ผลของแป้งต้านทานการย่อยและใยอาหารสกัดต่อการเจริญของเชื้อ *Lactobacillus plantarum* KL102 ในแบบจำลองไส้กรอกหมัก

EFFECT OF RESISTANT STARCH AND DIETARY FIBER EXTRACT ON GROWTH OF *LACTOBACILLUS PLANTARUM* KL102 IN FERMENTED SAUSAGE MODEL

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บทคัดย่อ

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาแบบจำลองไส้กรอกหมักแบบซินไบโอติกที่ประกอบด้วยแป้งต้านทานการย่อยสกัด (Resistant Starch Extract) ความเข้มข้นร้อยละ 1 หรือใยอาหารสกัด (Dietary Fiber Extract) ความเข้มข้นร้อยละ 1 ที่สกัดจากเนื้อหรือเปลือกกล้วย ตามลำดับ ร่วมกับเชื้อ *Lactobacillus plantarum* KL102 (KL102) ทำการวิเคราะห์ข้อมูลการเจริญและกิจกรรมสภาวะเป็นกรดของ KL102 ในอาหารเหลว MRS แบบจำลองไส้กรอกหมักที่เสริมและไม่เสริมแป้งต้านทานการย่อยสกัดหรือใยอาหารสกัดทำที่อุณหภูมิ 30 องศาเซลเซียส บ่มเป็นเวลา 72 ชั่วโมง จากผลการทดลองพบว่าแป้งต้านทานการย่อยสกัดและใยอาหารสกัดเป็นแหล่งของแป้งต้านทานการย่อยและใยอาหารทั้งหมด ตามลำดับ ในอาหารเหลว MRS และแบบจำลองไส้กรอกหมักที่มีการเสริมแป้งต้านทานการย่อยสกัดหรือใยอาหารสกัด KL102 สามารถเจริญภายใน 3 ชั่วโมงแรกของการบ่ม ในขณะที่ในแบบจำลองไส้กรอกหมักแบคทีเรียโพรไบโอติกชนิดนี้จะเจริญภายหลังการบ่ม 3 ชั่วโมง ซึ่งการเสริมแป้งต้านทานการย่อยสกัดหรือใยอาหารสกัดทำให้อัตราการเจริญสูงสุดเพิ่มและระยะเวลาหนึ่งชั่วอายุลดลง ($p < 0.05$) โดยเมื่อสิ้นสุดการบ่มแบบจำลองไส้กรอกหมักเสริมแป้งต้านทานการย่อยสกัดหรือใยอาหารสกัดมีจำนวน KL102 ที่รอดชีวิตมากกว่าอาหารเหลว MRS และแบบจำลองไส้กรอก ($p < 0.05$) นอกจากนี้ยังพบการลดลงของเชื้อ *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* และ *Listeria monocytogenes* ในแบบจำลองไส้กรอกหมักเสริมแป้งต้านทานการย่อยสกัดหรือใยอาหารสกัดเร็วกกว่าในอาหารเหลว MRS และแบบจำลองไส้กรอก อีกทั้งแบบจำลองไส้กรอกหมักเสริมแป้งต้านทานการย่อยสกัดหรือใยอาหารสกัดยังมีการลดลงของค่า pH และการเพิ่มขึ้นของปริมาณกรดเร็วกว่าแบบจำลองไส้กรอกหมัก ดังนั้น การวิจัยนี้แสดงให้เห็นว่าพรีไบโอติกที่มีศักยภาพ 2 ชนิด คือ แป้งต้านทานการย่อยและใยอาหารที่สกัดจากผลพลอยได้ของการแปรรูปกล้วย ซึ่งสามารถนำไปใช้ในการผลิตไส้กรอกหมักแบบซินไบโอติกในอนาคตได้

คำสำคัญ: แลคโตบาซิลัส แพลนทารัม แป้งต้านทานการย่อยสกัด ใยอาหารสกัด แบบจำลองไส้กรอกหมัก การใช้ประโยชน์ของผลพลอยได้จากการแปรรูปกล้วย

Abstract

The purpose of this research was to study synbiotic fermented sausage model (FSM) containing 1%(w/v) of resistant starch extract (RSE) or 1%(w/v) of dietary fiber extract (DFE), extracted from banana pulp or peel, respectively, with *Lactobacillus plantarum* KL102 (KL102). The growth profile and acidification activity of KL102 were monitored in MRS broth, FSM supplemented without and with RS or DF incubated at 30°C for 72 h. The results showed that RSE and DFE were determined as a rich source of resistant starch and dietary fiber, respectively. KL102 grew during the first 3 h in MRS broth and FSM supplemented with RSE or DFE, whereas this probiotic bacterium grew after 3 h in FSM. Similarly, RSE or DFE supplementation raised the maximum specific growth rate and lowered the generation time significantly ($p < 0.05$). In addition, FSM supplemented with RSE or DFE yielded KL102 survival with higher values of KL102 than FSM and MRS broth in the end of fermentation ($p < 0.05$). For pathogens, decrease of *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes* in MRS broth and FSM supplemented RSE or DFE had faster than those in FSM. Furthermore, the decrease in the extracellular pH and increase total acid had lower and higher, respectively, than in the case of the FSM containing RSE or DFE when compared with FSM. Therefore, this research examined the ability of two potential prebiotics; RSE and DFE extracted from by-products of banana processing. They can be used the production of synbiotic fermented sausage in future.

Keywords: *Lactobacillus plantarum*, Resistant Starch Extract, Dietary Fiber Extract, Fermented Sausage Model, Utilization of By-product from Banana Processing

Introduction

The waste of food is an unlucky reality worldwide. In particular, during the processing of fruit for pulp production, around 65-70% by weight of the raw material is lost, leading to serious environmental problems. However, it was demonstrated that some fibers of fruit by-products show functional properties such as water-holding, swelling, gel forming, bile acid binding, and cation-exchange capacities [1]. Low quality pulp of banana and the peels of banana are the fruit by-products. They content resistant starch (RS), insoluble and soluble dietary fibers (DF) and fructooligosaccharides. These prebiotics are in fact nondigestible compounds. They pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them, particularly lactobacilli and bifidobacteria [2]. Resistant starch extracted from green banana pulp contains high quantity of RS (approximately 45.71%). Dietary fibers extracted from green banana peel contains high quantity of DF (approximately 78.62%) [3]. Furthermore, peel of banana (Musa sp., Musaceae) contains around 43-

49 g of total DF per 100 g of dry matter, in addition to significant amounts of α-linolenic acid (ALA), essential amino acids and micronutrients [4-5].

Our previous researches reported that *L. plantarum* KL102 (KL102) was isolated from Thai fermented meat as natural starter culture. This bacterium should demonstrate antimicrobial activity against foodborne pathogenic bacteria [6] and survival from gastrointestinal condition [7] as candidate probiotic. In the USA, probiotic is considered as generally regarded as safe (GRAS) such as *L. plantarum* [8]. The intake of resistant starch extract (RSE), dietary fiber extract (DFE) and probiotics exert a positive impact on the development of the intestinal microbiota and are reported to relieve constipation and reduce the incidence of colon cancer [9]. Finally, the beneficial effects on probiotics viability exerted by some ingredients such as fruit by-product to pork sausage and fermented sausage have been reported [10].

Considering the continuous search for efficient, safe, and cost-effective RSE and DFE for application in the meat industry and the opportunities that RS and DF might open with regard to these concerns.

Objectives

The present study investigated the potential effects of RS and DF extracts from unripe banana by-products that are abundantly available in nature with regard to the quality characteristics of fermented sausage model. The effects of the addition of these RS and DF to fermented sausage model in term of probiotic viability enhancement are reported.

Methods

Extraction of resistant starch and dietary fiber

Edible green (unripe) banana (Kluai Namwa, ABB) pulps and peels were collected from a local banana processing factory in Chachoengsao province, Thailand. In processing into powder, the banana pulps (unapproved fruit) and peels were sliced into 1 mm thick pieces, spread evenly on a stainless steel tray, dried in a hot-air oven at 50°C for 8 h, and then milled and passed through a 1 mm sieve [11].

RSE from banana pulp prepared through two autoclaving-cooling cycles was dispersed in distilled water in a ratio of 1:4 (w/v). Hydrochloric acid was added until acid concentration was 0.1 mol/l. The mixture was stored at room temperature for 12 h, and then 1 mol/l sodium hydroxide solution was added to neutralize acid until pH 7.0. The neutralized mixture was stored at 4°C for 24 h, dried in a hot air oven (105°C) and then ground and passed through a 1 mm sieve [12].

DFE from banana peel was extracted using the method of Yoshimoto and others [13]. The banana peel powder samples were defatted for 12 h using hexane as a solvent (5 ml/g sample). The residue was dried at 50°C in a hot air oven to assure complete removal of the solvent. The defatted peel powder was mixed with water (1:20 w/v ratio). The pH was adjusted to 5.8 by adding 1 N HCl solution. An alpha-amylase was added (0.1 ml/g sample). The sample was incubated at 95°C for 30 min. after cooling down to 60°C, the pH was adjusted to 7.5

by adding 1 N NaOH. Neutrase was then added (10 mg/g sample) and incubated for 30 min at 60°C. After that, the pH was adjusted to 4-4.5 by using 1 N HCl solution. An amyloglucosidase solution was added (0.1 mg/g sample) at 60°C for 30 min. Finally, the mixture was filtered through Whatman No.4 filter paper and dried in the hot air oven at 50°C for 12 h. The dried samples were then powdered in an Udy cyclone mill (Udy cooperation, Colorado, USA) using a 1 mm sieve.

Chemical analysis

Moisture content of RSE and DFE was determined by using a moisture meter at 105°C [14]. Ash, protein, and lipid content were analyzed according to AACC methods [14]. Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) contents were determined by enzymatic and gravimetric method of AOAC, using a total dietary fiber assay kit (Megazyme, Ireland). Total starch (TR), resistant starch and non-resistant starch (NRS) were determined by enzymatic and gravimetric method of AOAC, using a resistant starch-100 kit (Megazyme, Ireland).

Bacterial strain

L. plantarum KL102 from Thai fermented meat product of culture collection of Meat Microbiology Laboratory, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang was used in this study. Its preliminary potential probiotic was determined by antagonistic activity of foodborne pathogen and survival from gastrointestinal model [6-7] as candidate probiotic. Furthermore, its identification was confirmed by 16S rRNA gene sequence analysis of *Lactobacillus*. This probiotic was maintained on de Man, Rogosa & Sharpe agar (MRS) (Merck, Darmstadt, Germany) in anaerobic condition. The overnight culture in anaerobic condition was prepared by inoculating approximately 10 ml of MRS broth adding NaCl (10.0 g/l) with 2-3 colonies taken from MRS agar that the count of bacteria was approximately 10^8 cfu/ml (McFarland standard of 0.5). Inoculum was adopted for growth profile and acidification activity of KL102 in fermented sausage model (FSM) supplemented with RSE and DFE.

For pathogens, *Escherichai coli* TUEC1, *Salmonella* Typhimurium TUST1, *Staphylococus aureus* TUSA1 and *Listeria monocytogens* TULM1 were used in the present study. These strains were previously isolated from pig carcasses in Southern Thailand abattoirs by the standard procedure [15-18] and theirs identity was confirmed by the Department of Medical Sciences, Ministry of Public Health of Thailand. These organisms were maintained on Mueller Hinton agar (MHA) (Merck, Germany). The overnight cultures were prepared by inoculating approximately 2 ml Mueller Hinton broth (MHB) (Merck, Germany) with 2-3 colonies taken from MHA that the counts of bacteria was approximately 10^8 cfu/ml (McFarland standard of 0.5). Inocula were adopted for growth profile of pathogens in FSM supplemented with RS and DF.

Growth condition

The growth profile and acidification activity were determined in FSM including meat extract powder (10.0 g/l); tryptone (5.0 g/l); glucose (10.0 g/l); NaCl (25.0 g/l); sodium tri-polyphosphate (3.0 g/l); sodium ascorbate (0.5 g/l); sodium nitrite (0.08 g/ml); and cooked rice (8.0 g/l). Unpeeled garlic was sterilized by soaking in 95%(v/v) ethanol for 20 min and then chopped under aseptic condition. After medium sterilization, sterilized chopped garlic (50.0 g/l) was added in the medium. *E. coli* TUEC1, *S.* Typhimurium TUST1, *S. aureus* TUSA1 and *L. monocytogens* TULM1 were inoculated in FSM at each final concentration of 10^2 cfu/ml. After that 300 ml of FSM were divided into 3 groups (100 ml/group) as follows: (1) non supplemented (FSM), (2) supplemented with RSE (10.0 g/l) and (3) supplemented with DFE (10.0 g/l). KL102 was inoculated in FSM and MRS broth (control). The initial count of bacteria in each group was approximately 10^5 cfu/g. The growths of the test inoculates were investigated by checking the evolution of viable cell counts of KL102 and all pathogen in all broth. The sampling for determination of cfu/g, pH and total acid was carried out in triplicate after 0, 3, 6, 12, 24, 36, 48 and 72 h of incubation at 30°C in anaerobic condition.

Growth profile

The sample suspensions were submitted to count for KL102. Enumeration of bacteria was done on MRS agar (de Man Rogosa and Sharp, Merck, Darmstadt, Germany) adding NaCl (10 g/l). The plates were incubated at 30 ± 2°C for 24-48 h in anaerobic condition before colonies were counted.

The results were transformed log cfu/ml. The maximum specific growth rate (μ_{max}) of KL102 was calculated during the exponential growth phase as $\mu_{max} = \ln(X_2/X_1)/t_2-t_1)$, being X_2 and X_1 the counts (cfu/ml) at time t_2 and t_1, respectively. The generation time ($\lambda = \ln 2/\mu_{max}$) was calculated for each culture from the corresponding value of μ_{max} [19].

For pathogens, the sample suspensions were submitted to count for *E. coli*, *S.* Typhimurium, *S. aureus* and *L. monocytogenes* according to standard procedures [15-18]. The results were transformed to log cfu/g. The plates were incubated at 35 ± 2°C for 24-48 h before colonies were counted. These pathogens were enumerated on violet red bile agar (Merck, Germany) for *E. coli* count, hektoen agar (Merck, Germany) for *S.* Typhimurium count baird parker agar added egg-yolk tellurite emulsion 20% (Merck, Germany) for *S. aureus* count and listeria selective agar added modified listeria selective supplement (Oxoid Limited, UK) for *L. monocytogenes* count.

Acidification activity

The pH of each sample suspension was determined at room temperature using electrodes of a pH meter (Mettler mini scan EZ, Germany) placed directly into each suspension. The determination was performed in triplicate to find the mean pH of the sample. The total acid was determined as described by Thomas; et al. [20] using colorimetric acidity titration.

Statistical analysis

All data were presented as means and standard deviations. Data of bacterial count, $\mu_{max,}$ λ, pH value and total acid were analyzed by the general linear model procedure. Least squares means were computed and separated ($p < 0.05$) using the PDIFF option of GLM. All statistical analyses were performed using SAS v. 9.0 (SAS Inst. Inc., Cary, NC, USA) [20].

Results

Chemical composition

The chemical compositions of RSE and DFE were showed in Table 1. The common components of RS and DF were carbohydrate, starch and total dietary fiber, respectively. Starch content of RSE had higher those of DFE ($p < 0.05$). The resistant starch and non-resistant starch fractions of RSE were in the range of 39.98-40.60 and 14.58-15.04 g/100 g dry matter, respectively. The soluble dietary fiber and insoluble dietary fiber fractions of DFE were in range of 15.23-15.60 and 70.02-71.30 g/100 g dry matter, respectively. On other hand, ash, fat and protein contents of RSE and DFE were minority components that in the range of 0.71- 4.72 g/100 g dry matter.

Table 1. Chemical composition of resistant starch and dietary fiber

Items (g/100 g dry matter)[1]	Resistant starch extract	Dietary fiber extract
Ash	2.39±0.08	4.58±0.21
Fat	1.04±0.02	0.77±0.06
Protein	3.17±0.11	1.94±0.02
Carbohydrate	93.40±0.09	92.61±0.11
Total dietary fiber	35.93±0.37	81.09±0.48
Soluble dietary fiber	7.31±0.16	15.44±0.17
Insoluble dietary fiber	28.62±0.29	70.65±0.64
Starch	55.24±0.42	5.42±0.66
Resistant starch	40.38±0.31	1.78±0.04
Non-resistant starch	14.86±0.29	3.64±0.11

[1]Values are means standard deviations of triplicate determinations. Treatments followed by different letters in same row were significant different ($P < 0.05$).

Growth and reduction profile

The growth of KL102 in fermented sausage model supplemented without and with 1% (w/v) RSE or 1% (w/v) DFE are shown in Figure 1. MRS broth was also included in this study for comparative purpose. The count of KL102 raised within the first 3 h of incubation time in MRS broth and FSM supplemented with RSE and DFE, whereas it increased after 3 h in FSM (Figure 1A). Higher growth rates were observed for FSM supplemented with RSE and DFE than FSM. According to Figure 1B and 1C, RS and DF supplementation increased the maximum specific growth rate (μ_{max}) and decreased the generation time (λ) significantly ($p < 0.05$).

In addition, MRS broth, FSM supplemented with RSE and DFE yielded bacterial growth with higher values of KL102 than FSM. This probiotic in all groups reached a stationary state within 24 h. In the end of fermentation, FSM supplemented with RSE and DFE yielded bacterial survival with higher values of KL102 than FSM and MRS broth ($p < 0.05$) (Figure 1A).

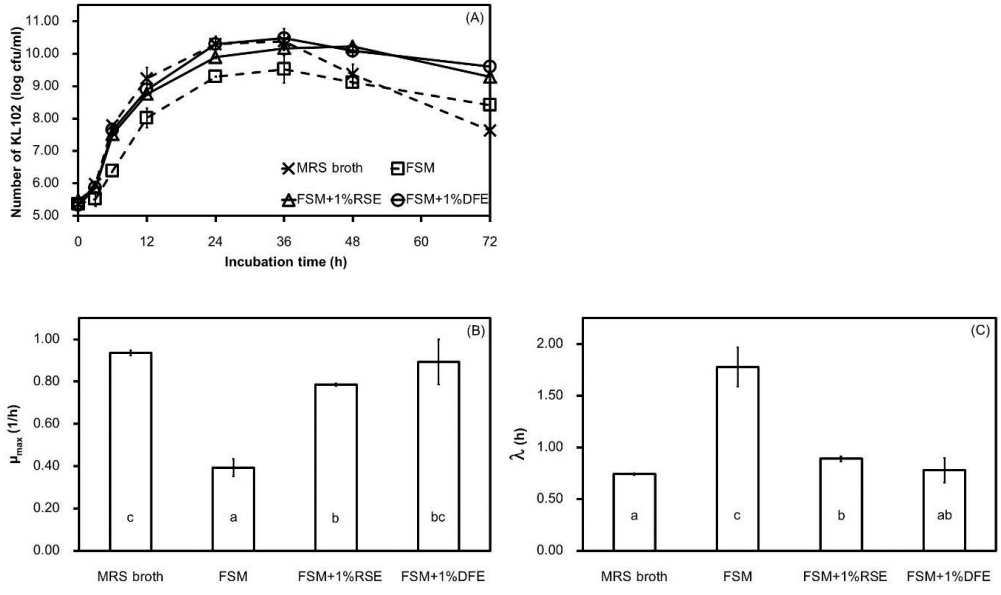

Figure 1. Growth (A), maximum specific growth rate (μ_{max}) (B) and generation time (λ) (C) of *L. plantarum* KL102 in MRS broth and fermented sausage model (FSM) supplemented without and with 1% (w/v) resistant starch extract (RSE) or 1% (w/v) dietary fiber extract (DFE). The results are presented as means of three independent experiments and standard deviations (bar). a-c Different letters indicate that values are significantly different ($p < 0.05$).

For reduction of pathogens, counts of all pathogens in MRS broth, FSM supplemented with RSE and DFE decreased following increase of incubation time. For FSM, they increased slightly during the first 6-12 h of incubation time, then decreased following increase of incubation time. Furthermore, reduction of all pathogens in MRS broth, FSM supplemented with RSE and DFE was faster than those in FSM. MRS broth, FSM supplemented with RSE and DFE could not detect *E. coli* and *S. Typhimurium* after 24 h, while FSM could not detect these pathogens after 48 and 36 h, respectively. Similarly, the counts of *S. aureus* and *L. monocytogenes* were not detected after 36 h for MRS broth, FSM supplemented with RSE and DFE and 48 h for FSM (Figure 2).

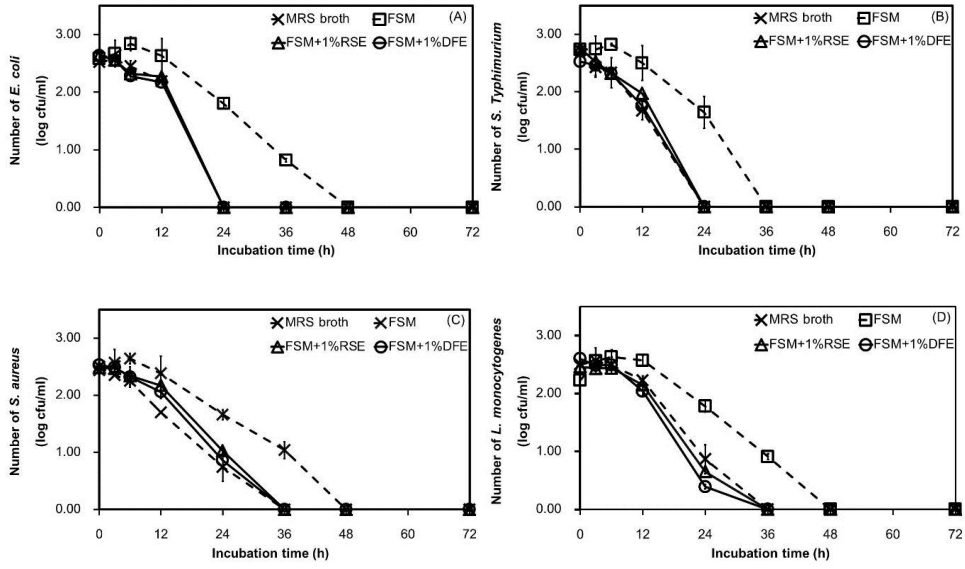

Figure 2. Reduction of *Escherichia coli* (A), *Salmonella* Typhimurium (B), *Staphycoccus aureus* (C) and *Listeria monocytogenes* (D) in MRS broth and fermented sausage model (FSM) supplemented without and with 1% (w/v) resistant starch extract (RSE) or 1% (w/v) dietary fiber extract (DFE). The results are presented as means of three independent experiments and standard deviations (bar).

Acidification activity of KL102

The pH and total acid content results in MRS broth and FSM supplemented without and with 1% (w/v) RSE or 1% (w/v) DFE are showed as the progressed fermentation (Figure 3.) MRS broth and FSM supplemented with RSE or DFE observed sharp decrease in pH within the 24 h of incubation time which were lower than 4. On the other hand, pH in FSM decreased less than 4 after 36 h of incubation time. After that, pH values of all broth continued to decrease throughout after 72 h of incubation time. Similarly, total acid contents in MRS broth and FSM supplemented with RSE or DFE were higher than those in FSM after 24 h of incubation time ($p < 0.05$).

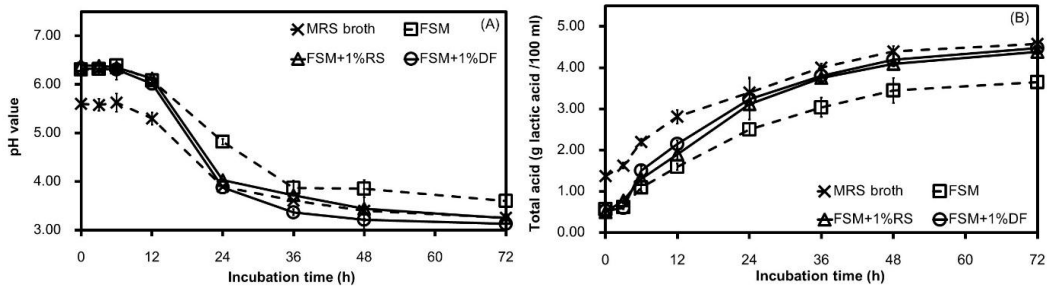

Figure 3. The pH value (A) and total acid (B) of changing by *L. plantarum* KL102 in MRS broth and fermented sausage model (FSM) supplemented without and with 1% (w/v) resistant starch extract (RSE) or 1% (w/v) dietary fiber extract (DFE).

Conclusions and Discussion

This study indicated the potential effects of RSE and DFE from banana by-products with regard to the quality characteristics of fermented sausage model. Table 1 showed starch, resistant starch and non-resistant starch contents were main compositions of RSE. The total dietary fiber, insoluble dietary fiber, soluble dietary fiber contents were main composition of DFE. DFE had more than 80 g/100 g dry matter of total dietary fiber. According to previous studies, DFE from unripe banana peel (KluayKhai) could be determined as a rich source of dietary fiber [3]. Furthermore, insoluble dietary fiber was the predominant fraction in DFE. The insoluble dietary fiber in banana peel fiber as reported by Suksathit and Tangwatcharin [3] includes hemicellulose (12.96 g/100 g dry matter), cellulose (23.60 g/100 g dry matter), and lignin (5.5 g/100 g dry matter). This class of dietary fiber is insoluble in water. It is possible that the DFE have pronounced effects on intestinal regulation and stool volume which are related to the probiotic consumption of insoluble dietary fiber [11, 22].

Figure 1. observed that adding of RSE or DFE in FSM supported growth of KL102 which means they exerted a prebiotic effect [19]. This effect was ascribed to the release in the FSM of additional nutrients or to the reduction of a negative environmental impact. KL102 grew rapidly within the first 3 h of incubation time in FSM supplemented with RSE and DFE. This reason affected to increase of the maximum specific growth rate and decrease of the generation time. The green banana flour is high RS content which is important on growth of probiotic bacteria. Generally, food with high RS content (green banana flour, retrograded rice flour, and RS standard) would be better to promote the survival of probiotic bacteria [23]. DFE extracted from green banana peel contains around 78.62 g of total DF, 1 g of inulin, 6 g of fructooligosaccharide and 10–20 g of pectin per 100 g of dry matter, in addition to significant amounts of α-linolenic acid (ALA), essential amino acids and micronutrients such as Mg, K, P and Ca [3-5]. Furthermore, KL102 counts in MRS broth and FSM decrease moderately at the end of incubation.

This decreased counts of KL102 resulted from low pH values in all broth (pH 3.20-3.91) after 36-72 h of incubation (Figure 3A), which was non-optimal pH of *L. plantarum* growth (pH < 4) [24]. *L. plantarum* has a facultatively heterofermentative metabolism and produce lactic acid which the acid decrease the pH [25]. While, KL102 counts in FSM supplemented with RSE and DFE were constant throughout the end of incubation. Due to prebiotic could protect *Lactobacillus* in low pH condition of fermentation [19].

For reduction of pathogens, Figure 2. showed that adding RSE or DFE in FSM decreased sharply all pathogen, possibly due to decrease in pH values and increase in total acid contents [26]. Moreover, various metabolic products of LAB, such as short-chain organic acid, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocin have antimicrobial potential [27].

Finally, the capabilities to enhance growth, survival and produce acid by *L. plantarum* KL102 are two of the reasons why RSE and DFE extracted from banana pulp and peel, respectively. These by-products of banana processing are two of the greatest prebiotics and can be applied to the fermented sausage inoculated probiotic lactic acid bacteria.

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