientum* was recorded as KNL-001 and kept in a herbarium at the Faculty of Pharmacy, Chiang Mai University, Thailand.

### 2.2. Chemical reagents

Magnesium stearate was purchased from Riedel-de Haen, Seelze, Germany. Strach1500® was purchased from Rama Production Co., Ltd, Bangkok, Thailand. *Escherichia coli* ATCC 25922, *Bacteroides vulgatus* ATCC 8482, *Bifidobacterium longum* ATCC BAA-999, and Total *Lactobacillus* (sp.) from *Lactobacillus casei* subsp. *rhamnosus* TISTR 047 were purchased from ATCC, Manassas, VA, USA. *Lactobacillus acidophilus* TISTR 450 and *Salmonella typhi* were purchased from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Megazyme Resistant Starch Assay Kit (Lot. K-RSTAR 08/17) was purchased from Megazyme International Ireland Ltd. Bray, Ireland. All other utilized chemicals were of analytical grade.

### 2.3. Evaluation of properties of pharmaceutical foundations

#### 2.3.1. Flowability

**2.3.1.1. Angle of repose.** An angle of repose was determined according to Shah et al. (2008). A glass funnel 10 mm orifice size was used. The height from the end of orifice to the bench surface was 100 mm and 50 g of powder was poured through a fixed funnel, resulting in a cone of the sample. The level of the funnel was then adjusted to 6 mm above the powder cone to determine the actual height of the cone (h); the radius (r) of the base was also measured. The angle of repose ( $\theta$ ) was calculated as the following Eq. (1):

$$\tan \theta = h/r \quad (1)$$

The angle of repose ( $\theta$ ) was the angle between the horizontal bases. The slope of a mound of granules was evaluated the flow properties corresponding to the angle of repose of the samples (Carr, 1965; USP41/NF36, 2018).

#### 2.3.1.2. Compressibility ratio

**2.3.1.2.1. Bulk density.** Approximately 50 ml of the powder sample was poured into a tared graduated cylinder and the initial volume and weight of the material was recorded. Then, the cylinder was held up 1 inch higher from a hard surface and dropped down on gravity force 3 times at 2 s intervals. The bulk density was calculated from the weight of sample and final volume of powder as following Eq. (2):

$$\text{Bulk density} = M/Vd \quad (2)$$

where M is weight of sample (g); Vd is volume of the sample after dropping 3 times (ml).

**2.3.1.2.2. Tapped density.** The graduated cylinder from the bulk density test was placed on a jolting volumeter (STAV 2003, JEL, Ludwigshafen, Germany). The tapping cycle was set at 500 times and the final volume (Vt) was then recorded. The tapped density was obtained from the following Eq. (3).

$$\text{Tapped density} = M/Vt \quad (3)$$

where M is weight of sample (g) and Vt is the volume of the sample after the final process (ml).

The bulk density and tapped density were calculated and used for the calculation of the compressibility ratio by following Eq. (4):

$$\text{Compressibility ratio} = (\text{Tapped density} - \text{Bulk density}) / (\text{Tapped density}) \times 100 \quad (4)$$

The compressibility ratio was indirectly associated with the relative flow rate, agglomeration, and powder particle size (Luangtaweepon, 2011).

#### 2.3.2. Pressure hardness profile

The powder of 1000 mg was compressed in a 13 mm punch and die by using a hydraulic hand press with a 3-second dwell-time. Magnesium stearate suspension (2% w/v in acetone) was used as a lubricant before each compression. Each 1000 mg of powder was compressed at different force levels: 1000, 2000, 3000, 4000, and 5000 kg. All tablets were stored in a desiccator for 24 h before hardness measurement (Suriyatem, 2011).

### 2.4. Preparation of tablets from dry powder URB, PRB, BS, and commercial banana starch (CBS)

Granules of dry powder and starch were prepared using the wet granulation method. First, powders were mixed with 10 % (w/v) starch 1500® (S1500) until the wet mass occurred. Next, the wet mass was pressed through a 16 mesh sieve. The obtained wet granules were dried in a hot air oven at 50 °C for 8 h. Then, the dried granules were sieved through a 20 mesh sieve to make suitable smaller size granules for further compression into tablets. Finally, 1000 mg of granules were compressed into a tablet 13 mm in diameter by using a hydraulic hand press. The compression's force was set at 5000 kg.

### 2.5. Quality control of tablets

#### 2.5.1. Weight variation

Weight variation was determined using the USP41/NF36 (USP41/NF36, 2018). In total, 20 tablets were randomized from each batch. Subsequently, individual tablets were weighed and the average weight was calculated and compared.

#### 2.5.2. Friability

Friability was determined using a friabilator (PTF 20E, Pharma Test, Hainburg, Germany). In total, 20 tablets were weighed ( $W_{pre}$ ) and rotated in the friabilator at 25 rpm for 4 min. After that, fine powder was removed from each tablet and the tablets were weighed ( $W_{post}$ ). Tablet

friability was calculated using Eq. (5) in percentage unit (%). Typical tablets should have a friability value of less than 1.0%:

$$\text{Friability}(\%) = 100 \times (W_{\text{pre}} - W_{\text{post}}) / W_{\text{pre}} \quad (5)$$

### 2.5.3. Hardness

The hardness of tablets was measured using a digital hardness tester (TBH 100, Erweka, Langen, Germany). Hardness of the tablets were reported in kilogram (kg) and should be sufficient to obtain the friability and disintegration time in the standard range.

### 2.5.4. Disintegration time

Disintegration time was measured using the disintegration tester (PTZ-AUTO 3, Pharma Test, Hainburg, Germany). Six tablets were tested in distilled water at  $37 \pm 2$  °C, according to USP41/NF36 (USP41/NF36, 2018).

### 2.6. Resistant starch determination

Powder samples before and after tablet compression were passed through an 80 mesh sieve. The content of resistant starch was analyzed according the AOAC official method 2002.02 with a Megazyme Resistant Starch Assay Kit (AOAC, 2012).

### 2.7. Prebiotic properties determination

The prebiotic properties of powder samples before and after tablet compression were reported as a prebiotic index (PI) according to the method previously described by Palframan et al. (2003) with some modifications. Briefly, the samples were tested on the growth promotion of representative bacteria in the digestive tract. *Lactobacillus paracasei* (Lac) and *Bifidobacterium longum* (Bif) were used as the representative probiotic cultures, while *Escherichia coli* (Ec) and *Clostridium perfringens* (Clos) were used as the representative enteric species. All representative strains were obtained from the culture collection units at the Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand. Inulin, a commercial prebiotic, was used as the reference standard for comparison. Each assay was performed in triplicate, measuring the number of viable Log10 colony forming units (Log10 CFU) per mL at T0 for 0 h and at T48 incubation for 48 h, respectively, on 1% w/v dextrose (standard carbon source in common medium as control), 1% w/v test banana samples, and 1% w/v inulin as the commercial prebiotic. The prebiotic index (PI) was then calculated using Eq. (6):

$$\text{PI} = (\text{Bif } T_{48} - \text{Bif } T_0 / \text{Total } T_{48} - \text{Total } T_0) + (\text{Lac } T_{48} - \text{Lac } T_0 / \text{Total } T_{48} - \text{Total } T_0) - (\text{Ec } T_{48} - \text{Ec } T_0 / \text{Total } T_{48} - \text{Total } T_0) - (\text{Clos } T_{48} - \text{Clos } T_0 / \text{Total } T_{48} - \text{Total } T_0) \quad (6)$$

where Bif T<sub>48</sub> is the number of *Bifidobacterium longum* at the sampling time; Bif T<sub>0</sub> is the number of *Bifidobacterium longum* at beginning time; Lac T<sub>48</sub> is the number of *Lactobacillus paracasei* at the sampling time; Lac T<sub>0</sub> is the number of *Lactobacillus paracasei* at the beginning time; Ec T<sub>48</sub> is the number of *Escherichia coli* at the sampling time; Ec T<sub>0</sub> is the number of *Escherichia coli* at the beginning time; Clos T<sub>48</sub> is the number of

*Clostridium perfringens* at the sampling time; Clos T<sub>0</sub> is the number of *Clostridium perfringens* at the beginning time; Total T<sub>48</sub> is the number of total bacteria at the sampling time; Total T<sub>0</sub> is the number of total bacteria at the beginning time. All experiments were performed in triplicate.

### 2.8. The study of prebiotic properties in promoting probiotic growth

The promoting probiotic growth of URB, PRB, and BS were recorded as the average number of probiotic bacteria (*Lactobacillus* sp.) according to the method previously described by Palframan et al. (2003) with some modifications. Each sample was tested for growth promotion of *Lactobacillus paracasei*, which sampled representative bacteria from the digestive tract. The number of viable Log10 colony forming units (Log10 CFU) per mL at T<sub>0</sub> for 0 h and at T<sub>48</sub> after incubation for 48 h was measured on 1% w/v glucose (standard carbon source in common medium as control; MRS) and 1% w/v sample test. The experiment was performed in triplicate.

### 2.9. The study of prebiotic properties in promoting probiotic growth and/or inhibiting pathogenic bacteria

The growth promoting effect on *Lactobacillus* and the inhibitory effect on pathogenic bacteria were previously studied by Palframan et al. (2003) and Moore (2011). Dextrose, a primary carbon source of bacterial growth in modified MRS broth, was replaced by the powder sample before and after compression into tablets. Broth samples were collected at various times to determine the amount of surviving bacterial on the plate culture to evaluate the promotion of probiotic growth (*Lactobacillus* sp.) and inhibition of pathogenic bacteria, *E. coli*, and *Salmonella* typhi. Probiotics and pathogenic bacteria were incubated in formulas from the powder samples to compare with a standard formula (MRS) with dextrose. First, the standard formula (MRS) with dextrose was prepared and 0.5% w/v powder samples was used without dextrose. Second, probiotic bacteria (*Lactobacillus* sp.) (5–6 log CFU/ml) were cultured in a standard formula (MRS) and powder samples of 0.5% w/v. Pathogenic bacteria (*E. coli* or *Salmonella* typhi) (5–6 log CFU/ml) were also cultured in a culture medium of 0.5% w/v. A controlled set was prepared as follows. Series 1: A set of bacteria *E. coli* or *Salmonella* typhi was cultured with probiotic bacteria (*Lactobacillus* sp.) in an MRS standard formula without powder samples. Series 2: Controls were specifically cultured with bacteria *E. coli* or *Salmonella* typhi in an MRS standard formula without powder samples and probiotic bacteria (*Lactobacillus* sp.). Sampling at various times was conducted to determine the type and amount of bacteria compared with the controlled set.

### 2.10. Statistical analysis

The results were determined in triplicates to confirm reproducibility. The data were given as mean ± S.D. Analysis of variance (ANOVA) was performed using statistical SPSS software version 17 (SPSS Inc, Chicago, Illinois, USA). Duncan's multiple range tests were performed to analyze the significant differences in physicochemical and prebiotic properties, and p < 0.05 was considered as significant.

**Table 1.** The evaluation of properties of pharmaceutical foundations.

Sample	Flowability		Pressure hardness profile
	Angle of repose (degree)	Compressibility Index (%)	
URB	Fair (39.20)	Fair (17.54)	Poor
PRB	Fair (37.86)	Fair (18.49)	Poor
BS	Good (33.75)	Poor (29.71)	Poor

URB = unpeeled raw *M. sapientum* fruit powder; PRB = peeled raw *M. sapientum* fruit powder; BS = *M. sapientum* starch.

### 3. Results and discussion

#### 3.1. The evaluation of properties of pharmaceutical foundations

##### 3.1.1. Flowability

**3.1.1.1. Angles of repose.** The average angles of repose for URB, PRB, and BS are shown in Table 1. It was found that the flowability of URB and PRB was fair while BS was good, thus corresponding to USP41/NF36 (USP41/NF36, 2018).

**3.1.1.2. Compressibility index.** The compressibility index of URB, PRB, and BS is shown in Table 1. When these values were expressed as flowability of powder, it was found that the flowability of URB and PRB was fair while BS was poor. To understand the characteristics of flowability for URB, PRB, and BS, many experiments, such as the compressibility ratio and angle of repose, should be conducted. The results of the different experiments were considered in terms of their relationships in order to get the more reliable outcomes. From this study, the flowability from the angle of repose and the compressibility ratio results of three powder samples were poor, which was in good agreement. The factors that caused poor flowability of the samples could be granule size, granule appearance, and distribution size. Consequently, the flowability of powder should be considered for uniformity of tablets.

##### 3.1.2. Pressure hardness profile

Table 1 shows that the high force levels with lower hardness indicates poor hardness-pressure profile. Regarding the pressure hardness profile, when compression force was implemented at the same level at each point, URB hardness was higher than PRB and BS.

Regarding the fundamental properties of URB, PRB, and BS, they all showed poor flowability and a poor pressure hardness profile. Thus, these limitations should be tackled by the wet granulation method in the production process (Luangtawepon, 2011). This method has been popular in tablet production since it can be applied with all types of formulas and active ingredients, especially those with poor flowability and a poor pressure hardness profile. In this study, the wet granulation method was used to prepare the tablets of URB, PRB, and BS in order to decrease the variations in terms of weight and active ingredients.

#### 3.2. Preparation of prebiotic tablets from dry powder URB, PRB, BS, and CBS

This study mainly focused on natural substances rather than synthetic ones, so 10% (w/v) S1500, a natural substance, was selected as a binder for the tablet preparation for further studies. Furthermore, to evaluate the potential of URB, PRB, and BS from *M. sapientum* as the supplementary foods on the market, CBS produced and obtained from local markets in Australia were made into tablets by using 10% (w/v) S1500 with the force of 5000 kg, and was compared to resistant starch and prebiotic property content. The appearance of the tablets was round with a smooth surface. The advantages of the tablet formulation over powder included an exact consumed amount of resistant starch, a consumer convenience, and higher stability due to low surface area.

#### 3.3. The quality control of tablets

From Table 2, weight variation of all tablets met the requirement of USP41/NF36 (USP41/NF36, 2018) because the weight of all tablets were in the range of 950.00–1050.00 mg, which was greater than 95%, but not more than 105% of the target weight of the tablet (1000 mg). In terms of tablet hardness, BS could generate the hardest tablet ( $10.04 \pm 1.33$  kg), which was comparable to the CBS tablet ( $9.04 \pm 0.62$  kg). The hardness of tablets from *M. sapientum* starch was twice of those from URB and PRB. The normal standard of tablet hardness was more than 4 kg, so these four formulas met the requirements. However, the hardness values with less force could be conducted if other substances, such as microcrystalline cellulose, were added [18], because this could decrease the content of resistant starch in tablets while increasing production cost. Regarding tablet friability, it was found that the friability of tablets was less than 1.00%, so these four formulas met the requirements. With regard to disintegration, tablets from URB, BS, and CBS had comparable disintegration time, whereas tablets from PRB demonstrated a significantly faster disintegration time of  $26.50 \pm 5.46$  s. The normal standard of disintegration time of uncoated tablets was less than 15 min, so these four formulas met the requirement. According to the results of quality control, all 4 formulas were acceptable according to the pharmacopeia, which were suitable to be used in other studies and the production industry.

#### 3.4. Resistant starch determination

According to the evaluation of resistant starch contents from 8 samples, as shown in Table 3, it was found that URB contained the highest resistant starch contents of  $78.58 \pm 0.31$  g/100 g (dry weight), with the statistically significant difference at a 95% confidence interval ( $p < 0.05$ ), while CBST contained the lowest resistant starch contents of  $38.54 \pm 0.03$  g/100 g (dry weight). When the contents of resistant starch prepared from *M. sapientum* were compared with those prepared from bananas in other varieties available in the markets (CBS and CBST), it was found that the contents of resistant starch in *M. sapientum* was higher than the other group ( $p < 0.05$ ). Thus, the variations in banana cultivars resulted in different amounts of resistant starch in the samples, which was in accordance with the research from Vatanasuchart et al. (2012). Moreover, the resistant starch contents of URB and PRB from *M. sapientum* were 70.69% and 78.58%, respectively. Those of *Musa cavendishii* were 35.06% and 42.04%, respectively (Bezerra et al., 2013). Therefore, the peeled bananas could yield more resistant starch than the unpeeled ones. The samples before compression (*M. sapientum* powder) and those after compression (*M. sapientum* tablets) were compared. It was found that the former group could yield higher contents of resistant starch than the latter group ( $p < 0.05$ ).

The decrease of resistant starch content after compression might result from the procedures of tablet preparation by means of wet granulation. This method requires heat to make wet granules to become dry granules. The process is similar to heat moisture treatment (HMT). According to the report from Hoyos-Leyva et al. (2015), it was found that HMT at 32.20% moisture content with a heating duration of 6.5 h could reduce the resistant starch of Morado bananas from 89.40 to 59.90 g/100

Table 2. The quality control of URBT, PRBT, BST and CBST.

Sample	Weight variation (mg)	Hardness (kg)	Friability (percent)	Disintegration Time (second)
URBT	$1,007.30 \pm 2.70$	$4.36 \pm 0.26$	0.66	$66.33 \pm 7.00$
PRBT	$1,006.60 \pm 2.00$	$5.67 \pm 0.48$	0.61	$26.50 \pm 5.46$
BST	$1,008.20 \pm 2.10$	$10.04 \pm 1.33$	0.46	$52.33 \pm 8.50$
CBST	$1,007.10 \pm 2.40$	$9.04 \pm 0.62$	0.52	$59.32 \pm 4.50$

URBT = unpeeled raw *M. sapientum* fruit tablets; PRBT = peeled raw *M. sapientum* fruit tablets; BST = *M. sapientum* starch tablets; CBST = commercial banana starch tablets.

**Table 3.** The RS content of samples.

Sample (g/100g dry weight)	Resistant starch $\pm$ SD
URB	70.69 $\pm$ 0.44 <sup>c</sup>
PRB	78.58 $\pm$ 0.31 <sup>a</sup>
BS	75.62 $\pm$ 0.01 <sup>b</sup>
CBS	59.09 $\pm$ 0.05 <sup>d</sup>
URBT	53.82 $\pm$ 0.13 <sup>e</sup>
PRBT	50.10 $\pm$ 0.70 <sup>f</sup>
BST	48.01 $\pm$ 0.03 <sup>g</sup>
CBST	38.54 $\pm$ 0.03 <sup>h</sup>

URB = unpeeled raw *M. sapientum* fruit powder; PRB = peeled raw *M. sapientum* fruit powder; BS = *M. sapientum* starch; CBS = commercial banana starch; URBT = unpeeled raw *M. sapientum* fruit tablets; PRBT = peeled raw *M. sapientum* fruit tablets; BST = *M. sapientum* starch tablets; CBST = commercial banana starch tablets.

Values are means of triplicate analysis (n = 3).

In a column, means not sharing a common letter are significantly different at  $p < 0.05$ .

g. Moreover, the increase in moisture level could lead to a significant decrease in resistant starch content. Besides, from the study of Aparicio-Saguilana et al. (2008), the solution of cross-linked starch was processed under an autoclave at 121 °C for 1 h, contributing to a decrease in the amount of resistant starch from samples (94%–85%). From the evidences above, it could be noted that the temperature and moisture could have a direct effect on the content of resistant starch.

### 3.5. Prebiotic properties

The PI of all samples is presented in Table 4. The numbers of several kinds of bacteria were analyzed and calculated to determine the PI according to Eq. (6). It was found that PI values of all samples were higher than bacterial culture with dextrose. The PI values of all samples were similar to that of the bacterial culture with commercial prebiotic (inulin) except for tablets from commercial banana starch (CBST), which was lower than bacterial culture with inulin. Besides, when compared with other samples and bacterial culture with dextrose, PRB had the highest PI value with a statistically significant difference ( $p < 0.05$ ;  $0.58 \pm 0.03$ ) that confirmed the ability to promote beneficial bacteria growth rather than non-beneficial bacteria growth.

When PI values from all samples were compared, it was found that PI values from *M. sapientum* powder were higher than those from *M. sapientum* tablets. Values of A3 and B3 did not show a statistically

**Table 4.** Prebiotic index of all samples.

Sample	Prebiotic index $\pm$ SD
Dextrose (Control)	0.18 $\pm$ 0.02 <sup>f</sup>
Inulin	0.47 $\pm$ 0.04 <sup>bc</sup>
URB	0.51 $\pm$ 0.07 <sup>b</sup>
PRB	0.58 $\pm$ 0.03 <sup>a</sup>
BS	0.48 $\pm$ 0.05 <sup>bc</sup>
CBS	0.40 $\pm$ 0.06 <sup>d</sup>
URBT	0.44 $\pm$ 0.05 <sup>c</sup>
PRBT	0.45 $\pm$ 0.03 <sup>c</sup>
BST	0.46 $\pm$ 0.06 <sup>bc</sup>
CBST	0.28 $\pm$ 0.02 <sup>e</sup>

URB = unpeeled raw *M. sapientum* fruit powder; PRB = peeled raw *M. sapientum* fruit powder; BS = *M. sapientum* starch; CBS = commercial banana starch; URBT = unpeeled raw *M. sapientum* fruit tablets; PRBT = peeled raw *M. sapientum* fruit tablets; BST = *M. sapientum* starch tablets; CBST = commercial banana starch tablets.

Values are means of triplicate analysis (n = 3).

In a column, means not sharing a common letter are significantly different at  $p < 0.05$ .

significant difference ( $p > 0.05$ ). The decrease of the PI value was related to the decrease of resistant starch in samples after compression. However, samples with a decreased value could still promote beneficial bacteria growth and perform better than bacterial culture with dextrose ( $p < 0.05$ ). According to the analysis of PI values for all samples, they had a statistically significant difference ( $p < 0.05$ ), showing that they could perform better than dextrose in terms of promoting *Lactobacilli* and *Bifidobacteria* bacteria, which are beneficial bacteria.

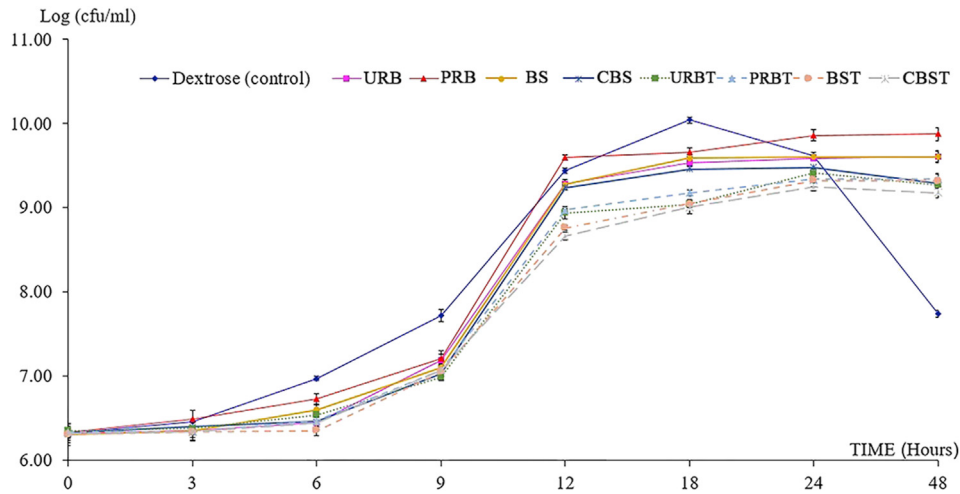
### 3.6. The study of prebiotic properties in promoting probiotic growth

The results on the ability of prebiotics to promote probiotic (*Lactobacillus* sp.) growth in eight culture mediums are shown in Figure 1. According to the experiment, when comparing the number of *Lactobacillus* sp. in *M. sapientum* powder and tablets from 6 to 24 h, the numbers of *Lactobacillus* sp. cultured in mediums with compressed powder samples were slightly lower than those of *Lactobacillus* sp. cultured in mediums with non-compressed powder samples with a statistically significant difference ( $p < 0.05$ ). However, after being cultured for 48 h, the numbers of *Lactobacillus* sp. cultured in mediums with powder samples before compression and after compression into tablets had no statistically significant difference ( $p > 0.05$ ). Moreover, when comparing all powder sample formula and standard MRS mediums containing dextrose after culturing for 48 h, it was found that the numbers of *Lactobacillus* sp. were statistically significantly different ( $p < 0.05$ ). Therefore, the ability to promote probiotic bacteria (*Lactobacillus* sp.) growth of powder after compression into tablets was similar to that of the powder before compression.

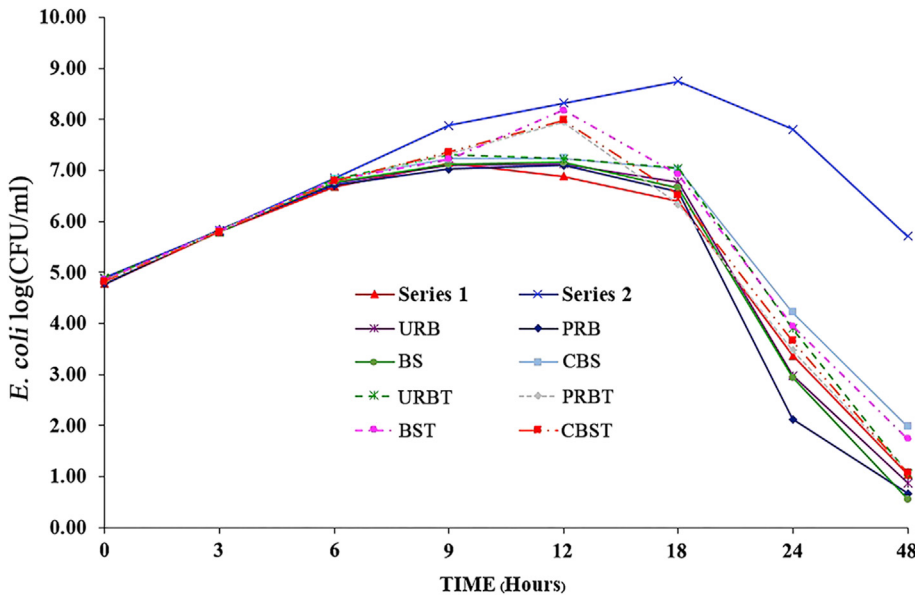
### 3.7. The study of prebiotic properties in promoting probiotic growth and/or inhibiting pathogenic bacteria

The objective of this section was to investigate the effects of inhibition on two pathogenic bacteria, i.e., *E. coli* and *Salmonella* typhi. The inhibition pathogenic bacteria was studied by filling these bacteria in the modified MRS liquid medium using each powder sample instead of dextrose, which is the primary carbon source of bacterial growth. The results are shown in Figures 2 and 3. In Figure 2, for the number of pathogenic bacteria (*E. coli*) in each powder sample in a standard MRS formula with probiotics (*Lactobacillus* sp.), the number of *E. coli* increased rapidly after 3–12 h but was still lower than the controlled groups (Series 2) with only *E. coli* in standard MRS. However, when compared to controlled groups (Series 1), with *E. coli* and *Lactobacillus* sp. in standard MRS mediums, the number of *E. coli* with the MRS culture medium in 8 powder samples was slightly higher. After 12 h, the number of *E. coli* in each sample decreased rapidly, except for a controlled group (series 2). Additionally, the numbers of *E. coli* with the starch samples was lower than the controlled group (Series 2) until 48 h of culture, indicating that starch did not promote *E. coli* growth. Therefore, all 8 powder samples can increase probiotics (*Lactobacillus* sp.) to reduce *E. coli*, which is suitable for the development of prebiotics to promote future health.

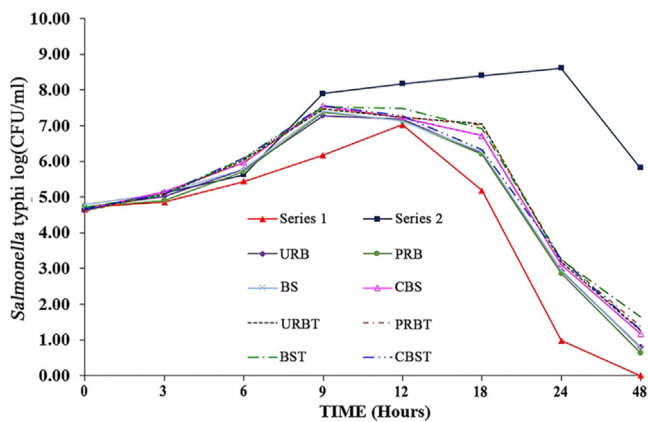
In Figure 3, it was found that the number of *Salmonella* typhi in each powder sample with probiotics increased considerably from 3–9 h but was still lower than the controlled group (Series 2) with only *Salmonella* typhi in a standard MRS formula containing dextrose at 9 h. However, when compared to a controlled group (Series 1) with *Salmonella* typhi and *Lactobacillus* sp. in a standard MRS medium, the numbers of *Salmonella* typhi with the MRS culture media containing 8 powder samples were slightly higher at 9 h. After 12 h, the number of *Salmonella* typhi in each sample decreased rapidly, except for the controlled group (Series 2). Moreover, the numbers of *Salmonella* typhi with 8 powder samples and probiotics (*Lactobacillus* sp.) were lower than the controlled group (Series 2) until 48 h. At 48 h, the numbers of *Salmonella* typhi in all 8 samples were approximately 1–2 log CFU/mL, while the controlled group (series 2) was about 6 log CFU/mL. Therefore, it can be concluded that all 8 powder samples could promote probiotics (*Lactobacillus* sp.) to reduce



**Figure 1.** The number of *Lactobacillus* sp. in different culture media at different times. URB = unpeeled raw *M. sapientum* fruit powder; PRB = peeled raw *M. sapientum* fruit powder; BS = *M. sapientum* starch; CBS = commercial banana starch; URBT = unpeeled raw *M. sapientum* fruit tablets; PRBT = peeled raw *M. sapientum* fruit tablets; BST = *M. sapientum* starch tablets; CBST = commercial banana starch tablets.



**Figure 2.** The number of *Lactobacillus* sp. in different culture media at different times. URB = unpeeled raw *M. sapientum* fruit powder; PRB = peeled raw *M. sapientum* fruit powder; BS = *M. sapientum* starch; CBS = commercial banana starch; URBT = unpeeled raw *M. sapientum* fruit tablets; PRBT = peeled raw *M. sapientum* fruit tablets; BST = *M. sapientum* starch tablets; CBST = commercial banana starch tablets, Series 1: *E. coli* with probiotic bacteria in MRS standard formula, Series 2: only *E. coli* in MRS standard formula.



**Figure 3.** The number of *Salmonella typhi* in different culture media at different times. URB = unpeeled raw *M. sapientum* fruit powder; PRB = peeled raw *M. sapientum* fruit powder; BS = *M. sapientum* starch; CBS = commercial banana starch; URBT = unpeeled raw *M. sapientum* fruit tablets; PRBT = peeled raw *M. sapientum* fruit tablets; BST = *M. sapientum* starch tablets; CBST = commercial banana starch tablets, Series 1: *Salmonella typhi* with probiotic bacteria in MRS standard formula, Series 2: only *Salmonella typhi* in MRS standard formula.

*Salmonella typhi* better than the standard MRS medium containing dextrose.

#### 4. Conclusions

The tablets from *M. sapientum* starch was suggested for use as a health product to promote *Lactobacillus* sp. growth, which would be beneficial for human digestive function. *M. sapientum* tablets produced using the direct compression method met a requirement of USP41/NF36 because it had narrow weight variation and higher hardness than 4 kg. Interestingly, *M. sapientum* tablets demonstrated a fast disintegration time within 1 min. These findings suggested that *M. sapientum* tablets were suitable to be produced on the industrial scale.

#### Declarations

##### Author contribution statement

Patthanakorn Jaiturong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nachtharinee Laosirisathian: Performed the experiments.  
 Busaban Sirithunyalug, Sukum Eitssayeam: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.  
 Sasithorn Sirilun: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.  
 Wantida Chaiyana: Analyzed and interpreted the data; Wrote the paper.  
 Jakkapan Sirithunyalug: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

#### Funding statement

This work was supported by the National Research Council of Thailand (NRCT); The Graduate School, Chiang Mai University, Thailand; Faculty of Pharmacy, Chiang Mai University, Thailand; and Faculty of Science, Chiang Mai University, Thailand.

#### Competing interest statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

#### References

- AOAC, 2012. Association of Official Analytical Chemist Official Methods of Analysis. Methods 2002.02, nineteenth ed. AOAC International, Rockville, MD, USA.
- Aparicio-Saguilana, A., Gutiérrez-Meraza, F., García-Suárez, J.F., Tovar, J., Bello-Pérez, A.L., 2008. Physicochemical and functional properties of cross-linked banana resistant starch, Effect of pressure cooking. *Starch/Stärke* 60, 286–291.
- Asp, N.G., Van Amelsvoort, J.M.M., Hautvast, J.G.A.J., 1996. Nutritional implications of resistant starch. *Nutr. Res. Rev.* 9 (1), 1–31.
- Bezerra, C.V., Amante, E.R., de Oliveira, D.C., Rodrigues, A.M.C., da Silva, L.H.M., 2013. Green banana (*Musa cavendishii*) flour obtained in spouted bed – effect of drying on physico-chemical, functional and morphological characteristics of the starch. *Ind. Crop. Prod.* 41, 241–249.
- Bird, A.R., Brown, I.L., Topping, D.L., 2000. Starches, resistant starches, the gut microflora and human health. *Curr. Issues Intest. Microbiol.* 1, 25–37.
- Carr, R., 1965. Evaluation flow properties of solid. *Chem. Eng. J.* 72, 163–168.
- Englyst, H.N., Kingman, S.M., Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 46, 33–50.
- Ford, A.C., Quigley, E.M., Lacy, B.E., Lembo, A.J., Saito, Y.A., Schiller, L.R., et al., 2014. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. *Am. J. Gastroenterol.* 109 (10), 1547–1561.
- Fuentes-Zaragoza, E., Riquelme-Navarrete, M.J., Sanchez-Zapata, E., Perez- Alvarez, J.A., 2010. Resistant starch as functional ingredient: a review. *Food Res. Int.* 43, 931–942.
- Gao, H., Huang, S., Dong, T., Yang, Q., et al., 2016. Analysis of resistant starch degradation in postharvest ripening of two banana cultivars: focus on starch structure and amylases. *Postharvest Biol. Technol.* 119, 1–8.
- Goldstein, E.J.C., Tyrrell, K.L., Citron, D.M., 2015. *Lactobacillus* species: taxonomic complexity and controversial susceptibilities. *Arch. Clin. Infect. Dis.* 60 (Suppl 2), 98–107.
- Heydaria, Z., Rahaiea, M., Alizadeh, A.M., 2019. Different anti-inflammatory effects of *Lactobacillus acidophilus* and *Bifidobacterium bifiduum* in hepatocellular carcinoma cancer mouse through impact on microRNAs and their target genes. *J. Nutr. Intermed. Metab.* 16.
- Hoyos-Leyva, D.J., Bello-Perez, A.L., Agama-Acevedo, E., Alvarez-Ramirez, J., 2015. Optimising the heat moisture treatment of Morado banana starch by response surface analysis. *Starch/Stärke* 67, 1026–1034.
- Johnson, J.D., Cocker, K., Chang, E., 2015. Infantile colic: recognition and treatment. *Am. Fam. Physician* 92 (7), 577–582.
- Le, B., Yang, S.H., 2018. Efficacy of *Lactobacillus plantarum* in prevention of inflammatory bowel disease. *Toxicol. Rep.* 5, 314–317.
- Liyuan, S., He, Z., Limei, L., Xinyuan, W., Qiuyue, G., Yundong, Z., 2018. Effects of *Lactobacillus* on the inhibition of *Helicobacter pylori* growth. *Biotechnol. Biotechnol. Equip.* 32 (6), 1533–1540.
- Luangtaweepoon, I., 2011. Effect of Herbal Powder Composition on Tablet Formulation Development, Master's Thesis. The graduate school, Chiang Mai University, Chiang Mai, Thailand.
- Martinez, R.M., Hulten, K.G., Bui, U., Clarridge, J.E., 2014. Molecular analysis and clinical significance of *Lactobacillus* spp. recovered from clinical specimens presumptively associated with disease. *J. Clin. Microbiol.* 52, 30–36. <https://jcm.asm.org/content/52/1/30>.
- Mcfarlane, G., Cummings, J.H., 1999. Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health. *BMJ* 318, 999–1003.
- Moore, K.E., 2011. Biological Analysis Of Prebiotics in Various Processed Food Matrices. Master's thesis. The graduate college, University of Nebraska, Nebraska, USA.
- Palfaman, R., Gibson, G.R., Rastall, R.A., 2003. Development of a quantitative tool for the comparison of the prebiotic effect of dietary oligosaccharides. *Lett. Appl. Microbiol.* 37 (4), 281–284.
- Sajilata, M.G., Singhal, R.S., Kulkarni, P.R., 2006. Resistant starch-A review. *Compr. Rev. Food Sci. Food Saf.* 5 (1), 1–17.
- Shah, R.B., Tawakkul, M.A., Khan, M.A., 2008. Comparative evaluation of flow for pharmaceutical powders and granules. *AAPS PharmSciTech* 9, 250–258.
- Sung, V., D'Amico, F., Cabana, M.D., Chau, K., Koren, G., Savino, F., et al., 2018. *Lactobacillus reuteri* to treat infant colic: a meta-analysis. *Pediatrics* 141 (1).
- Suriyatem, R., 2011. Development of Cross-Linked Carboxymethyl Modified rice Starch as Tablet Disintegrant. Master's thesis. The graduate school, Chiang Mai University, Chiang Mai, Thailand.
- The United States Pharmacopeia 41, 2018. The National Formulary 36. Pharmacopeial Convention, Maryland, United States.
- Topping, D.L., Clifton, P.M., 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* 81, 1031–1064.
- Vanderhoof, J.A., Whitney, D.B., Antonson, D.L., et al., 1999. *Lactobacillus GG* in the prevention of antibiotic-associated diarrhea in children. *J. Pediatr.* 135, 564–568.
- Vatanasuchart, N., Niyomwit, B., Wongkrajang, W., 2012. Resistant starch content, in vitro digestibility and physico-chemical properties of flour and starch from Thai bananas. *Maejo Int. J. Sci. Technol.* 6 (2), 259–271.
- Wang, Z.K., Yang, Y.S., Stefa, A.T., Sun, G., Peng, L.H., 2014. Review article: fungal microbiota and digestive diseases. *Aliment. Pharmacol. Ther.* 39 (8), 751–766.
- Yuea, Y., Yec, K., Lub, J., Wangd, X., Zhangb, S., Liub, L., et al., 2020. Probiotic strain *Lactobacillus plantarum* YYC-3 prevents colon cancer in mice by regulating the tumour microenvironment. *Biomed. Pharmacother.* 127.