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THESIS

PRODUCTION OF PROBIOTIC BEVERAGE FROM RICEBERRY RICE

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Recently, a number of lactose intolerant consumer and demand of the vegetarian probiotic products is concerned. Therefore, this research aimed to develop non dairy probiotic beverage from Riceberry rice. It was germinated for five days to maximize GABA content (270 mg/ kg) dried malt and extracted with hot water (1:3) for probiotic malt-based beverage production. The five probiotic bacteria (*L. plantarum* TC24, *L. acidphillus* TISTR450, *L. reuteri* KUB-AC5, *L. Johnsonii* KUN119-2, *E. fecalis* N1-33) were tested their ability to grew in rice malt extract, survival of bacteria in acid and bile salt condition, antimicrobial activity, and preference test. *L. plantarum* TC24 was the most preferred by panelist and appropriated for using as probiotic bacteria. Inulin was suitable prebiotic for enhancing growth in the host intestinal more than FOS by prebiotic activity score 0.2. The probiotic beverage was formulated and cell encapsulation by alginate was applied for improving bacterial survival in gastrointestinal tract. However, encapsulated cell was leaked from the bead by acid degeneration and the survival of encapsulated and free cell were decreased to 10^6 cfu/ml after kept at 8 and 30 °C for 26 and 21 days, respectively. The sensory test confirmed that the product preferences was changed after storage for 14 days. The marketing survey with one hundred consumers indicated that the honey flavor was recommended and the shelf life of honey flavored beverage was 21 days at 8°C. One hundred milliliter of final product contained 6.84 mg GABA, vitamin A 163 μg and 10^{11} cfu/ml probiotic cell. The consumer test by fifty persons rated degree of preferences in between neither like nor dislike to like slightly and the product acceptance was increased significantly after they knew product benefit information. This research suggested that Riceberry rice malt extract was high potential media for probiotic or synbiotic beverage production.

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Atchareeya Nakkarach

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INTRODUCTION

Nowadays, consumers concern the important of having good health, therefore many functional foods are selling and rising their market share gradually. One of them is the probiotic food containing live microorganism such as live-cultured yogurt. Typically, probiotic food mainly contain dairy ingredient and some people are sensitive to dairy products, experiencing a host of symptoms including flatulence, diarrhea, skin rash and fatigue when they consume milk and other dairy products (Shah, 2001). Moreover, some vegetarians demand nearly 33% of the board market (LFI, 2006) also aware of dairy ingredient in any of functional food. Therefore, non-dairy probiotic product is a novel alternative product for ordinary people and specific choice for those of lactose intolerant victims and vegetarians.

Probiotics are beneficial bacteria that can be found naturally in various foods, or in the form of dietary supplements. They are defined as selected, viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their function in the intestinal tract (Dimer and Gibson, 1998; Zimmer and Gibson, 1998; Sanders, 1998; Vaughan *et al.,* 1999; Zubillaga *et al.,*2001; Holzapfel and Schillinger, 2002). Thus, amount of live probiotic passes the human gastrointestinal tract and colonization, is one key point to achieve benefit from their function. Healthy selected probiotic will adhere to human intestinal where a few or none of sufficient nutrient can be found, thus to prolong their resident time, the dietary fiber must be ingested by the host. Once it is utilized and promote growth of probiotic, it is defined as prebiotic. Prebiotics promote the growth of beneficial bacteria in the digestive system, particularly probiotic bacteria. Thus, consuming both of prebiotic and probiotic could regulate the intestinal flora and bring favorably effects to consumer through their synergism. However, number of live probiotic bacteria must high enough and the recommended dose reach 10^6 cfu/ml (Shan, 2001).

The unproper transportation method, variation of shelf temperature and acid and bile salt of gastrointestinal track cause decreasing of living cell number and finally, cell concentration not enough to overcome pathogenic microorganism or even the local micro flora. Therefore, cell encapsulation could be one method to enhance number of living cell. Microencapsulation techniques have been developed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment.

The objective of this study is to develop probiotic beverages from non dairy resource. The novel product was developed base on basic of non-dairy probiotic beverage (Granato *et al.,* 2010) and only six steps used in this research, firstly was assembling an idea to have a beverage tasted of smooth sweet and sour, harmony with flavor, conferred benefit of GABA and other nutrition from Riceberry rice malt and potentially restored digestive system of consumer. Secondly, the microorganism screening was done and thirdly was formulation development by selection of prebiotic, the fourth step was process development by using encapsulation to enhance microbial survival, fifth was sensory and market studies and finally is nutrition claims. These were the steps of novel probiotic product development used in this research.

OBJECTIVES

This research, aims to produce probiotic beverage from Riceberry rice malt, which has sub objective as following:

1. Selection and evaluation of probiotic bacteria culture in Riceberry malt beverage

2. Improvement of bacterial survival in gastrointestinal tract condition by encapsulation

3. Product shelf life evaluation by chemical, microbiological method and consumer preference test.

LITERATURE REVIEW

1. Probiotics

Probiotics are live microorganisms defined as selected, viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their function in the intestinal tract (Dimer and Gibson, 1998; Zimmer and Gibson, 1998; Sanders, 1998; Vaughan *et al.,* 1999; Zubillaga *et al.* ,2001; Holzapfel and Schillinger, 2002). There are some ideal properties of the probiotic strains, which would benefit the human health and could be used in probiotic industry: resistance to acid and bile; attachment to the human epithelial cells; colonization in the human intestine; production of antimicrobial substances, called bacteriocins (Jack *et al.,* 1995) good growth characteristics and provide beneficial effects on the human health as illustrated in Figure 1.

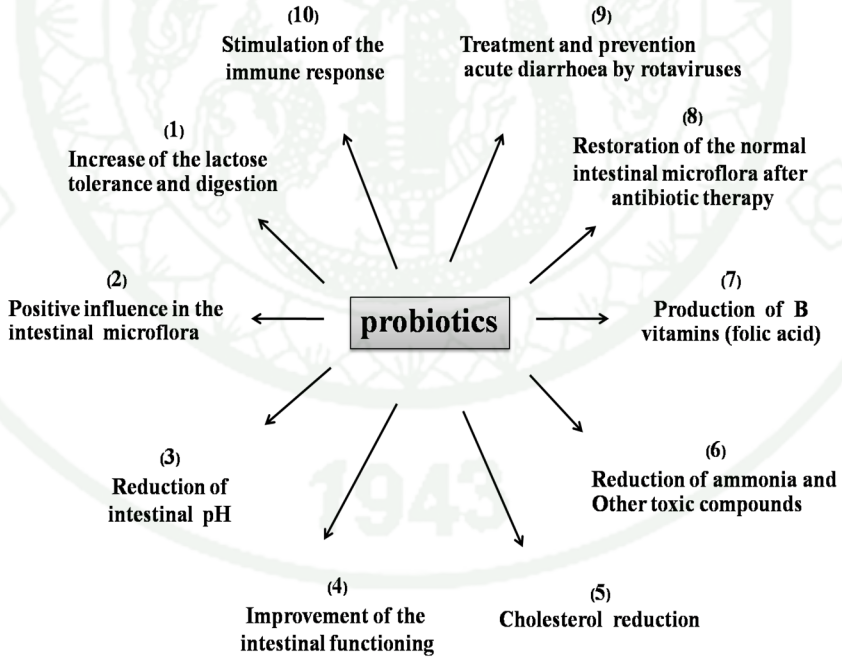


Figure 1 Probiotic beneficial effects on human health.

Source: Gibson and Roberfroid (1995)

1.1 Health benefit of probiotics

1.1.1 Intestinal tract health

Diarrhea is a major cause of infant death worldwide and could be found in young children which caused by rotaviruses. Probiotic consumption could be useful for treatment of many types of diarrhea, including antibiotic-associated diarrhea in adults and travelers. Particularly in developing country, which lack of good practice and high risk of pathogenic contamination in food. Probiotic bacteria have also been shown to preserve intestinal integrity and mediate the effects of inflammatory bowel diseases, irritable bowel syndrome, colitis, and alcoholic liver disease (Vasudha and Mishra, 2013). In addition, lactic acid bacteria may improve intestinal mobility and relieve constipation, particularly in seniors.

1.1.2 Immune system

The evidence from *in vitro* systems and the animal models suggested that probiotics can enhance both the specific and nonspecific immune responses, possibly by activating macrophages, increasing levels of cytokines, increasing natural killer cell activity, and/or increasing levels of immunoglobulin (Flávera *et al.,* 2008). In spite of limited testing in humans, these results may be particularly important to the elderly, who could get benefit from an enhanced immune response.

1.1.3 Allergy

Probiotics may exert a beneficial effect on allergic reaction by improving mucosal barrier function. Probiotics such as *Lactobacillus* may be helpful in alleviating some of the symptoms of food allergies such as those associated with milk protein. Probiotic consumption may thus be a means for primary prevention of allergy in susceptible individuals. This could play a key role in minimizing allergy at

a time when the prevalence of allergic disease in Western societies has increased dramatically over the past 40 years (Delcenserie *et al.,* 2010)

1.1.4 Lactose intolerance

Several of evidences showed that the appropriate strains of lactic acid bacteria, such as *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and other lactobacilli in fermented milk products, can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine and stomach. Because lactose intolerance affects almost 70% of the population worldwide (Council of California, 2000), consumption of these probiotic products may be a good way to heal or relieve of lactose intolerance syndrome.

1.1.5 Cancer

A few epidemiological studies indicate that consumption of fermented dairy products containing *Lactobacilli* or *Bifidobacteria*, lowers the incidence of colorectal cancer, which shows that probiotics reduce the risk of colon cancer. A case-control study conducted in Japan with 180 cases and 445 controls revealed that habitual intake of lactic acid bacteria reduces the risk of bladder cancer (Rafter, 2004).

1.1.6 Nutrient synthesis and bioavailability

Fermentation of food with lactic acid bacteria has been shown to increase folic acid content of yogurt and kefir, and to increase niacin and riboflavin levels in yogurt, vitamin B12 in cottage cheese and vitamin B6 in Cheddar cheese. In addition to nutrient synthesis, probiotics may improve the digestibility of some dietary nutrients such as protein and fat. Short-chain fatty acids such as lactic acid, propionic acid and butyric acid produced by lactic acid bacteria may help maintain an

appropriate pH and protect against pathological changes in the colonic mucosa. (Council of California, 2000).

2. LAB (Lactic Acid Bacteria)

Lactic acid bacteria are Gram-positive and they are non-spore forming, fermentative bacteria that grow anaerobically. The main function of these bacteria is the fermentative conversion of sugars present in raw materials into lactic acid. There are a large number of probiotics currently used and available in dairy fermented foods. One of the most significant groups of probiotic organisms are the lactic acid bacteria (Table 1) because they constitute a diverse group of organisms providing considerable benefits to human. LAB have been used as probiotics to manage intestinal disorders such as lactose intolerance, acute gastroenteritis, constipation, and inflammatory bowel disease. In addition, lactic acid bacteria was used in the food industry for imparting flavor, texture and possessing preservative properties. Food applications for probiotics are found mostly in dairy products, with yogurts, kefir, and cultured drinks.

The global market for probiotic ingredients, supplements, and foods was worth $14.9 billion in 2007 and reached US$16 billion in 2008. Estimate target a total of US$19.6 billion on sales in 2013, a compound annual growth rate (CAGR) of 4.3%. Probiotics of the *Lactobacillus* genus accounted for the largest share, representing 61.9% of total sales in 2007 (Food Processing, 2009).

Table 1 The most commonly used species of lactic acid bacteria in probiotic

Lactobacillus sp.	*Bifidobacterium sp.*	*Enterococcus sp.*	*Streptococcus sp.*
L. acidophilus	*B. bifidum*	*Ent. faecalis*	*S. cremoris*
L. casei	*B. adolescentis*	*Ent. faecium*	*S. salivarius*
L. delbrueckii ssp. (bulgaricus)	*B. animalis*		*S. diacetylactis*
L. cellobiosus	*B. infantis*		*S. intermedius*
L. curvatus	*B. thermophilum*		
L. fermentum	*B. longum*		
L. lactis			
L. plantarum			
L. reuteri			
L. brevis			

Source: Fook *et al.* (1999)

2.1 *Lactobacillus plantarum*

L. plantarum is a gram positive bacterium that is found in a variety of niches. These niches include dairy, meat, and much vegetable fermentations, it is also found in the human gastrointestinal tract. It is a facultative heterofermentative lactic acid bacterium that utilizes an extensive range of fermentatable carbon sources. *L. plantarum* also produces anti-microbial peptides and exopolysaccharides. It has the ability to maintain a pH gradient between the inside and outside of the cell in the presence of large amounts of acetate or lactate (Maaike *et al*., 2006).

The ability of this microbe to adapt and thrive in a range of environments, its ability and capacity to be genetically manipulated, as well as its ability to ferment and degrade different materials makes *L. plantarum* a very interesting and important bacteria to study.

Kyung and colleague (2006) studied production of probiotic cabbage juice by lactic acid bacteria. *L. plantarum* was examined for their ability to utilize cabbage juice for cell synthesis and lactic acid production without nutrient supplement. From the results of this study, *L. plantarum* could be used as probiotic cultures for production of a healthy beverage from cabbage for vegetarians or consumers who are allergic to lactose present in probiotic dairy products.

2.2 *Lactobacillus acidophilus*

Early studies of *L. acidophilus* were performed on strains isolated from fecal of humans, pigs and chickens. Since then *L. acidophilus* has been further characterized as a short Gram-positive rod (2-10μm), is homofermentative and has optimal growth at temperatures of 37-42˚C. Of the *Lactobacillus* species and *L. acidophilus* is the most well known and is commercially distributed as a probiotic., for example, the *L. acidophilus* strain, NCFM, was isolated from a human in 1970 and characterized at North Carolina State University. NCFM has been commercially available in the United States as a probiotic strain since the mid-1970s. NCFM is also used for formula, yogurt and fluid milk production (Altermann *et al.,* 2005). The physiological, biochemical, genetic, and fermentative properties have been widely explored in both humans and animals.

Amal and colleague (2012) investigated of cereal-based probiotic beverages. Rice and millet grains were fermented with *L. acidophilus*. The fermentation improved the color, flavor, texture and overall acceptability of the beverages. Slightly changes in the number of probiotic, pH and acidity during storage were observed. The shelf-life of the rice and millet fermented beverages was to be 15 days under refrigerated storage.

2.3 *Lactobacillus johnsonii*

Lactobacillus johnsonii is one of many microorganisms that reside in the human intestine. Like all species of the *Lactobacillus* genus, it is an anaerobic, Gram-

positive bacterium, which has a rod-like shape and does not undergo spore formation (Falsen *et al.,* 1999). *L. johnsonii* and other GI tract microbes aid in polysaccharide and protein digestion and also generate a variety of nutrients, including vitamins and short-chain fatty acids that make up 15% of a human's total caloric intake. In addition, because *L. johnsonii* is able to undergo fermentation and can therefore make lactic acid, it plays a major role in the fermentation and preservation of various food items, such as dairy, meat, vegetable products, and cereal (Falsen *et al*., 1999; Pridmore *et al.,* 2004).

Maja and colleague (2012) selected appropriate *Lactobacillus* strains for production of functional whey-based beverage. The study showed that *L. johnsonii* was top candidate for the functional whey based beverage production. This strain attained titratable acidity of 9.2 °SH after 10 h of fermentation, appropriate odor and cell number of 6.8 log (cfu/ml).

2.4 *Lactobacillus reuteri*

Lactobacillus reuteri are Gram-positive, rod-shaped, and anaerobic. These heterofermentatic lactic acid bacterium naturally inhabit the gut of a wide range of organisms, including in humans, pigs, chickens and mice. They can also be isolated from human breast milk. In vitro, *L. reuteri* grows optimally on MRS media at 37 ˚C (Morita *et al.,* 2008). They have also been found to grow in biofilms (Jones *et al.,* 2009). *L. reuteri* produces reuterin, an antimicrobial that inhibits growth of harmful bacteria, fungi, and protozoa. Due to these probiotic properties, *L. reuteri* is believed to be a promising therapy for the alleviation and reduction of certain illnesses related to gastrointestinal health, oral health, and urogenital health, including infantile colic, eczema, and *Helicobacter pylori* infection (Jones *et al.,* 2009).

Adrian and colleague (2007) studied of a whey-based probiotic product with *L. reuteri*, that inoculated into reconstituted whey containing sucrose and pectin in order to prepare a fermented probiotic product. The treatment with the highest bacterial counts and sensory scores was selected and stored. The result was found

titratable acidity and pH values as well as sensory properties did not change appreciably during storage. At the end of the storage period, slight acidification was detected, although the beverage still retained an acceptable flavor.

2.5 *Enterococcus faecalis*

Enterococci are Gram-positive cocci that can survive in harsh natured conditions. They can be found in soil, water, and plants. Some strains are used in the manufacture of foods whereas others are the cause of serious human and animal infections (e.g. they are known to colonize the gastrointestinal and genital tracts of humans). They are associated with both community and hospital acquired infections. *Enterococci* can grow at a temperature range of 10 to 42°C and in environments with broad pH values. Some are known to be motile. While there are over 15 species of the *Enterococcus* genus, 80-90% of clinical isolates are *E. faecalis* (Gilmore *et al.,* 2002). *Enterococci* are typically catalase negative, and are anaerobic. They are able to grow in 6.5% NaCl, can hydrolyze esculin in the presence of 40% bile salts and are pyrrolidonyl arylamidase and leucine arylamidase positive (Gilmore *et al.,* 2002). *Enterococci* have been proven to present a therapeutic challenge because of their resistance to many antimicrobial drugs, "including cell-wall active agents; aminoglycosides, penicillin and ampicillin, and vancomycin" (Paulsen *et al.,* 2003).

3. Prebiotic

Prebiotics are defined as " non digestible food ingredients that benefit the host by selectively stimulating the growth and/or activity of one or more of a limited number of bacteria in the colon and thus improve health" (Gibson and Roberfroid, 1995). The function of prebiotics is to basically stimulate existing metabolisms in the colon as show in Figure 2 (Coussement, 1996). Thus, the prebiotic approach advocates administration of non-viable entities and therefore overcomes survival problems in the upper gastrointestinal tract. The prebiotic concept considers that many potentially health-promoting micro-organisms, such as *bifidobacteria* and *lactobacilli*,

are already resident in the human colon. To be an effective prebiotic an ingredient must:

- Neither be hydrolysed nor absorbed in the upper part of the gastrointestinal tract;
- Have a selective fermentation such that the composition of the large intestinal microbiota is altered towards a healthier composition;
- Maintenance of viability in the product and harsh condition such as gastric acidity and bile salts

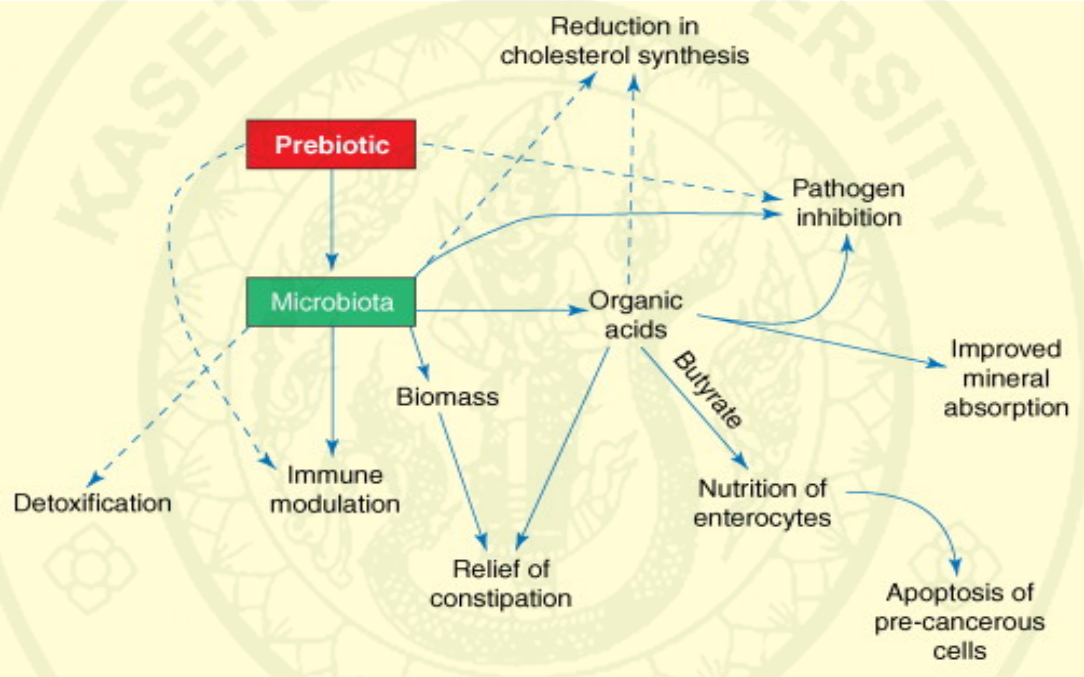


Figure 2 Schematic showing the possible mechanisms of prebiotic action

Source: Ouwehand *et al.* (2005)

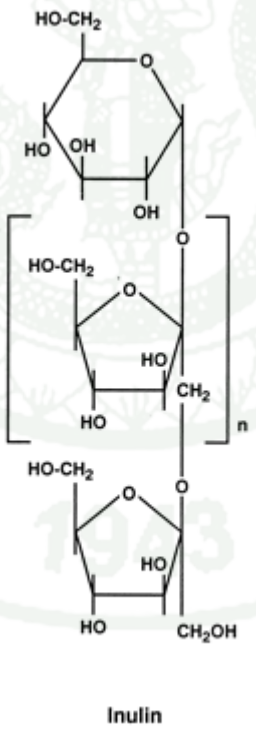
4. Common prebiotic

4.1 Inulin

Inulin is a polydisperse β(2-1) fructan. The fructose units in this mixture of linear fructose polymers and oligomers are each linked by β(2-1) bonds. A glucose molecule typically resides at the end of each fructose chain and is linked by an α(1-2)

bond, as in sucrose. The chain lengths of these fructans range from 2-60 units. The unique aspect of the structure of inulin is its β(2-1) bonds. These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fiber effects (Kathy, 1999). The structure of inulin is shown in Figure 2.

Inulin containing in plants such as onions, leeks, Jerusalem artichokes, and garlic are shown in the Table 2. There are many ways that plants store energy for themselves, with inulin being one of them. As such, it can be found in the roots and rhizomes of many plants. When eaten, this substance does not increase blood sugar, which makes it an option for those with diabetes. Many manufacturers use inulin in processed foods because it has tremendous health benefits. It can also be used to replace higher calorie ingredients such as fat, sugar, and flour.



Inulin

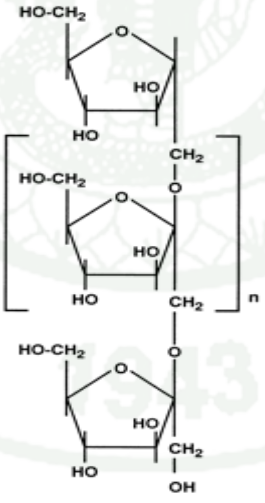
Figure 3 The structure of inulin

Source: Anatoly (2008**)**

There are many benefits of inulin. It has one-third to one-fourth less food energy than that sugar and a sixth to a ninth less energy than fat. It is also a soluble fiber which means that when it passes through the body, it creates a gel. Since fiber is not digested in the human body, it passes through to the intestine largely intact. Therefore, it feeds the beneficial bacteria that live there. It also helps to reduce the absorption of bad cholesterol in the body (Shelly, 2009).

4.2 Fructo-oligosaccharides (FOS)

Fructo-oligosaccharides (FOS) are oligosaccharide containing 2-10 monosaccharide residues connected by glycosidic linkages Oligofructose derived from chicory contains both fructose chains (F_m) and fructose chains with terminal glucose units (GF_n). Synthesized oligofructose contains only fructose chains with glucose end units or GF_n molecules. Both types of oligofructose contain β(2-1) linkages between the fructose molecules, and they both carry essentially the same nutritional benefits (Kathy, 1999). The structure of FOS is shown in Figure 4.



Fructooligosaccharide

Figure 4 The structure of fructo-oligosaccharides

Source: Anatoly (2008)

FOS exhibits sweetness levels between 30 and 50 percent of sugar in commercially prepared syrups (Joseph, 2012). It is accepted that FOS are not degraded or absorbed in the upper human gastrointestinal tract. As such, they enter the colon intact where they are susceptible to metabolism by the resident microbiota. The β configuration of anomeric C2 in fructose monomers, is thought to make FOS resistant to hydrolysis by human digestive enzymes which display a high degree of specificity for glycosidic linkages (Gibson and Roberfroid, 1995).

Table 2 Inulin and fructo-oligosaccharides content in plants used for human nutrition

Source	Edible	Inulin content (% on fresh weigh)	fructo-oligosaccharides content (% on fresh weigh)
onion	bulb	2-6	2-6
jeusalem	tuber	16-20	10-15
chicory	root	15-20	5-10
leek	bulb	3-10	2-5
garlic	bulb	9-16	3-6
artichoke	leaves-heart	3-10	<1
banana	fruit	0.3-0.7	0.3-0.7
rye	cereal	0.5-1.0	0.5-1.0
barley	cereal	0.5-1.5	0.5-1.5
wheat	cereal	1-4	1-4

Source: Modified from Shelly (2009**)**

5. Encapsulation

There are different methods such as physical entrapment in polymeric networks, attachment or adsorption to a preformed carrier, membrane entrapment,

microencapsulation have been used for immobilizing LAB. The purpose of these techniques is either to retain high cell concentrations within the bioreactor or to protect cells from a hostile environment. For industrial applications in the food industry, the carrier material must be non-toxic, readily available and affordable. It should also lead to high-cell loading and the cells should have a prolonged viability in the support. Thermal (κ-carrageenan, gellan, agarose and gelatin) or ionotropic (alginate and chitosan) gelation of the droplets are used to produce spherical gel biocatalysts. These polymers are readily available and widely accepted for use as additives in the food. For food applications, the most widely used immobilization technique is the entrapment of cells within a food-grade porous polymeric matrix (Table 3).

Table 3 Lactic acid bacteria immobilization by entrapment techniques

Support	Species	Maximum cell concentration[a]	Reference
Ca-alginate	*Lactococcus lactis* ssp.	2.0 x 10^{11} cfu/ml	Prévost and Diviès 1992
Ca-alginate	*Lactococcus lactis* ssp.	3.8 x10^{11} cfu/g	Prévost and Diviès 1992
κ-carrageenan	*Lactobacillus casei*	5.1 x10^{11} cfu/ml	Arnaud *et al.,* 1992
κ-carrageenan	*Lactococcus lactis*	1.3 x10^{11} cfu/g	Lamboley *et al.,* 1997
Gellan gum	*Bifidobacterium longum*	6.8 x10^{10} cfu /g	Doleyres *et al.,* 2002

[a] cfu /ml or g of support

[b] LBG = locust bean gum

Source: Yann *et al.* (2011**)**

In many applications, controlled-size polymer droplets are produced using extrusion or emulsification, under mild conditions. A careful selection of polymer composition is necessary to achieve high mechanical stability of gel biocatalysts

during long-term fermentation, according to the conditions of the fermentation (Artignan *et al.,* 1997). Gel entrapment is a relatively simple method resulting in usually spherical beads with diameters ranging from 0.3 to 3.0 mm with high biomass concentration. However, the large scale production of beads under aseptic conditions necessary for food applications still remains an important issue for the industrialization of immobilized cells in the food sector.

Encapsulation is a process whereby cells are retained within an wall material to reduce cell injury. Encapsulation in hydrocolloid beads has been investigated as a means to protect and improve viability of probiotic microorganisms in food products and in the intestinal tract. Other benefits of encapsulation includes: protection of probiotics from bacteriophage, increased survival during freeze-drying and freezing, and greater stability during storage (Bhagya, 2009). Hou and colleague (2003) demonstrated that encapsulation of *Lactobacillus delbrueckii* ssp. *bulgaricus* increased their bile tolerance, and viability was elevated by approximately four log units after encapsulation within artificial sesame oil emulsions. Capela and colleagues (2006) improved viability of probiotic organisms encapsulation in 3% w/v sodium alginate in freeze-dried yogurt after 6 months of storage at 4°c (~8 log cfu/g) and 21°C (~6 log cfu/g).

6. Non-dairy probiotic beverages

There are a wide variety of traditional non-dairy fermented beverages produced around the world. Many of them are non-alcoholic beverages manufactured with cereals as principal raw material. Despite potential sensory challenges, there is a genuine interest in the development of fruit-juice based functional beverages, fortified with the probiotic and prebiotic ingredients. The fruit juices have been suggested as an ideal medium for the functional health ingredients, because they inherently contain beneficial nutrients, they have taste profiles that are pleasing to all the age groups, and because they are perceived as being healthy and refreshing (Tuorila and Cardello, 2002).

Evidently, there are already some relatively new nondairy probiotic beverages in the market. Grainfields Wholegrain Liquid (non-dairy product in Australia) is a refreshing, effervescent liquid that delivers active, friendly lactic acid bacteria and yeasts as well as vitamins, amino acids, and enzymes. It is fermented with Lactobacilli and yeasts cultures: *L. acidophilus*, *L. delbreukii, Saccharomyces boulardii* and *S. cerevisiae.* The liquid is dairy-free, contains no genetically modified ingredients and has no added sugar. Grainfields wholegrain liquid is made from organic ingredients including the grains, beans, and seeds such as the malted organic oats, maize, rice, alfalfa seed, pearl barley, linseed, mung beans, rye grain, wheat and millet. The liquid is fermented to achieve high levels of active probiotic bacteria sustained in a liquid medium immediately available for the use within the digestive system. The consumer demand for non dairybased probiotic products has increased (Shah, 2001) and the application of probiotic cultures in non-dairy products and environments represents a great challenge (Mattila *et al.,* 2002).

Thus, scope of this study was to develop non-dairy probiotic beverage from Riceberry rice malt. Riceberry rice is a cross bred rice producing dark violet grain contains three times more iron than other varieties and high level of antioxidants. Therefore, the premium selected rice varieties was malted and mashed with hot water to extract the sweet wort containing mono, di and tri-saccharide, free amino acid which available for lactic acid bacteria and GABA. The five of lactic acid bacteria were evaluated for some important probiotic properties and selected prebiotics was used for product formulation. Product shelf-life, chemical composition and sensory evaluation were conducted for ensuring this product was safe for consumption.

MATERIALS AND METHODS

Materials

1. Microorganism

There are 5 lactic acid bacteria for probiotic selection, including of *Lactobacillus plantalum* TC24, *Lactobacillus johnsonii* KUNN19-2, *Enterococcus faecallis* N1-33 were supported by Assoc. Prof. Vichien Leelawatcharamas. *Lactobacillus reuteri* KUB-AC5 was supported by Assoc. Prof. Sunee Nitisinprasert And *Lactobacillus acidophilus* TISTR450 obtained from Thailand Institute of Scientific and Technological Research (TISTR).

2. Rice for malt-base beverage production

Rice for malting in this research is Riceberry from Kasetsart University, Kamphaeng Saen Campus, was used for rice malt-based beverage production.

Methods

1. Study of appropriate germination time for rice malt production

Malting of rice was carried out by steeping and air rest for intervals up to 7 days which steeping and air rest 8 and 4 hours respectively, in second days, steeping and air rest 4 and 8 hours respectively, in fourth days, steeping and air rest 2 and 10 hours respectively, in fifth days and air rest 24 hours in last day in order to achieve high GABA rice malt. Temperature of germination was controlled at 30 °C and relative humidity was 99%. After each day, geminating rice was taken to dry using temperatures program as following, 40°C (14 hours), 50 °C (10 hours), 60 °C (14 hours) 80 °C (6 hours), 105 °C (4 hours) in the tray dryer. Root and shoot of dried malt were eliminated and finished malt was kept at room temperature.

2. Rice malt extract preparation for probiotic beverage production

Riceberry malt was milled to 0.5 mm particle size by using Hammer mill and mashed with water (1:3) in steam jacket pan. Temperatures was raise to 45°C and hold for 30 min, then 60°C for 60 min ,70°C for 30 min and 85°C for 10 min. Then rice malt extract was separated by filltration through white thin cloth and sterlied by autoclave at 121°C for 15 min. The cleared malt extract was obtained by filtration through filter paper whatman No.1 and stored at 4°C. Qualities of wort including of acidity, pH and ºBrix were analyzed.

3. Bacteria culture preparation

Five bacterial strains, *L. plantalum* TC24, *L. reuteri* KUB-AC5, *L. johnsonii* KUNN19-2, *L. acidophilus* TISTR450 and *E. faecallis* N1-33 were grown at 37°C for 48 hours in MRS broth and 10^7 cfu/ml living cells were used as inoculums. The cell pellet was collected by centrifugation at 5000 rpm for 30 min at 4°C, and washing twice with rice malt extract before using.

4. Selection of appropriate bacteria for malt-base probiotic beverage production

4.1 Test of bacterial growing in Riceberry malt extract media

Rice berry malt extract was inoculated with 10^7 cfu/ml of *L. plantalum* TC24*, L. reuteri* KUB-AC5*, L. johnsonii* KUNN19-2, *L. acidophilus* TISTR450 and *E. faecallis* N1-33, separately. Fermentation was carried out at 37°C for 48 hours. The cell concentration was determined every 4 hours during incubation period.

4.2 Determination of antimicrobial activity

The antimicrobial activity was determined by agar diffusion method (Dobner *et al.,* 2003 and Baydar *et al.,* 2004). Nutrient broth cultures of *Bacillus subtilis* TISTR025, *E. coli* 010, *Samonella enteritidis* DMST17368 and *Staphylococcus aureus* TISTR118 were grown at 37°C for 24 h. Afterward culture was spread plate. Wells were punched out of the solid agar using sterile cork borer, and 50 µl of the cell-free culture broth (after16 hours of fermentation) which wasn't adjusted and adjusted to pH 2-12 with HCl or NaOH, was dropped in each wells and using phosphate buffer solution pH 2-12 as a control. After 24 h, the diameter of inhibition zones were measured.

4.3 Acid - bile tolerance test

L. plantarum TC24*, L. reuteri* KUB-AC5*, L. johnsonii* KUNN19-2, *L. acidophilus* TISTR450 and *E. faecallis* N1-33 grown in MRS broth at 37°C for 48 hours were collected by centrifugation at 5000 rpm for 30 min at 4°C. Cell pellet was washed twice with rice malt extract and resuspended into 10 ml of phosphate-buffered saline (PBS) before addition to steriled PBS pH 2.5 (adjusted using 5 M HCl) and then incubated at 37°C for 3 hours, to determine the viable cell (Charalampopoulos *et al.,* 2004; Zhao *et al.,* 2012).

The survival cell pellet from acid test was washed twice with rice malt media and 5 ml of 3% oxgall solution and wort were added. The tubes were then incubated at 37°C, the assay had a final bile concentration of 1.5 %, and pH 7.5. The viable bacteria was counted after exposure for 0 and 3 hours (Patel *et al.,* 2004). The percentage of survival was determined according to equation.

%survival cell = (last cell concentration / initial cell concentration) x 100

4.4 sensory test of five probiotic beverages

The sensory test were performed on five probiotic fermentation beverages. Panelists were asked to taste each sample and to rate its overall, aroma and taste using 5 hedolic scale : the scores ranged from 1= dislike very much, to 3 = like moderately, to 5= like very much i.e. higher sensory scale indicated better overall acceptance.

5. Improvement of bacteria survival by encapsulation

5.1 Encapsulation

The selected probiotic was encapsulated to enhance survival cell number in acid and bile condition and prolong their shelf life in storage condition. The concentrated cell suspension was mixed with steriled 2% sodium alginate, 5.5 % rice malt media, 5% glycerol, 1% of selected prebiotic and 0.1% tween 80. Alginate-bacteria mixture was extruded from a needle (No. 25), 10 cm height from calcium chloride solution using a peristaltic pump. The droplets were dropped into a steriled 0.1M calcium chloride solution at room temperature and stored in the solution for 30 min and then washed gel beads with rice malt extract. Fermentation was carried out at 37°C for rate log phase. The encapsulation cell was evaluated the potential to protect cell in in vitro GI condition and morphology of alginate bead was observed by SEM (Sohail *et al.,* 2011).

5.2 Simulated GI tract

Survival of the encapsulate cell was tested in the simulated GI tract condition. Simulated gastric and bile juices were prepared freshly. Simulated gastric juices was prepared by suspending pepsin (0. 592 µl/ml) (Zhao *et al.,* 2012) in sterile buffer saline (0.5%, w/v) to a final concentration of 3 g/l and adjusting the pH to 2.5 with concentrated HCl or steriled 0.1 mol/l NaOH using a pH meter.

Simulated bile juices was prepared by suspending pancreatin USP (P-1500, Sigma, St. Louis, MO, USA) in the steriled saline to a final concentration of 1 g/l, with 4.5% bile salts and adjusting the pH to 7.5 with sterile 0.1 mol/l NaOH using a pH meter. The viable bacteria was counted after exposure for 0 and 3 hours and the percentage of survival was determined according to equation(Haruhito *et al.,* 2006).

% survival cell = (last cell concentration / initial cell concentration) x 100

5.3 Morphology of microencapsulate bead

The Morphology of bead was observed by scanning electron microscopy (SEM). The beads were first washed with a phosphate solution (15.25 g/l Na_2HPO_4, 5.85 g/l KH_2PO_4), fixed by 2.5% glutaraldehyde, dehydrated by sequential ethanol extraction, and dried by a critical point dryer. The beads were then cut and coated with gold for analysis using a scanning electron microscope (JSM-5600LV, JEOL) (Kourkoutas *et al.,* 2005).

6. Determination of prebiotics activity score by lactic acid bacteria

Prebiotic activity reflects the ability of a given substrate to support the growth of a beneficial microorganism relative to other micro-organisms and relative to growth on a non-prebiotic substrate such as glucose. Therefore carbohydrates have a positive prebiotic activity score if they are metabolised as well as glucose by probiotic strains and are selectively metabolised by probiotics but not by other bacteria.

The assay was performed by adding 10^7 cfu/ml of an overnight incubated culture of *L. plantarum* TC24 to tubes containing modified MRS (half the usual concentration of potential growth substrates such as peptone, meat extract and yeast extract), modified MRS + 1% inulin contain 0.98 g/100ml, modified MRS + 1% FOS contain 0.95 g/100ml, modified MRS + glu 0.02 g/100ml, modified MRS + glu 0.05 g/100ml. The sugar concentration found in inulin powder (2%) and FOS (5%) were

taken into account and the growth of bacteria in MRS + 0.02 g and MRS + 0.05 g were compared to MRS containing 0.98 g inulin/100 ml and 0.95 g FOS/100 ml, separately. In addition, overnight cultures of *Escherichia coli, Bacillus subtilis, Samonella enteritidis* were mixed and added to the test tubes containing M9 medium24, M9 medium24 + 1% inulin contain 0.98 g/100ml, M9 medium24 + 1% FOS contain 0.95 g/100ml, M9 medium24+ glu 0.02 g/100ml, M9 medium24+ glu 0.05 g/100ml. The cultures were incubated at 37 ˚C. Each assay was replicated three times. The prebiotic activity score was determined according to equation (Marotti *et al.,* 2012).

$$[(A- B)/(C - D] - [(E - F)/(G - H)]$$

A = probiotic log cfu/ml on prebiotic at 24 h
B = probiotic log cfu/ml on prebiotic at 0 h
C = probiotic log cfu/ml on glucose at 24 h
D = probiotic log cfu/ml on glucose at 0 h
E = enteric log cfu/ml on prebiotic at 24 h
F = enteric log cfu/ml on prebiotic at 0 h
G = enteric log cfu/ml on glucose at 24 h
H = enteric log cfu/ml on glucose at 0 h

7. Influence of storage condition on living bacteria cell concentration

The most preference sample from period experiment was stored at 8°C and 30 °C for 4 weeks, the viable cell number was counted every day in the first week and every 3 days in next further weeks. The consumer preference test of storage product was carried out every 7 days, due to change of their chemical, microbiological propertied might influence consumer perception. Qualities of product including of acidity, pH and ºBrix was analyzed along the storage time. In addition the changes of bead morphology was observed by SEM (5.3).

8. Sensory and market study

The hundred consumers were asked to answer the questions and tested the ordinary probiotic beverage. Their opinion was considered for product improvement and reprocesses. Then 50 consumer were asked to rate the degree of preferences using 9 points hedonic scales. Three attributes of overall acceptance, flavor and taste were evaluated. The scores ranged from 1= dislike very much, to 5 = neither like nor dislike, to 9= like extremely well, i.e. higher sensory scale indicated better overall acceptance.

9. Product nutrition analysis

Ash, fat and moisture were analyzed by AOAC (2005). Carbohydrate and Total energy were analyzed by In house method TE-CH-076 based on AOAC (2010) 985.29. Protein was analyzed by In-house method base on AOAC (2005) 981.10. Iron and calcium were analyzed by In-house method TE-CH-134 base on AOAC (2005) 999.10. Vitamin A and B were analyzed by In-house method TE-CH-057 base on AOAC (2010) 942.23. Fiber was analyzed In house method TE-CH-169 based on Compendium of method for food Analysis Thailand. All information nutritious were analyzed by Central Laboratory (Thailand) Co. Ltd.

10. Microbial and chemical analysis methods

10.1 Dissolved cell

For immobilized cell, alginate gel beads containing cells was depolymerized in steriled 1% (w/v) sodium citrate solution with shaking 20 min at room temperature. The cells were serially diluted with Ringer's solution and counted as the same manner done with free cells.

10.2 Bacterial counting method

The number of viable cells was determined by double layer pour plate method using MRS media, incubation at 37°C for 48 hours.

10.3 Ethanol

Ethanol analysis was established using High Performance Liquid Chromatography (HPLC) with Refractive Index (RI) detector (RI detector 2400, KNAUER Advanced scientific instruments Co., Ltd). Standard ethanol concentration of 0.2, 0.4, 0.6, 0.8, and 1.0% v/v was prepared. Samples were diluted with deionized water at ratio 1:50 and filtered through filter paper No.1 (Whatman®, 125 mm diameter) before analysis. Ten µl of the sample was injected through a Column Rezex-organic Acid H+ (8%) (250x4.60 mm) using deionized H_2SO_4 as a mobile phase at 0.6 ml/min flow rate. The temperature of column set at 60°C. Quantity of ethanol in samples was calculated.

10.4 GABA

GABA was analyzed by Central Laboratory (Thailand) Co., Ltd.

10.5 Acidity

Lactic, citric and acetic concentration were measured using High Performance Liquid Chromatography (HPLC) with Refractive Index (RI) detector (RI detector 2400, KNAUER Advanced scientific instruments) that analyzed by Suranaree university.

10.6 Total acid

Total acid was analyzed by titration method according to with AOAC (2000).

RESULTS AND DISCUSSION

1. Effect of germination time on GABA content in malt and rice malt preparation for cereal based probiotic beverage

The appropriate germination time for high GABA rice malt production was determined in order to achieve more nutritious cereal malt based beverage. Nowadays GABA (γ-Aminobutyric acid) enriched food becomes a popular healthy food, since GABA has a major inhibitory neurotransmitter function, inhibits cancer cell proliferation and also reduces blood pressure (Panatda *et al.,* 2010). In this research, Riceberry rice grains were subjected to germinate up to 7 days under temperature and humidity control. The GABA concentrations of Riceberry rice malt were increased gradually along the first five days of germination time, 42.3, 75.94, 230.1, 251.45, 270.32 and then slightly decreased to 237.83, 223.01 mg/kg dry malt, on day sixth and seventh, respectively (Figure 1). GABA concentrations was increased between 1-5 days because the accumulation of GABA during germination by the action of glutamate decarboxylase (GAD), which converts glutamate to GABA. (Lea *et al.,* 1990). And after 5 days GABA concentrations was decreased because GABA could be used for plant growing. Riceberry rice malt was germinated for a longer time but starch and protein digestibility in endosperm were increased and easily digested by hydrolysis enzyme, produced in malting process. These hydrolysis enzyme were necessary for sweet malt extract preparation.

Then, Riceberry rice malt was milled and mashed with water ratio 1:3 to activate hydrolysis enzyme activities. Sweet wort contains sugars and amino acids which are benefit for consumer (Preet and Punia, 2000). Moreover, wort contained GABA 6.84 mg/100ml, total soluble solid was 18 degree Brix and pH 4.8 and then it was stored at 4°C for next experiment.

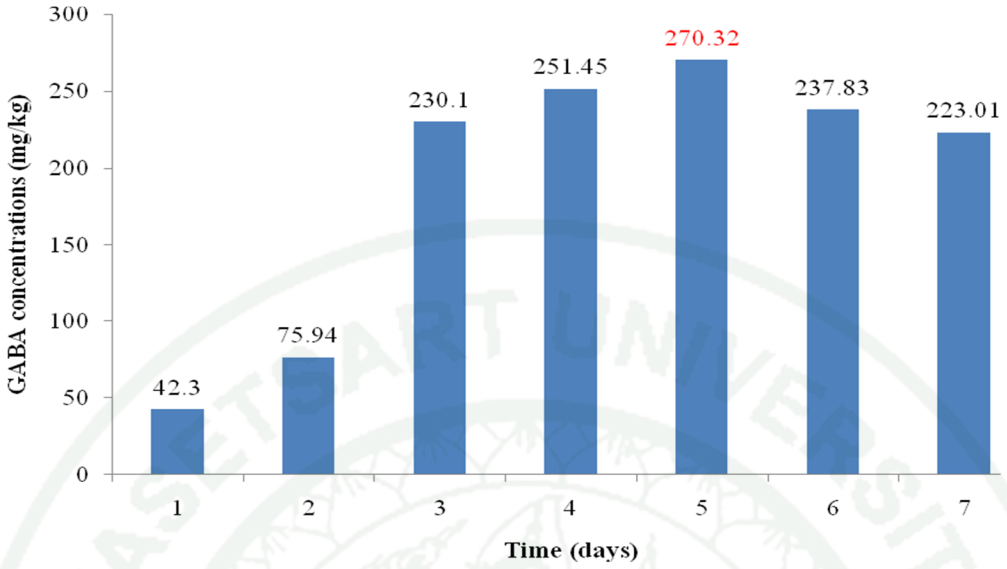


Figure 5 The GABA content found in cleaned dried Riceberry malt which germinated for one to seven days.

2. Selection of appropriate bacteria for malt-base probiotic beverage production

2.1 Growths of five strains lactic acid bacteria in Riceberry rice malt extract.

The growths of lactic acid bacteria (*L. plantarum* TC24, *L. acidphillus* TISTR 450, *L. reuteri* KUB-AC5, *L. Johnsonii* KUNN19-2, *E. fecalis* N1-33) in Riceberry rice malt extract were shown on Figure 6. *L. acidphillus* TISTR450, *L. reuteri* KUB-AC5, *L. Johnsonii* KUNN19-2, *E. fecalis* N1-33 have been reported their potential as probiotics for human (Melaku *et al.,* 2006, Supichar, 2007, Panward *et al.,* 2012 and Rodklongtan, *et al.,* 2014). All probiotic strains were capable of growing well on rice malt extract and the cell concentration reached 10^9 cfu/ml after 16 hours of fermentation which above the minimum dose recommended for health benefit promotion (10^6-10^7 cfu/ml based on a 100 ml daily dose) (Sanders and Huis 1999). Similar with other research, the *L. plantarum* and *L. acidphillus* strains could grow in cereal media (barley malt) and increased cell concentration to 10^8 cfu/ml after 24 hours fermentation (Sorbhi *et al*., 2012).

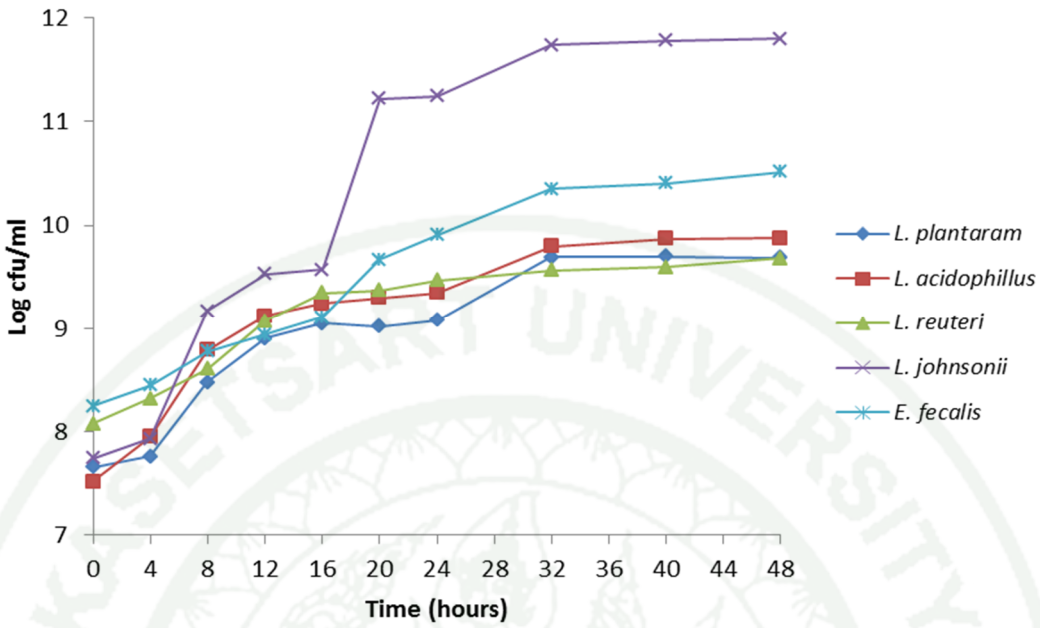


Figure 6 The growth curves of bacteria culture in Riceberry rice malt extract at temperature 37°C for 48 hours

The growth of bacteria implied that Riceberry malt extract is enriched media for microbial culture as well as other malt extract attributed to the simultaneous presence of mono saccharides (glucose and fructose) and disaccharides (maltose and sucrose) in the malt medium (approximately 3 and 12 g/l, respectively) (Charalampopoulos *et al.,* 2002). *L. Johnsonii* KUNN19-2 was showed that great growth in Riceberry malt extract. This was apparently due to the utilization of sugars and oligosaccharides contained in Riceberry malt extract served as the main carbon source during fermentation which is then used as energy source for the growth of *L. Johnsonii* KUNN19-2.

The changes of pH, acidity and % Brix during fermentation were shown in Table 4. Five bacteria cultures were vigorously fermented the Riceberry malt extract. Probiotic bacteria consumed sugars and raised acidity from 0.2-0.3 g/100 ml to 0.8-0.9 g/100 ml and as well as titratable acidity. These results are in agreement with other report, that the titratable acidity of fermented rice milk with *L. case*i and *L. acidophilus* was produced up to 0.86 g/100 ml acidity as lactic and pH 3.48 after 16

hours **fermentation** (Wongkhalaung and Boonyartanakronkit, 2012). Organic acid was produced from sugar fermentation by lactic acid bacteria; particularly, lactic acid was obtained by homofermentative or heterofermentative. Homofermentative used sugar through Embden Meyerhof Parnas pathway to produce lactic acid about 95% conversion and heterofermentative produce equimolar amounts of lactate, CO_2 and ethanol from glucose using the phosphoketolase pathway (Axelsson, 2004).

Table 4 The changes of total soluble solid, pH and acidity of fermented Riceberry malt extract by five lactic acid bacteria

	pH		Acidity g/100ml		°Brix	
	0 hr	48 hr	0 hr	48 hr	0 hr	48 hr
L. plantarum TC24	4.88	2.45	0.29	0.98	18	15.9
L. acidphillus TISTR450	4.8	2.4	0.26	0.88	18	16
L. reuteri KUB- AC5	4.7	2.8	0.29	0.78	18	17
L. Johnsonii KUNN19-2	4.86	2.26	0.22	0.68	18	15.6
E. fecalis N1-33	4.85	2.7	0.24	0.82	18	16.7

The pH 4.8 were decreased markedly at the first 8 hours of fermentation period and further decreased afterward. At 48 hours *L. plantarum* TC24, *L. acidphillus* TISTR450*, L. reuteri* KUB-AC5*, L. Johnsonii* KUNN19-2 and *E. fecalis* N1-33 were pH 2.45, 2.4, 2.8, 2.26 and 2.7, respectively. *L. plantarum* produced more acid during fermentation than the other bacteria. The different fermentation rates could be attributed to strain specificities (Amal *et al*., 2012). The increase in acidity, decrease in pH when fermentation proceed, that might discourage the growth of most spoilage and pathogenic microorganisms that cannot withstand such condition, consequently increase of shelf life of probiotic beverage. However, it was reported that acid production ability by lactic acid bacteria, especially post incubation, also effected the cell viability of probiotic bacteria such as *L. acidophilus* (Kyung *et al.,* 2004). Therefore, the appropriate fermentation time must be considered and controlled for optimal acidity.

2.2 Acid and bile tolerance test

The acids such as the hydrochloric acids (HCl) found also in the human stomach disrupt the biomolecules of cells, such as fatty acids, proteins and DNA. Thus, low pH environments can inhibit the metabolism and reduce the growth and viability of lactic acid bacteria (Chan *et al*., 2011). However, probiotic bacteria was expected to tolerate to acid and bile salt and serve health benefit to the host, thus the ability to survive under such condition was determined in this research.

Table 5 The acid and bile tolerance test of probiotic bacteria

	Acid tolerance			Bile tolerance		
	cfu/ml		%	cfu/ml		%
	hour		survival	hour		survival
	0	3		0	3	
L. plantarum	$95.5x10^9$	$57 x10^9$	59.68	$58 x10^9$	$6.8 x10^9$	11.72
L. acidphillus	$8.4 x10^9$	$6.5 x10^9$	77.38	$8 x10^9$	$6.1 x10^8$	7.62
L. reuteri	$18.2 x10^9$	$11.5 x10^9$	63.1	$10 x10^9$	$1.35 x10^9$	13.5
L. Johnsonii	$67.5 x10^9$	$12.25 x10^9$	18.14	$13 x10^9$	$4 x10^8$	3.07
E. fecalis	$6.1 x10^9$	$2.4 x10^9$	39.34	$3.3 x10^9$	$2.04 x10^8$	6.18

In this study, The initial cell concentration were in a range of 10^9-10^{10} cfu/ml, after 3 hours exposure of acidic condition (pH 2.5), the viability of five probiotic bacteria was decreased, whist cell concentration still was nearly to 10^9-10^{10} cfu/ml. The result indicated that, all of these bacteria were tolerated to acid. Other studies also confirmed that, six *Lactobacillus* strain (*L. fermentum, L. crispatus, L. brevis* 211*, L. brevis* 218*, L. brevis* 23*, L. acidophilus*) can tolerate to acid, no number of probiotic decreased after 3 hour exposure to pH 2 and 3 (Jin *et al.,* 1998). And concur with other research found that *L. plantarum* was the most endured cell compared to other 8 strains of bacteria culture at pH 3 (Ding *et al.* 2007).

The resistance to bile is an important characteristic that enables Lactobacillus to survive in the intestinal tract (Gilliland *et al.,* 1984). The result of bile tolerance was shown in Table 5. All strain of probiotic bacteria was shown a loss of viability when exposure to 1.5% oxgall. After 3 hours viable cell of *L. plantarum* TC24 and *L. reuteri* KUB-AC5 was slightly decreased, that cause final cell concentration of 10^8-10^9 cfu/ml. And viable cell of *L. acidophilus* TISTR450, *E. fecalis* N1-33, *L. Johnsonii* KUNN19-2 were decreased to 10^8 cfu/ ml.

The results suggested that *L. plantarum* TC24, *L. acidphillus TISTR*450, *L. reuteri* KUB-AC5 were the most tolerant cell which *L. plantarum* TC24, *L. acidphillus* TISTR450, *L. reuteri* KUB-AC5 have survival cell in acid condition 59.68%, 77.38% and 63.10% respectively, and declined in bile condition to 11.72%, 7.62% and 13.5% respectively.

2.3 Antimicrobial activity

The zones of inhibition (diameter in millimeters) against the tested bacteria (*Staphylococcus aureus* TISTR118*, Bacillus subtilis* TISTR024*, Samonella enteritidis* DMST17368 and *E. coli*) were shown in Figure 7. In present study, all probiotic bacteria could inhibit pathogenic bacteria and the broad activity found in supernatant derived from *L. plantarum* was the most obviously (Table 6). The previous report mentioned that *Lactobacillus* sp. strain GG inhibited pathogenic bacteria (*Enterobacteriaceae, Staphylococcus* spp., *Pseudomonas* spp., and *Streptococcus*) by producing antimicrobial substances (Silva *et al.,* 1987). In addition *L. acidophilus*, *L. plantarum* and *L. casei* produced a greater inhibitory effect towards causing pathogens *E. coli, Staphylococcus, Streptococcus, Klebsiella* and *Pseudomonas* (Selvamohan and Sujitha, 2010).

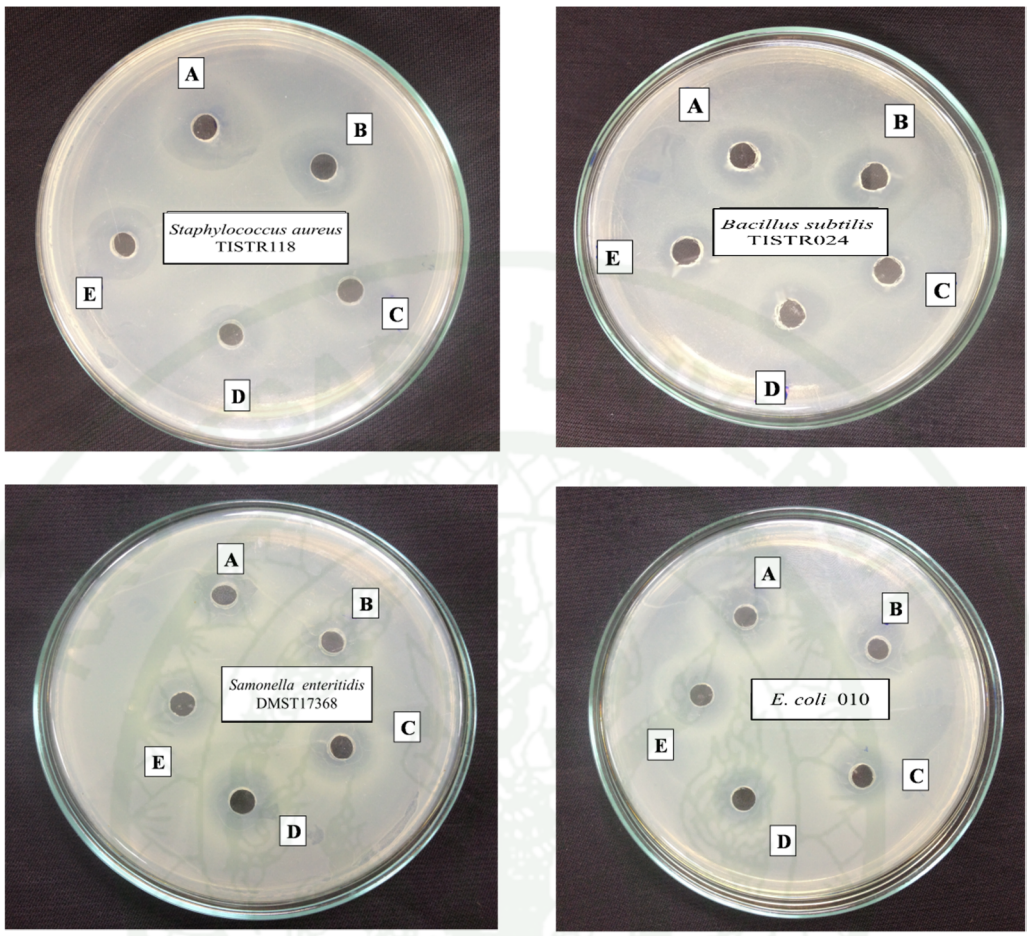


Figure 7 Inhibition zone of supernatant of probiotic bacteria against *Staphylococcus aureus* TISTR118, *Bacillus subtilis* TISTR 024, *Samonella enteritidis* DMST17368 and *E. coli* 010 by agar well diffusion method. A: *L. plantarum* TC24, B: *L.acidophilus* TISTR450, C: *L.reuetri* KUB-AC5, D: *E. fecails* N1-33, E: *L. johnsonii* KUNN19-2

Table 6 Antibacterial activity of supernatant derived from probiotic bacteria

	Inhibition zone (mm)			
	Staphylococcus aureus TISTR 118	*E. coli* 010	*Bacillus subtilis* TISTR 024	*Samonella enteritidis* DMST17368
L. Reuteri KUB-AC5	15	14	12	15
L. Johnsonii KUNN19-2	16	15	11	15
L. acidophilus TISTR 450	17	15	11	14
E. fecalis N1-33	14	14	12	14
L.plantarum TC24	20	15	14	16

The pH of supernatant was adjusted to 2-12 before assay, antibacterial activity of all probiotic bacteria against all pathogenic bacteria was found at pH 2, 3 and 4 while at pH 5, *Bacillus subtilis* TISTR024 was inhibited by supernatant derived from *L. plantarum* TC24 and *E. fecalis* N1-33, whereas *Streptococcus aureus* TISTR118 was only inhibited by *E. fecalis* N1-33. At pH 6-12 were not found antibacterial activity of all probiotic bacteria (Figure 8, 9, 10 and 11). These results indicated that inhibition was found in acidic condition which might be effect of organic acid in supernatant, particularly lactic acid. It is weak acid meaning it partially dissociate in water, once 50% of acid is dissociated in those pH called pKa and pKa of lactic acid was approximately 3.86. The environmental pH below the pKa, the higher of undissociated form and this form was dissolved well in fat molecule associated in bacterial membrane, then causing weak acid anions to accumulate in the cytoplasm, there by effecting metabolic process and destruction. However, not only the effect of organic acid but also other compound produced by bacteria had inhibitory ability such as bacteriocin, a proteinaceous compound exhibited antimicrobial activity. There was report of bacteriocin activity activated at pH 2-12 and at pH more than 7, this molecule was inactive form and degraded (Osmanagaoglu *et al*., 2001). Maldonado-Barragán and colleague (2013) indicated that *L. plantarum* produced bactericin by co-culture induction with a range of specific bacteria.

Therefore, the further intensive study of antibacterial activity by *L. plantarum* TC24 is needed for more precise characterization.

The bacteriocin were characterized with respect to thermal and pH stability, susceptibility to denature by enzymes. Ogunbanwo and colleague (2003) studied the characterization of bacteriocin produced by *L. plantarum.* Determined heat Resistance, pH sensitive, enzyme treatments. The results found that *L. plantarum* TC24 isolated produced bacteriocins that had broad spectrum of inhibition against both pathogenic, food spoilage organisms and various lactic acid bacteria. Bacteriocin produced by *L. plantarum* was heat stable at 121°C for 10 min, pH 2 to 6 and active principle was proteinaceous in nature since the bacteriocins was inactivated by proteolytic enzymes, but not by other non proteolytic enzymes.

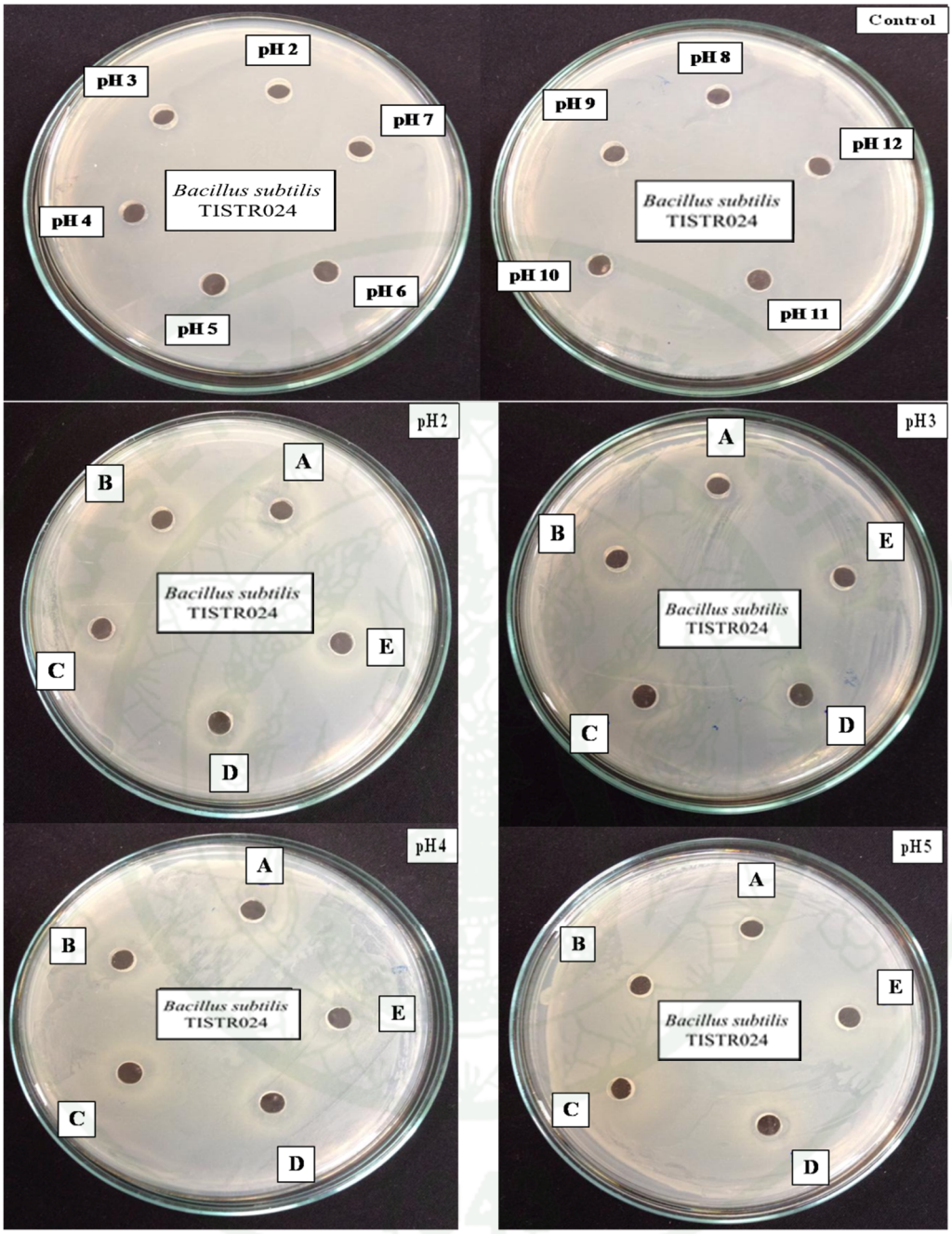


Figure 8 Inhibition zone of supernatant of probiotic bacteria at pH 2-12 against *Bacillus subtilis* TISTR 024 by agar well diffusion method. A: *L. plantarum* TC24, B: *L.acidophilus TISTR*450, C: *L.reuetri* KUB-AC5, D: *E. fecails* N1-33, E: *L. johnsonii* KUNN19-2

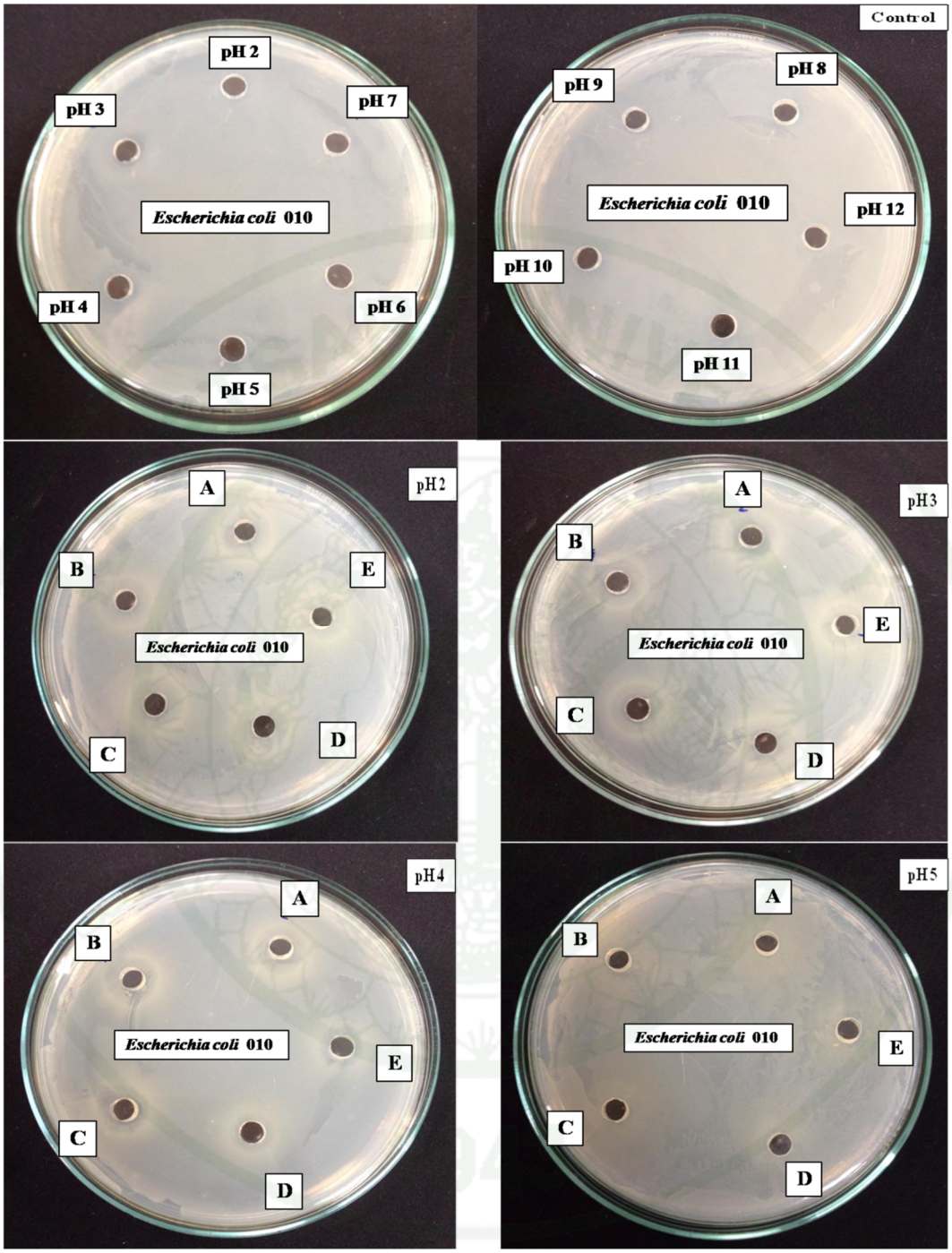


Figure 9 Inhibition zone of supernatant of probiotic bacteria at pH 2-12 against *E. coli* 010 by agar well diffusion method. A: *L. plantarum* TC24, B: *L.acidophilus TISTR*450, C: *L.reuetri* KUB-AC5, D: *E. fecails* N1-33, E: *L. johnsonii* KUNN19-2

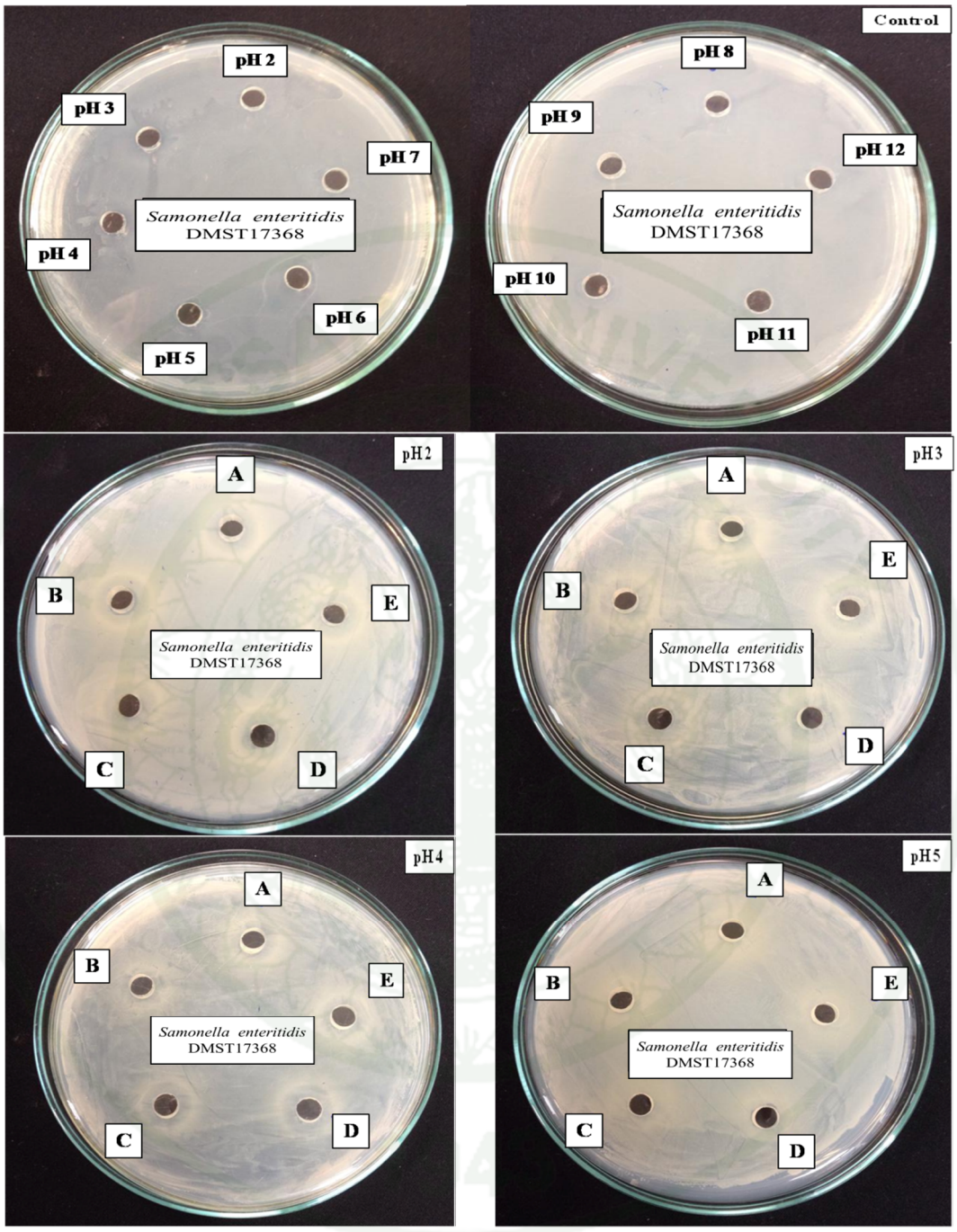


Figure 10 Inhibition zone of supernatant of probiotic bacteria at pH 2-12 against *Samonella enteritidis* DMST17368 by agar well diffusion method. A: *L. plantarum* TC24, B: *L.acidophilus TISTR*450, C: *L.reuetri* KUB-AC5, D: *E. fecails* N1-33, E: *L. johnsonii* KUNN19-2

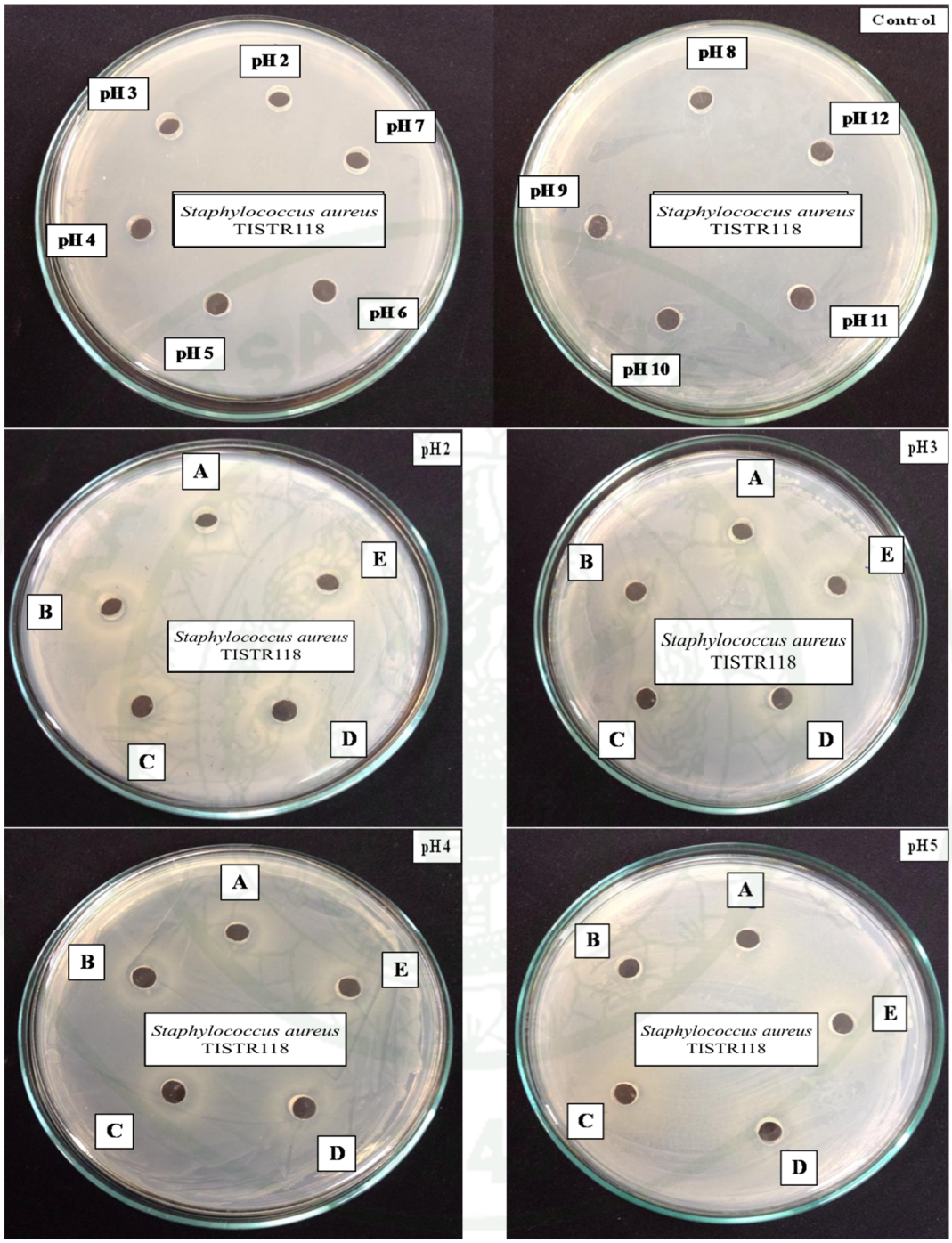


Figure 11 Inhibition zone of supernatant of probiotic bacteria at pH 2-12 against *Staphylococcus aureus* TISTR118 by agar well diffusion method. A: *L. plantarum* TC24, B: *L.acidophilus TISTR*450, C: *L.reuetri* KUB-AC5, D: *E. fecails* N1-33, E: *L. johnsonii* KUNN19-2

2.3 Sensory test of five probiotic beverages

The sensory test were performed on five probiotic fermentation beverages due to the consumer perception is also one important factor for buying decision as well as product benefit. Panelists were asked to taste each sample which fermented for 16 hours and to rate its degree of overall, aroma and taste (Figure 12). The result showed that *L. plantarum* TC24 was scored 4.1-4.4 (where 4 = "like slightly" and where 5 = "like very much") for overall, aroma and taste which more preferable than other bacteria. Thus, *L. plantarum* TC24 was the most preferred for consumption; additionally, other results of acid and bile tolerance test, antagonistic activity to pathogenic bacteria suggested that *L. plantarum* TC24 was likely to be probiotic bacteria and high potential probiotic bacteria which was selected for beverage production and study forward.

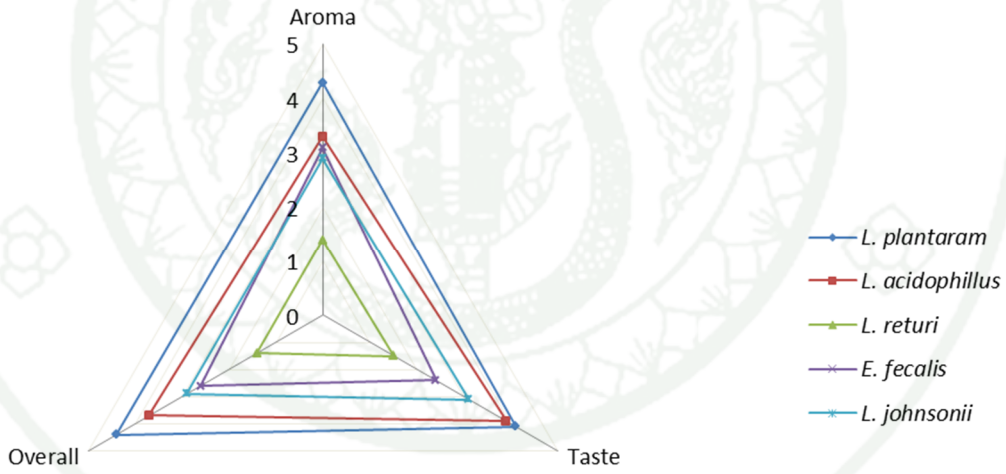


Figure 12 Sensory test of probiotic products on 5 point hedonic scale

3. Prebiotic activity score

One important prerequisite for carbohydrate to have prebiotic activity is that it should be metabolized by a tested probiotic strain as well, or nearly as well as glucose is metabolized (Huetoner *et al*., 2012). In this experiment used inulin 98% and FOS 95% purity and the rest of 2% and 5% were sugars. In order to find out, weather these bacteria could utilize inulin and FOS by monitory growth of bacteria, the effect of sugar found in both of prebiotics, must be taken into account. Thus, growth of probiotic bacteria were tested in modified MRS + 1% inulin (containing 0.98 g/100ml) compared to modified MRS + glu 0.02 g/100ml, and between modified MRS + 1% FOS (containing 0.95 g/100ml) and modified MRS + glu 0.05 g/100ml. In addition, the growth of pathogenic bacteria under the same conditions were tested in M9 medium 24 and compared to probiotic bacteria by using the equation of prebiotic activity score.

The result showed that bacteria could utilize inulin and FOS, thus higher cell concentration were found in medium containing inulin and FOS. The probiotic activity score showed in figure 13 were derived from the cell density reported in Table 7 and 8. Moreover, the prebiotic must support growth of benefit bacteria rather than pathogenic microorganism found in gastrointestinal tract. The results in Table 8 indicated that these prebiotic supported growth of pathogenic bacteria as well. Finally, the prebiotic activity score was calculated and found that inulin was the proper prebiotic for *L. plantarum* TC24 at 0.2 prebiotic activity score. Similar with other research, prebiotic activity score of *L. plantarum* L12 grown on soluble dietary fiber fraction. The result found that *L. plantarum* L12 had prebiotic activity score at 0.25 (marotti *et al.,* 2012).

Table 7 Probiotic (*Lactobacillus plantarum* TC24) growth in different mediums.

Time (Hour)	Cell concentration (cfu/ml)			
	Inulin 0.98 g/100ml	Glu 0.02 g/100ml	FOS 0.95 g/100ml	Glu 0.05 g/100ml
0	$1x10^7$	$1.5x10^7$	$2 x10^7$	$3.2 x10^7$
24	$3 x10^9$	$3x10^8$	$1.2 x10^9$	$4.1 x10^8$

Table 8 Pathogen (mixed culture of *E.coli* , *Staphylococcus aureus* and *Salmonella enteritidis*) growth in different mediums.

Time (Hour)	Cell concentration (cfu/ml)			
	Inulin 0.98 g/100ml	Glu 0.02 g/100ml	FOS 0.95 g/100ml	Glu 0.05 g/100ml
0	$3.1x10^6$	$2.4x10^6$	$2.1 x10^6$	$5 x10^6$
24	$1.84x10^9$	$2.4 x10^8$	$2x10^9$	$3.3 x10^8$

The prebiotic activity score indicated that metabolic capacity apparently exits (Marotti *et al.* 2012). The effectiveness of a prebiotic depends on its ability to be selectively fermented by and to support growth of specific targeted organisms (Huebner *et al.,* 2006). Inulin showed higher prebiotic activity score than that found in FOS. Utilization of prebiotic by probiotic bacteria requires the presence of specific hydrolysis and transport systems for the specific prebiotics. Both fructose and fructo oligosaccharides can be produced from inulin by using inulinase. Inulinases can be divided into exo- and endo- acting enzymes according to their modes of action on inulin. Endoinulinases (2,1-β-D-fructan fructanohydrolase) are specific for inulin and hydrolyse the internal b-2,1-fructofuranosidic linkages to yield inulo oligosaccharides as the main products, e.g. inulotriose, inulotetraose, and inulopentaose. Exoinulinases (β-D-fructan fructohydrolase) successively split off terminal fructose units from the non-reducing end of inulin (Han *et al.,* 2009).

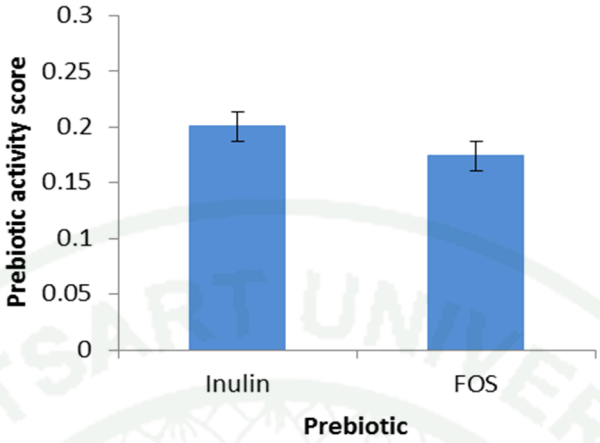


Figure 13 Prebiotic activity score

Moreover, effect of prebiotic on growth of *L. plantarum* TC24 in Riceberry malt extract were not found in cell cultured for 48 hours due to the plenty of available simple carbon molecules such as monosaccharide, di and trisaccharide in wort must be earlier consumed. Our research revealed that Riceberry malt extract contain sufficient nutrient for probiotic bacteria growing (Figure 14).

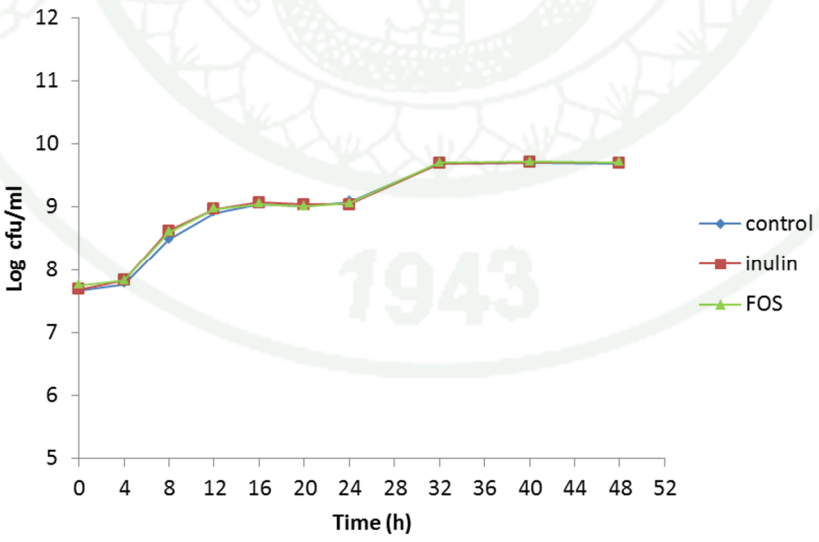


Figure 14 Effect of prebiotics on growth of *L. plantarum* TC24

4. Improvident of cell survival by encapsulation

4.1 Growth of *L. plantarum* TC24 in fermenting beverage

Figure 15 showed growth of both free and encapsulated *L. plantarum* TC24 in rice berry malt extract during fermentation. The initial viable cell number of free cell and encapsulated cell were 4.57×10^7 cfu/ml and 3.29×10^7 cfu/ml-gel respectively, then reached the final concentration 4.91×10^9 cfu/ml and 5.72×10^9 cfu/ml-gel, respectively. The viable cell number of encapsulation cell was found to be slightly higher than that of free cells. After 4 hours, some bacteria leaked from beads and grew in the Riceberry malt extract which initially contained no bacteria and reach 10^3 cfu/ml after 48 hours. This might be because the encapsulation protected cell from oxygen and high concentrations of substrates and fermentation products (Talwalkar *et al.,* 2003); therefore, better growth were achieved by encapsulated cell fermentation.

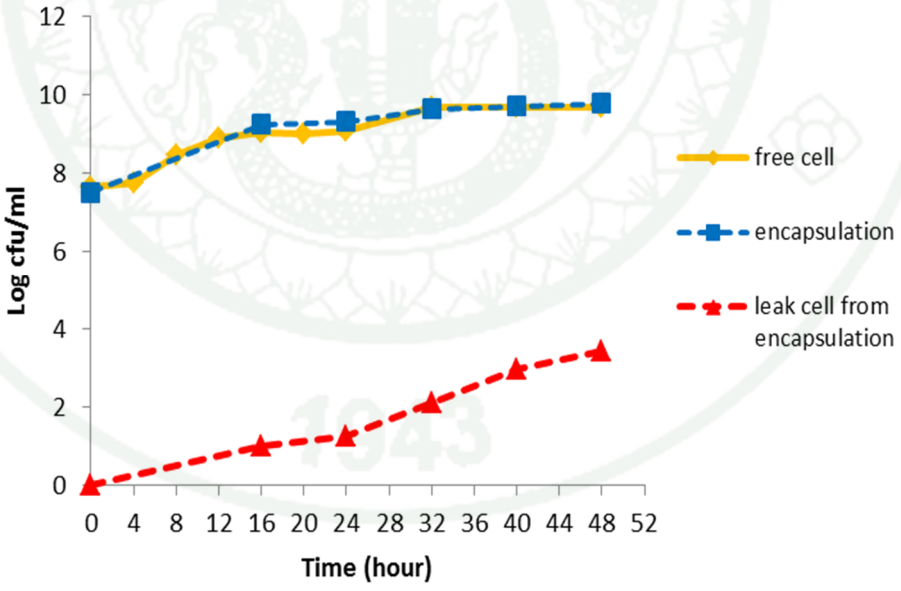


Figure 15 Growths of free and encapsulated cell of *L. plantarum* TC24

4.2 Survival of *L. plantarum* TC24 in GI tract condition

Probiotics are live microbial food which beneficially affect the host by improving intestinal microbial balance (Fuller, 1989). The most important aspect of probiotics research is the survival of probiotic bacteria in the gastrointestinal tract. The gastric tract contains gastric juice of low pH due to the high hydrochloric acid concentration of the secreted gastric acid (Holzapfel *et al.,* 1998), while the intestinal tract contains bile salts and pancreatin and has a pH of around 8.0 (Le Vay, 1988). The presence of food and food ingredients has been reported to improve the viability of microorganisms during gastric transit (Heller, 2001).

The survival of free cell and encapsulate cell on the simulated gastric juice after exposure for 3 hours were 51.51% and 77.45% and the simulated bile juice were 46.52% and 65.35%, respectively. Indicated that encapsulation improved survival rate of bacteria and maintain living cell at 10^9 cfu/ml. These results was similarly with acid and bile tolerance test of five probiotic (Table 9).

Table 9 Survival of *L . plantarum* TC24 in the simulatedgastrointestinal tract condition

	Gastric cfu/ml		% survival	Bile juice cfu/ml		% survival
	hour			hour		
	0	3		0	3	
Free cell	$2.97x10^9$	$1.53x10^9$	51.51	$1.15x10^9$	$5.35x10^8$	46.52
Encapsulated cell	$3.06x10^9$	$2.37x10^9$	77.45	$2.54x10^9$	$1.66x10^9$	65.35
leak cell	15	86		23	77	

The encapsulation technique improved survival rate of lactic acid bacteria in harsh condition such as strong acid and bile salt condition. Similarly, other research studied survival of free and encapsulated probiotic bacteria in yoghurt added prebiotic (Hi-maize starch) and found that encapsulated probiotic bacteria survived in simulated

acid and bile juice better than free probiotic cells by preventing the diffusion of acid contents in to the capsule (Kailasapathy, 2005).

6. Effect of storage condition on shelf life of probiotic beverage

6.1 Effect of storage condition to bacterial cell concentration

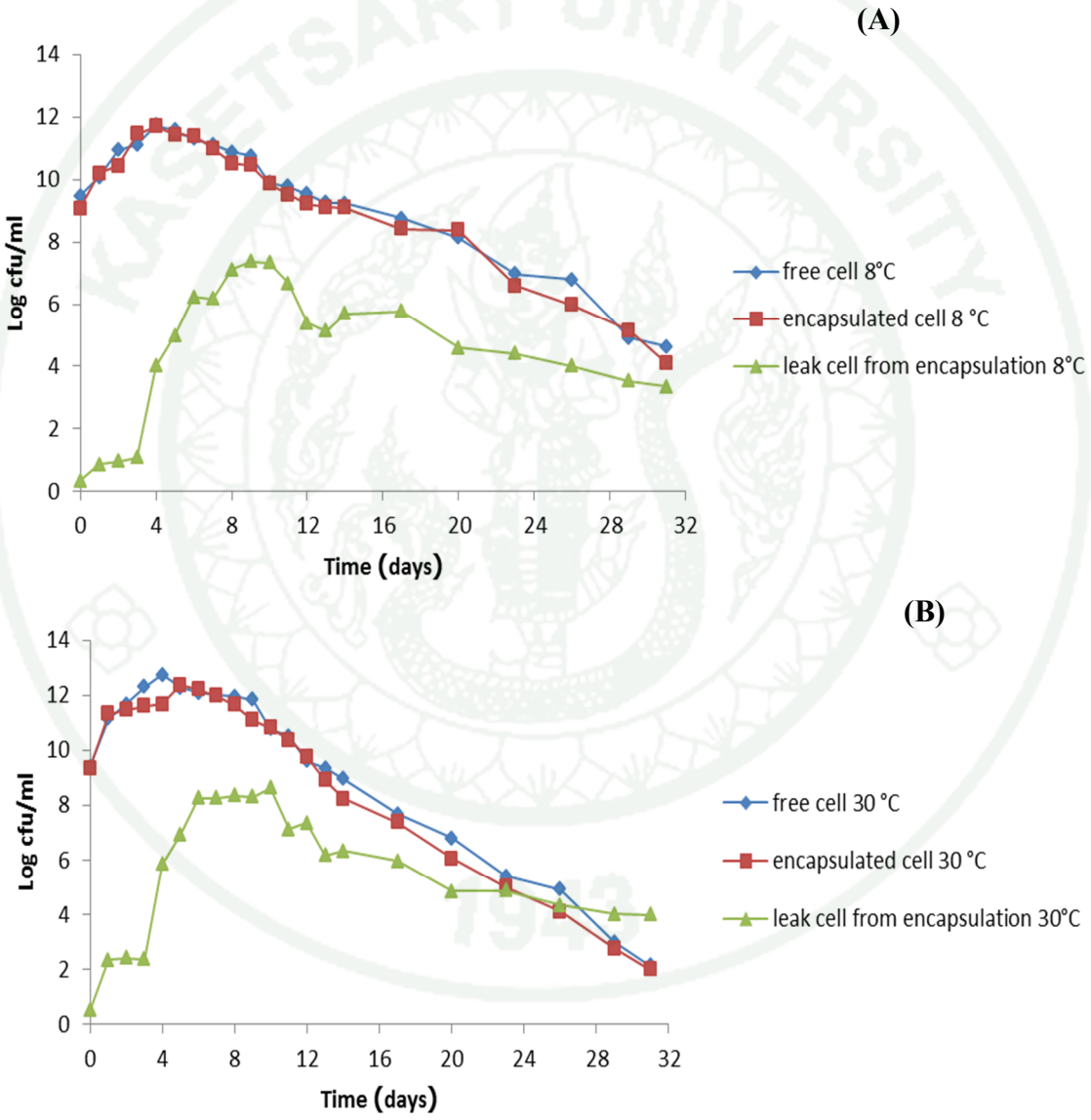


Figure 16 Growth of *L. plantarum* TC24 in beverage kept at (A) 8 °C and (B) 30 °C for 30 days

The cell viability of *L. plantarum* TC24 in fermented Riceberry rice extract during storage at 8ºC and 30 ºC were shown in Figure 16. In the first 4 days cell concentrations were increased from 9 log cfu/ml to 12 log cfu/ml, after 14 days growth was declined nearly to the initial cell concentration. The same trend was also observed by other research found that the viable cell counts of *L. acidophilus* in rice beverage was decreased about 1 log cycle during storage for 15 days, the viable cell counts of *L. acidophilus* was ranged from 9.78 log cfu/ml to 8.04 log cfu/ml (Amal *et al.,* 2012). After 4 week, the viable cell count of free cell were remained $4.34x10^4$ and $1.37x10^2$ cfu/ml by storage at 8ºC and 30 ºC, respectively, and the viable cell count of encapsulated cell remained $7.46x10^4$ and $3.64x10^2$ cfu/ml-gel of storage by 8 ºC and 30 ºC respectively. The results of this research suggested that storage temperatures influenced bacterial survival cell concentration. However, the beneficial cell concentration was recommended for 10^6 cfu/ml for daily consumption, thus storage at 8 ºC could be applied to maintain beneficial viable cell for at least 26 days while 30 ºC was 21 days. The reduction of bacteria during the cold storage could be influenced by many factors such as oxygen sensitivity and metabolites such as hydrogen peroxide, acid, ethanol and bacteriocins produced by lactic acid bacteria.

Figure 17 displayed the changes of acidity, pH and total soluble solid in probiotic beverage for free and encapsulated cell during storage at 8ºC and 30 ºC. Encapsulated cell was found to possess lower acidity value while pH was a bit higher than free cell and these were correlated to the reduction of cell concentration. Since, higher temperature stimulated bacterial metabolism, thus at 30 ºC the total soluble solid in Riceberry malt extract was lower than those found at 8 ºC and more acid was released to the beverage. Comparison between the free and the encapsulated cell, the encapsulated cell concentration was not significantly different but pH of encapsulated cell condition was higher and acidity was lower than those found in the free cell.

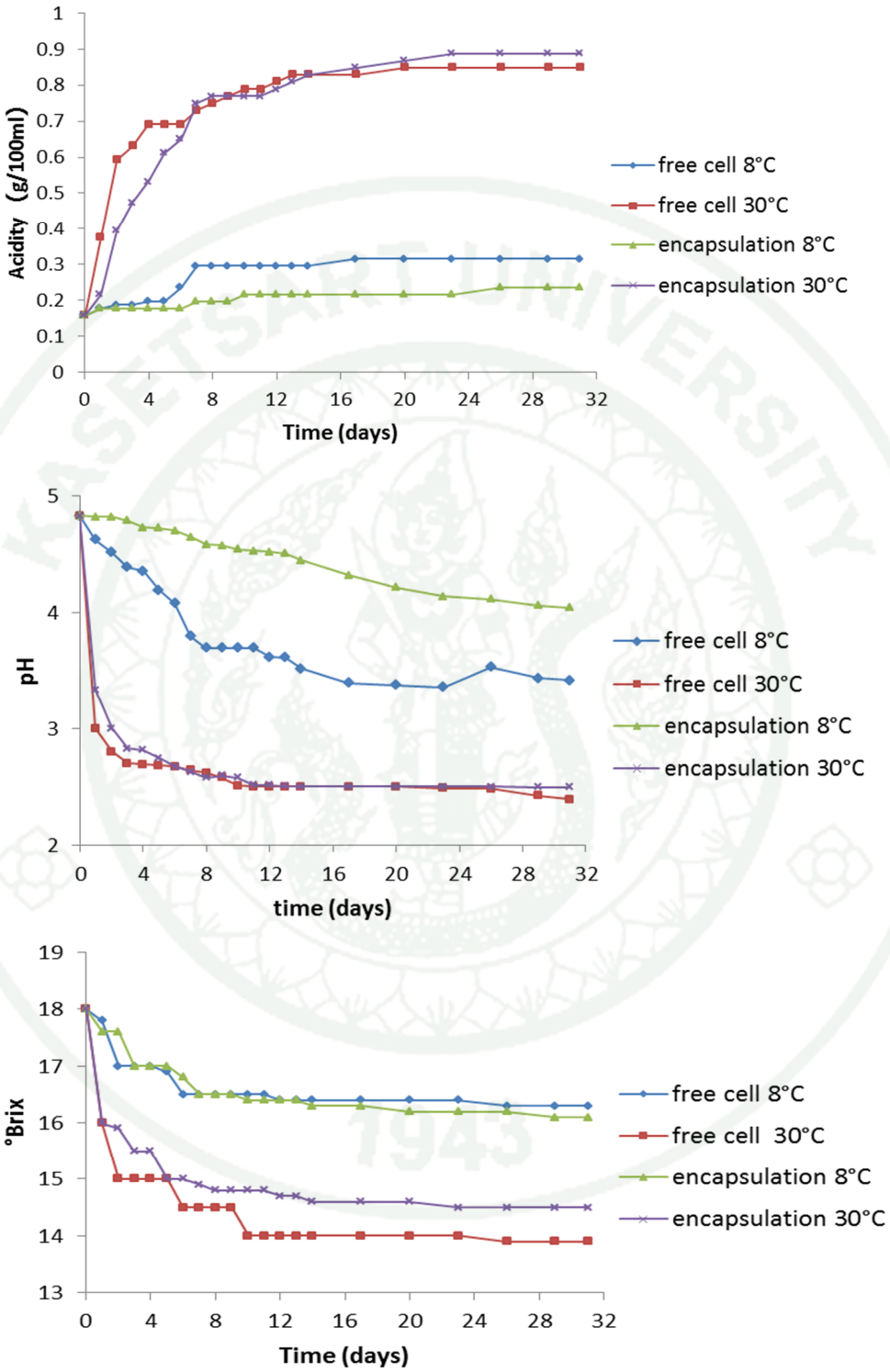


Figure 17 The change of acidity, pH and total soluble solid of free cell and encapsulation cell during storage at 8 °C and 30 °C

Scanning electron microscopic (SEM) showed the shape, the surface and cross sectional of calcium alginate bead before and after storage at 8 °C for 2 weeks (Figure 18). External appearance of the alginate bead before storage (A, B, C) and the degenerated alginate bead (D, E, F) were different. The initial alginate bead presented a more compact and closed matrix, the thin film of alginate layer covered the globular packed cell. The alginate was anionic polysaccharide and egg-box structure which this binding was a co-operative process that predominantly involves consecutive guluronate (Grant *et al.,* 1973). *L. plantarum* TC24 was a gram positive bacteria thus bacteria might be transferred into an egg-box of alginate structure and bacteria was grown in fixed area by binary fission, thus bacteria was group appeared. Whist, the degenerate alginate exhibited a more porous and relaxed encapsulating matrix due to bacteria was increased and alginate bead was degenerated by acid. As a result it exhibited a more porous and leaked of cell after storage for 3 days and the concentration of leaked cell was increased a long with storage time.

On degenerated bead found that bacteria was spread because the thin layer of alginate surface was decayed that unable to encapsulate cell. Encapsulation from alginate wasn't protect the cell in high acid environment which pH lower than pKa of β-D-mannuronic acid 3.7) that alginate was changed to alginic acid and then was not crossed link with Ca+, resulting alginate bead was degenerated. The similar research was observed in fermented rice beverage samples. Data indicated that storage time significantly affected the acidity level in the rice beverage; titratable acidity increased, whilst the pH was decreased during storage (Amal *et al.,* 2012).

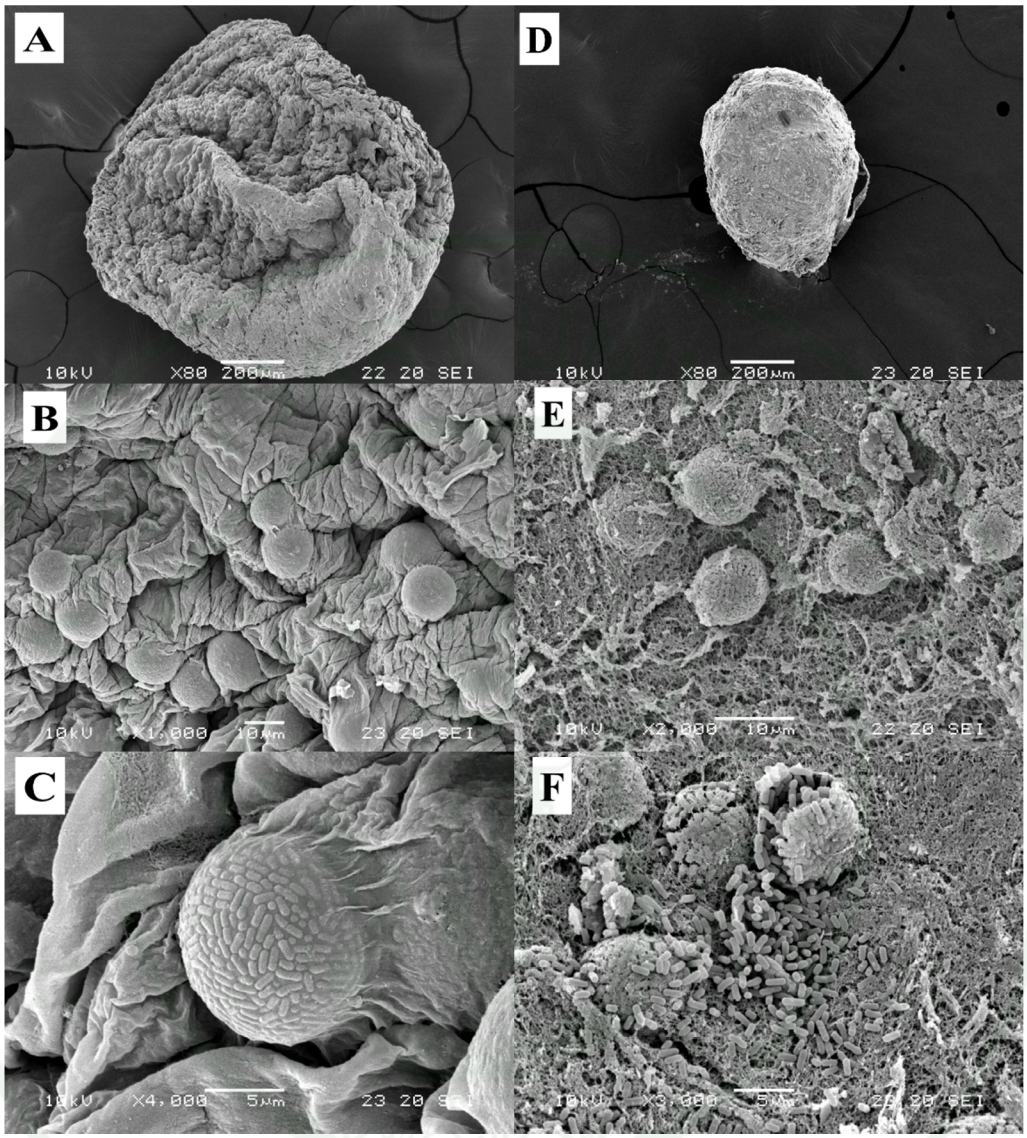


Figure 18 The bead at 16 hours fermentation; (A) Completed alginate bead, (B) Globular structure embedded on the surface of alginate bead (C) The group of bacterial cell pecked in globular structure under the smooth, thin film of alginate layer. After 2 week; (D) Whole structure of degenerated bead (E) The rough and porous globular structure embedded on the surface of degenerated alginate bead (F) The unpacked of bacterial cell and broken globular structure shown on the porous surface of alginate bead.

6.2 Sensory test

The mean score of each attribute of all products were compared and analysis of variance (ANOVA) was tested by SPSS version 14. The study of consumer preference test was conducted with laboratory members using 9 point hedonic scale found the mean score of aroma in sample of 0-7 days of the free cell and the encapsulation cell kept at 8 and 30 °C were not significantly different in addition, the sample kept for 14-28 days were not significantly different (Table 10).

Table 10 Sensory test of probiotic beverages

	days	Storage temperature			
		Free cell		Encapsulation cell	
		8°C	30°C	8°C	30°C
Aroma	0	6.5^{a}	6.3^{a}	6.3^{a}	6.2^{a}
	7	6.3^{a}	6.4^{a}	6.2^{a}	6.1^{a}
	14	4.3^{b}	4^{b}	3.9^{b}	3.4^{b}
	21	4.2^{b}	3.7^{b}	3.6^{b}	3.4^{b}
	28	4^{b}	3.6^{b}	2.8^{b}	2.9^{b}
Taste	0	6.1^{a}	6^{a}	5.9^{a}	5.5^{a}
	7	6^{a}	5^{ab}	5.8^{a}	4^{bc}
	14	4.1^{bc}	3.4^{cd}	3.2^{cde}	2.6^{def}
	21	3.7^{cd}	3.4^{cd}	2.6^{def}	1.9^{ef}
	28	3.6^{cd}	3.2^{cde}	2.4^{def}	1.6^{f}
Overall	0	6.5^{a}	6.3^{ab}	5.3^{abcd}	5.2^{abcde}
	7	6.3^{ab}	5.1^{bcde}	5.8^{abc}	4.8^{cdef}
	14	4^{defg}	3.9^{efg}	3.6^{fgh}	2.7^{ghi}
	21	3.7^{fgh}	3^{ghi}	2.4^{hi}	2.1^{i}
	28	3.2^{ghi}	2.9^{ghi}	2.1^{i}	1.9^{i}

Different letters in the each attribute indicated a significant difference between the means($p<0.05$)

The study of consumer preference test was conducted by laboratory member using 9 point Hedonic scale and mean score of each attribute for products were compared and analysis of variance test by SPSS version 14. The freshly prepared products of free and encapsulated cell beverage were not significantly different in term of taste and aroma, where as a slightly different of overall quality was shown in Table 12. After storage for 2 weeks, the significant changes of aroma, taste and overall quality were detected by panelist, The effect of storage temperature on sensory test was found even though temperature influence number of living cell. Therefore, this result suggested that the product could be stored for 26 days at 8°C and 21 days at 30°C. But better consume it before 14 days.

6.3 Consumer test and shelf-life of flavored beverage

The one hundred questionnaires and sensory test of probiotic beverage was used to survey the consumer preferences in Kasetsart University, found that consumer recommended to modify the flavor of probiotic beverage as honey flavor. Thus, probiotic beverage was added with 6% honey and bacterial cell concentration, pH and total soluble solid were monitored at 8°C compared with original probiotic beverage for 1 month. The final bacterial cell concentration in honey flavored beverage was slightly less than original flavored probiotic. Although, there was a report about the antibacterial effect of honey, mostly against gram-positive bacteria (Bogdanov *et al.,* 2008). Both bacteriostatic and bactericidal effects have been reported for many strains, some of them are pathogenic (Al-waili *et al,* 2005). But both of probiotic beverages maintain live bacteria nearly 10^6 cfu/ml after stored for 3 weeks (Figure 19, 20) which was the minimum dose recommended for health benefit promotion in host gastrointestinal tract (Sanders and Huis, 1999).

The development of probiotic beverage added 6% honey was tasted again by 50 trained panelist which on age was in range 15-60 years and gross revenues over 10,000 Bath per month. The sensory test of probiotic beverage using 9 point Hedonic scale; where, 9 and 1 represent “extremely good” and “extremely poor”, respectively, and a score of 5 represents “Neither Like nor dislike” was used for product

evaluation. The result of sensory test found that overall property of the honey flavored probiotic beverage was rated in a range between 5.5-6 (between neither like nor dislike and like slightly). Approximately, 70% of consumer preferred probiotic beverage before they were informed the product information and increased to 94% after they were educated some of product information "Probiotic beverage made from Riceberry malt extract added probiotic was a plenty of vitamins, amino acids and minerals. Probiotic bacteria is beneficial microorganism balancing digestive system and prebiotic is added for enhancing probiotic function in the colon system.

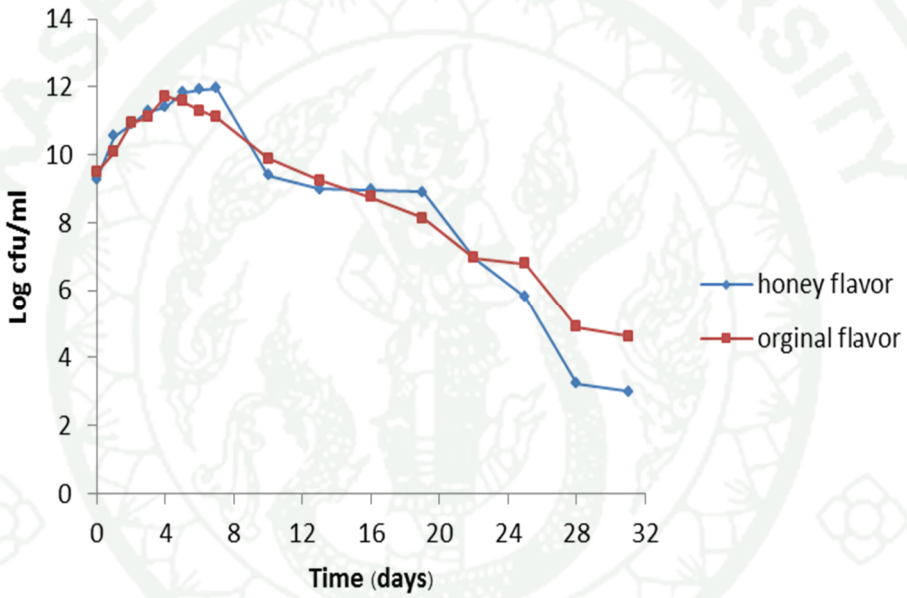


Figure 19 Growth of bacteria cultured in honey flavored beverage and original flavor beverage

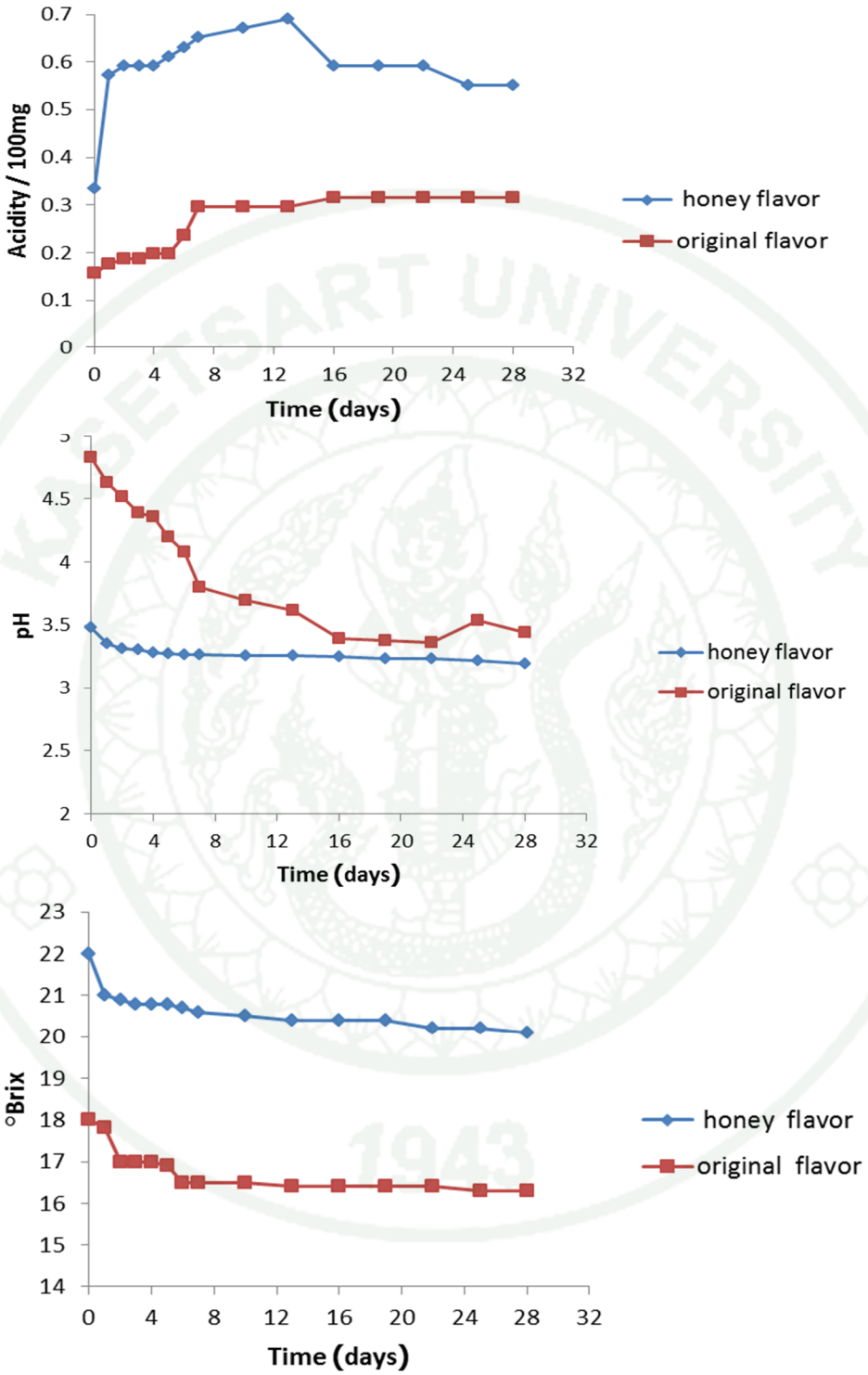


Figure 20 The changes of total soluble solid, pH and acidity of honey flavored beverage and original flavor beverage

8. Product safety and nutrition

The composition of Riceberry paddy rice and Riceberry rice malt was examined to ensured that the product was safe and served consumer needed. Riceberry rice contained protein 6 g/100g, carbohydrate was 68 g/100g and fat 2.63 g/100g at moisture content 17g/100g. Riceberry rice was acknowledged as high iron rice which was 476 mg/kg and this content was decreased after malting process to 248 mg/kg and the quantity of iron in probiotic beverage was only 0.51 mg/100 ml. This reduction might be some iron in rice was released from solid part during soaking rice with water in malting processes. But the most of iron must be embedded in rigid rice husk which was separated out of the extract then only a few of iron was dissolved and diluted in Riceberry malt extract. The fungi contamination was critical for malting and in this study was not found any of alflatoxins which analyzed by HPLC at LOD 0.39 ng/g. GABA in Riceberry rice malt was 270 mg/kg and GABA in probiotic beverage was 6.84 mg/100ml while vitamin A was 163 μg/100 ml and contains dietary fiber derived from inulin accounted for 2% of daily intake. A bottle of probiotic beverage give energy 33.3 kCal (Table 11).

One serving of probiotic beverage contain GABA 2.74 mg/40ml which less than GABA in Taiwan tea that have GABA 72 mg/40ml (Wang *et al.,* 2006). In addition, other research used acidulated malt for sourdough which was added lactic acid bacteria for digestion of protein, and generation GABA to 162 mg/kg from glutamate decarboxylase (Stomek *et al.,* 2011). Therefore it could be improved GABA content in beverage with selected probiotic bacteria that able to produce GABA. The free alpha amino nitrogen of probiotic beverage was compared with commercial product found that free alpha amino nitrogen of probiotic beverage was 46.5 mg/l which more than drinking yoghurt but less than essence of chicken (Figure 21).

Table 11 Composition of Riceberry rice, malt and probiotic beverage

Compound	Riceberry[1] Rice /100 g	Malt[2] Riceberry/100g	Probiotic beverage		
			100 ml	serving (40 ml)	%RDI
Moisture (g)	17.07	5.97	86.97	-	-
Ash(g)	5.61	7.02	0.25	-	-
Carbohydrate(g)	68.55	77.34	20.15	8.06	3
Fat(g)	2.63	3.18	0.04	0	0
Protein(g)	6.14	6.49	0.58	0	-
Iron(mg)	476.36	248.21	0.51	0.204	< 2
Calcium(mg)	-	-	4.68	1.87	0
GABA(mg)	-	27	6.84	2.74	-
Vitamin A (μg)	-	-	163.9	65.56	10
Vitamin B1(mg)	-	-	< 0.03		0
Fiber(g)	-	-	1.27	< 1	2
Total energy			83.28	33.3	-
Ethanol			ND	ND	-

[1] Fine ground paddy Riceberry (0.9 mm)

[2] Fine ground Riceberry malt (0.9 mm)

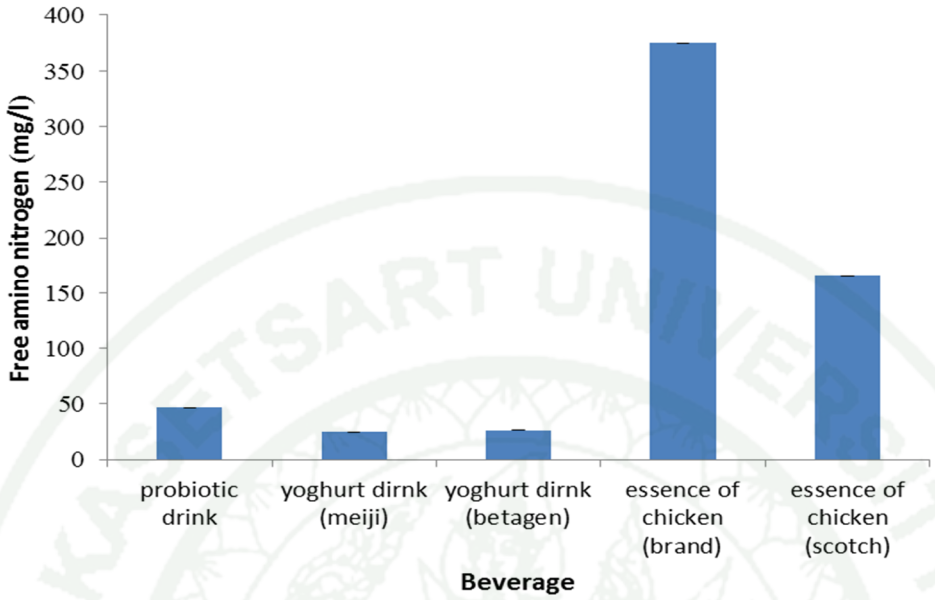
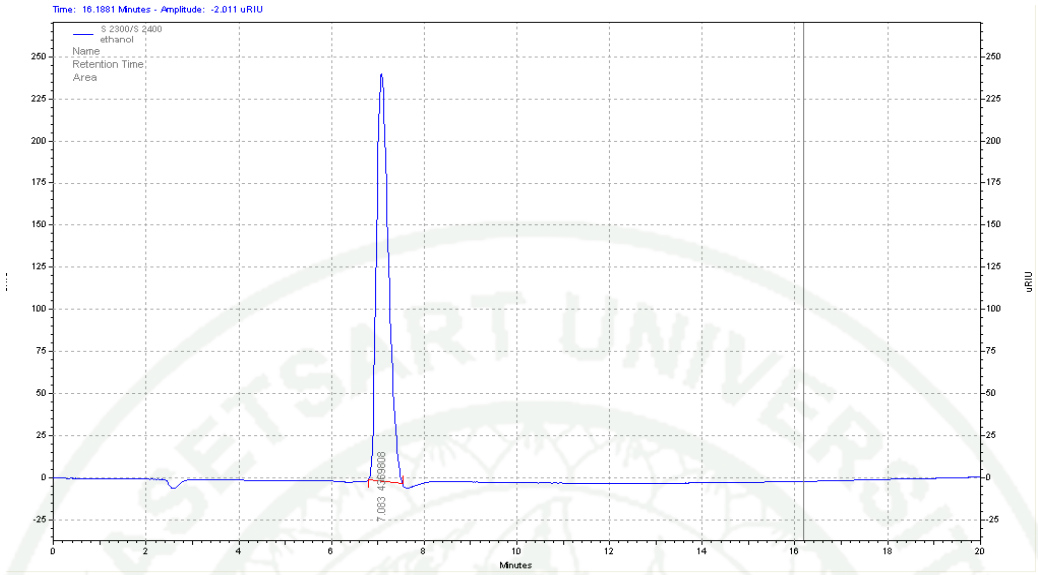


Figure 21 Content of free amino nitrogen (mg/l) in probiotic beverage and commercial products.

The fermentation pathways of lactic acid bacteria were the Embden Meyerhof (EM) pathway or phosphoketolase pathway. Which acetyl phosphate in phosphoketolase pathway was changed to ethanol. Thus, ethanol in probiotic beverage was analyzed for confirmation that *L. platrarum* TC24 and the product was safe for all consumer who aware of alcohol. The research revealed that probiotic beverage from *L. plantarum* TC24 was not found any of ethanol during the fermentation process (Figure 22).

(A)



(B)

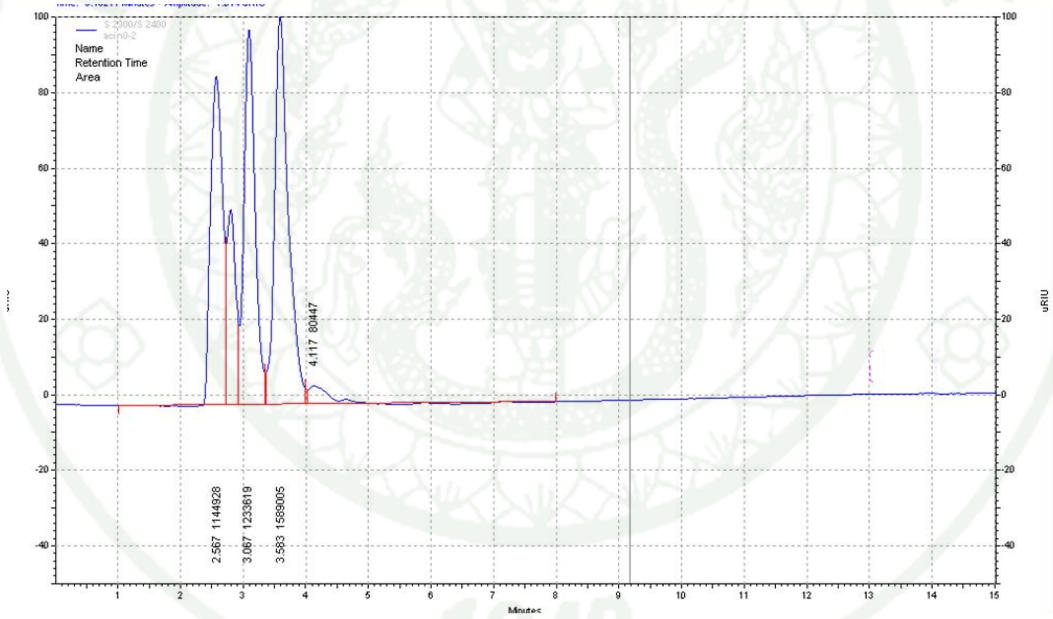


Figure 22 (A) The chromatogram of standard ethanol 20 g/l and (B) The chromatogram of probiotic beverage from *L. plantarum* TC24

Originally, lactic, acetic and citric acid in riceberry malt extract were 559, 216.4 and 12.7 mg/100ml, respectively. *Lactobacilli* utilized oligosaccharides and simple sugars and produced by-products, namely acids upon fermentation. The concentration of lactic and acetic acid were increased (Table 12) and the results also

showed that the production of lactic acid was higher than acetic acid in fermentation at 16 hours. *L. plantarum* TC24 was facultative heterofermentative that lactic acid producers and produce lactic acid as the main metabolite via the phosphoketolase pathway(Vinee, 2010). Phosphoketolase is a pathway that degrades hexose to lactic acid, CO_2 and ethanol (Xu *et al.,* 2008). *L. plantarum* TC24 was produced acetic acid and ethanol by phosphoketolase pathways. For a different route to take place, there must be either excess pyruvate in the medium or and addition electron acceptor either formed by a different pathway or internally added to the growth medium. For example, adding an exogenous electron acceptor changed the end-products profile for *L. plantarum* generating acetate at the expense of lactate. Example of pyruvate alternative electron acceptors include O_2, citrate, and fructose (Lahtinen *et al.,* 2012) Citric acid was decreased because citric acid is also used as an energy source by lactic bacteria (Davis *et al.,* 2004), or can be used as and electron acceptors in phosphoketolase pathways previously mentioned.

Table 12 Organic acid in Riceberry malt extract and probiotic beverage after 16 hours of free cell fermentation

	Acid mg/100ml		
	Lactic	Acetic	Citric
Riceberry malt extract	289.41	21.83	20.32
Probiotic beverage	559	216.4	12.7

CONCLUSION

Riceberry rice is one interesting new rice cultivar developed by Rice Science Center and Rice Gene Discovery, Kasetsart University, Kumpansan Campus. The nutritious components found in this rice was remained in rice malt extract, additionally, GABA molecule was synthesized through the malting processes by 5 days germination. The sweet wort containing free alpha amino nitrogen, sugar displayed as total soluble solid were important for bacterial growing and serving nutritious molecule to the consumer with the concept of novel non-dairy probiotics beverage for brain and balancing of digestive system. Probiotic bacteria was selected through ability to resist harsh environment of GI tract and antibacterial activity as major property needed living in host body. *L. plantarum* TC24 resisted to acid and bile salt or even enzymes in digestive system and it showed the great inhibitory effect against pathogens bacteria more than other probiotic bacteria. In addition, sensory test of probiotic beverage from *L .plantarum* TC24 was more preferable than other bacteria in overall, aroma and taste thus it was selected for beverage production. Prebiotic activity score of inulin was 0.2 that higher than FOS which indicated that bacteria utilized inulin better than FOS. Encapsulation was technique used to protect cell and improve the survival rate in host gastrointestinal system. Storage test of beverage found that encapsulation cell could be applied to rise viable cell rather than free cell; however, leaking of cell could be found after stored for 3 days and the degeneration of bead was obviously observed from bead stored for 14 days using SEM. Storage at 8ºC could be applied to maintain number of viable cell longer than storage at 30 ºC; however, the sensory score of stored product was significantly changed after kept for 14 days. The free cell beverage was flavored with 6% honey and could be stored at 8 ºC for 21 days at cell concentration nearly to 10^6 cfu/ml. The consumer test by fifty persons rated degree of preferences in between of neither like nor dislike and like slightly, moreover, product description influenced product acceptance. Therefore, product description must be clarified and straightly communicate to the consumer. This research suggested that probiotic drink from

Riceberry rice malt extract is a novel functional beverage for specific consumer who are vegetarian or lactose intolerant consumer and for all health-conscious consumer.

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APPENDICES

Appendix A

Methods

Appendix A1 Determination of the amount of lactic acid (AOAC, 2000)

1 Preparation of reagents

1.1 Free CO_2 water

Preparation of free CO_2 water by boiling of distilled water for 20 min.

1.2 Standard solution of 0.1 N NaOH

4 g of NaOH was dissolved in distilled water and adjusted to a final volume of 1 l. Determination of standard concentration of 0.1 N NaOH by weighing 0.3 g of acid potassium phthalate ($KHC_8H_4O_4$) into 250 ml flask, then 100 ml of free CO_2 water was added. Phenolphthalein (2 droplets) was dropped in acid potassium phthalate solution which then was titrated with 0.1 N NaOH. The standard concentration of 0.1 N NaOH could be calculated using equation below:

$$\text{Standard concentration (N)} = \frac{KHC_8H_4O_4 \text{ (g)} \times 1000}{\text{NaOH (ml)} \times 204.229}$$

1.3 Phenolphthalein solution

Dissolved 1 g phenolphthalein in 95 % alcohol and adjusted to a final volume of 100 ml.

2.2 Procedure

Pipetted 1 ml supernatant of samples into flask (250 ml) and then 40 ml of free CO_2 water was added. Dropped phenolphthalein (2 droplets) into diluted sample solution flask and then titrated with 0.1 N NaOH standard solutions until to the end point (sample solutions became light pink). The amount of acetic acid was calculated s below:

$$\text{The amount of lactic acid (g/100 ml)} = \frac{N \times V \times 90 \times 100}{1000 \times 1}$$

By N = Standard concentration of 0.1 N NaOH

V = Volume of 0.1 N NaOH standard solution

Appendix A2 Free amino nitrogen (FAN)

Free amino nitrogen will be determined by Ninhydrin method according to EBC 8.10 (1998). One ml of sample will be diluted with water to 100 ml. Then, 2 ml of the diluted sample will be taken into test tube and 1 ml of color reagent (100 g Disodium hydrogen phosphate (Na_2HPO_4), 60 g Potassium Dihydrogen Phosphate (KH_2PO_4), 5 g of Ninhydrin and 3 g of fructose in 1 L of distilled water) will be added. The test tube will be heated for exactly in boiling water for 16 min and then cooled in a water bath at 20°C for 20 min. Five ml of diluting solution (2 g Potassium iodate (KIO_3) in 600 ml distilled water and 400 ml of 96% ethanol) will be added and measure the observe density at 570 nm. Blank will be prepared from reagents with 2 mL of distilled water. Glycine standard solution will be checked by using 2 mL of glycine solution. FAN content will be estimated the using the formula

$$FAN(mg/L) = \frac{A_1 \times 2 \times d}{A_2}$$

Where;

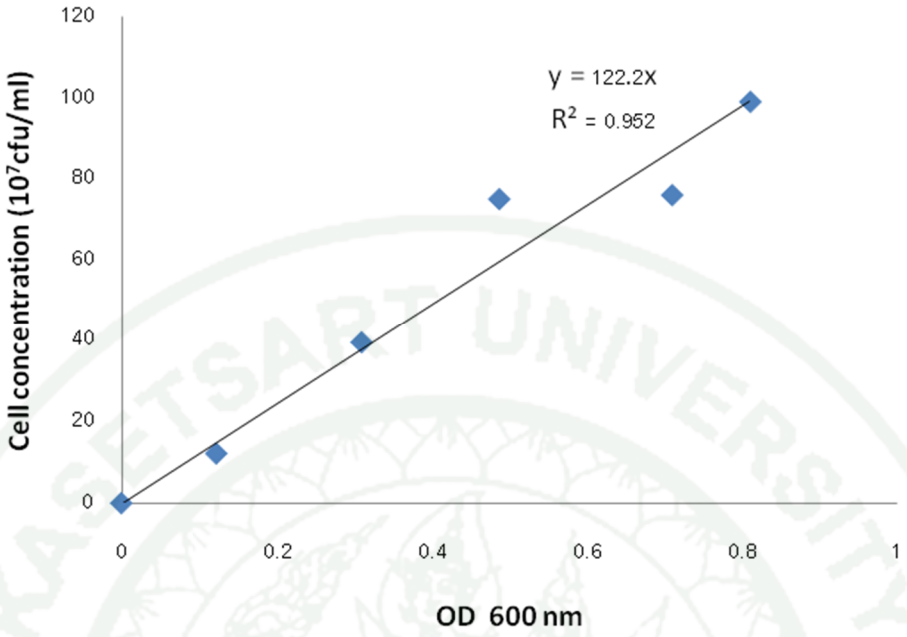
A_1 = Observe density of test solution at 570 nm

A_2 = Mean observe density of standard solution at 570 nm

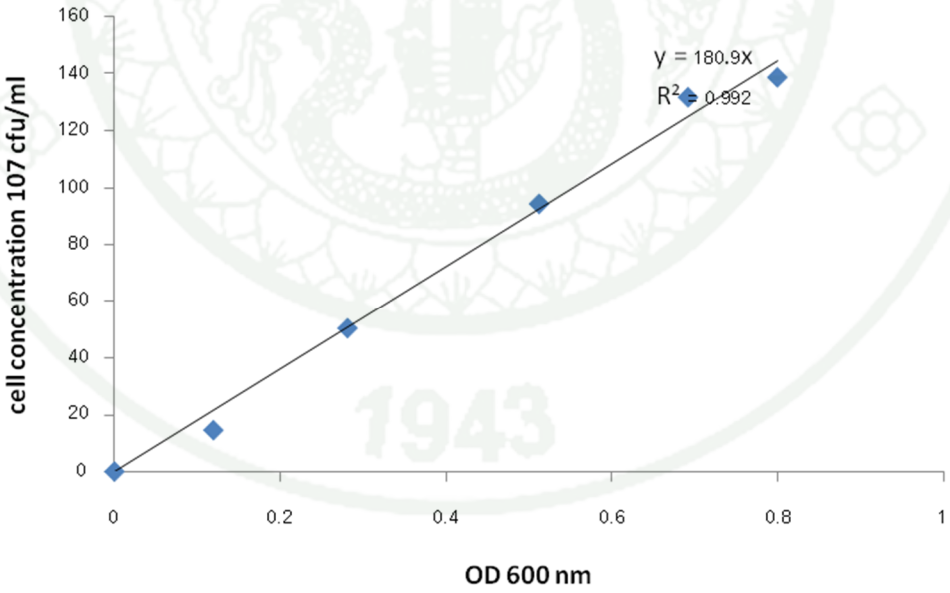
d = Dilution factor of sample

Appendix B

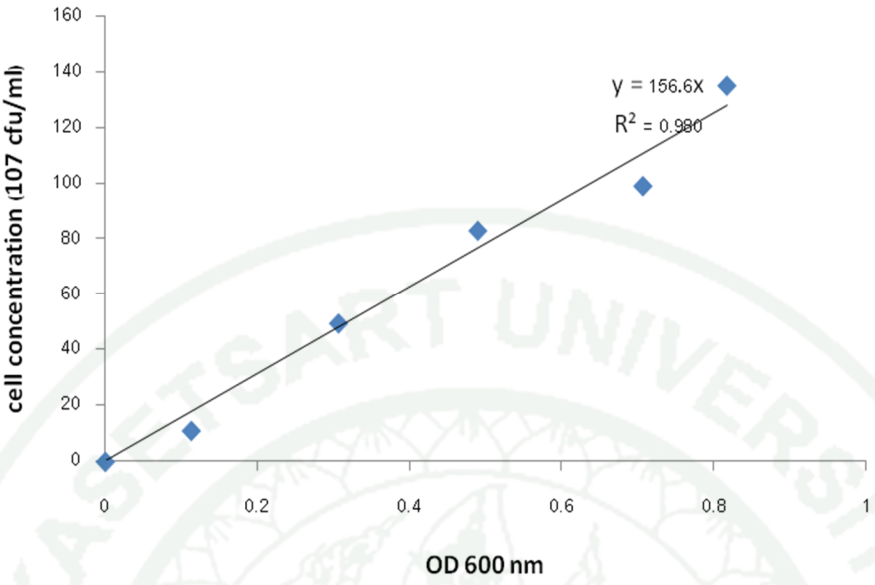
Results



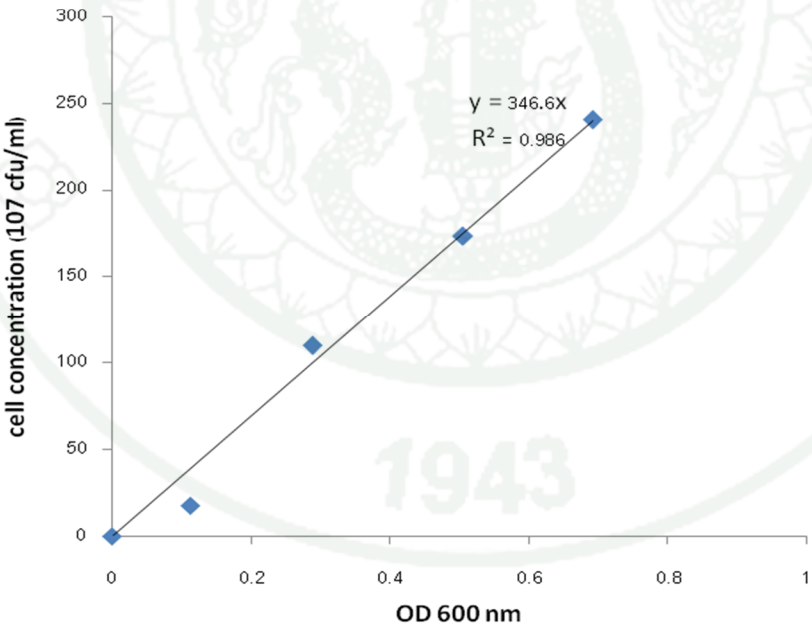
Appendix Figure B1 Standard curve of *L. plantarum* TC24



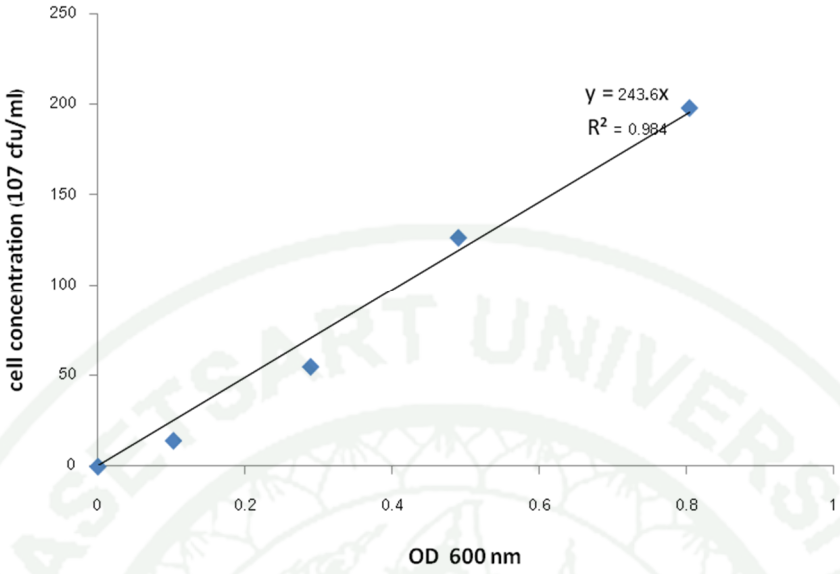
Appendix Figure B2 Standard curve of *L. acidophilus* TISTR450



Appendix Figure B3 Standard curve of *L. reuteri* KUB-AC5



Appendix Figure B4 Standard curve of *L. johnsonii* KUNN19-2



Appendix Figure B5 Standard curve of *E. faecallis* N1-33

Appendix Table B1 Growth of *L.plantarum* TC24 in Riceberry malt extract

Time (hours)	rep		Ave.
	1	2	
0	5.27×10^7 cfu/ml	3.87×10^7 cfu/ml	4.57×10^7 cfu/ml
4	6.32×10^7 cfu/ml	5.3×10^7 cfu/ml	5.81×10^7 cfu/ml
8	2.862×10^8 cfu/ml	3.188×10^8 cfu/ml	3.025×10^8 cfu/ml
12	7.177×10^8 cfu/ml	8.913×10^8 cfu/ml	8.045×10^8 cfu/ml
16	1.00×10^9 cfu/ml	1.26×10^9 cfu/ml	1.13×10^9 cfu/ml
20	1.01×10^9 cfu/ml	1.07×10^9 cfu/ml	1.04×10^9 cfu/ml
24	1.24×10^9 cfu/ml	1.17×10^9 cfu/ml	1.20×10^9 cfu/ml
32	4.99×10^9 cfu/ml	4.86×10^9 cfu/ml	4.92×10^9 cfu/ml
40	5.01×10^9 cfu/ml	5.08×10^9 cfu/ml	5.04×10^9 cfu/ml
48	4.85×10^9 cfu/ml	4.96×10^9 cfu/ml	4.91×10^9 cfu/ml

Appendix Table B2 Growth of *L. acidphillus* TISTR450 in Riceberry malt extract

Time (hours)	rep		Ave.
	1	2	
0	2.42×10^7 cfu/ml	4.16×10^7 cfu/ml	3.29×10^7 cfu/ml
4	8.75×10^7 cfu/ml	9.11×10^7 cfu/ml	8.93×10^7 cfu/ml
8	5.55×10^8 cfu/ml	6.73×10^8 cfu/ml	6.14×10^8 cfu/ml
12	1.29×10^9 cfu/ml	1.35×10^9 cfu/ml	1.32×10^9 cfu/ml
16	1.78×10^9 cfu/ml	1.72×10^9 cfu/ml	1.75×10^9 cfu/ml
20	1.9×10^9 cfu/ml	2.1×10^9 cfu/ml	2×10^9 cfu/ml
24	2.3×10^9 cfu/ml	2.14×10^9 cfu/ml	2.22×10^9 cfu/ml
32	6.28×10^9 cfu/ml	6.3×10^9 cfu/ml	6.29×10^9 cfu/ml
40	7.69×10^9 cfu/ml	7.27×10^9 cfu/ml	7.48×10^9 cfu/ml
48	7.24×10^9 cfu/ml	7.8×10^9 cfu/ml	7.52×10^9 cfu/ml

Appendix Table B3 Growth of *L. reuteri* KUB-AC5 in Riceberry malt extract

Time (hours)	rep		Ave.
	1	2	
0	1.12×10^8 cfu/ml	1.26×10^8 cfu/ml	1.19×10^8 cfu/ml
4	1.99×10^8 cfu/ml	2.21×10^8 cfu/ml	2.1×10^8 cfu/ml
8	4.39×10^8 cfu/ml	3.61×10^8 cfu/ml	4×10^8 cfu/ml
12	1.26×10^9 cfu/ml	1.10×10^9 cfu/ml	1.18×10^9 cfu/ml
16	2.3×10^9 cfu/ml	2.18×10^9 cfu/ml	2.24×10^9 cfu/ml
20	2.5×10^9 cfu/ml	2.22×10^9 cfu/ml	2.36×10^9 cfu/ml
24	2.87×10^9 cfu/ml	2.99×10^9 cfu/ml	2.93×10^9 cfu/ml
32	3.87×10^9 cfu/ml	3.47×10^9 cfu/ml	3.67×10^9 cfu/ml
40	4.01×10^9 cfu/ml	3.87×10^9 cfu/ml	3.94×10^9 cfu/ml
48	4.68×10^9 cfu/ml	4.88×10^9 cfu/ml	4.78×10^9 cfu/ml

Appendix Table B4 Growth of *L. johnsonii* KUNN19-2 in Riceberry malt extract

Time (hours)	rep		Ave.
	1	2	
0	5.67×10^7cfu/ml	5.35×10^7 cfu/ml	5.51×10^7 cfu/ml
4	8.88×10^7 cfu/ml	8.16×10^7 cfu/ml	8.52×10^7 cfu/ml
8	1.51×10^9 cfu/ml	1.47×10^9 cfu/ml	1.49×10^9 cfu/ml
12	3.46×10^9 cfu/ml	3.3×10^9 cfu/ml	3.38×10^9 cfu/ml
16	3.8×10^9 cfu/ml	3.7×10^9 cfu/ml	3.75×10^9 cfu/ml
20	1.74×10^{11} cfu/ml	1.56×10^{11} cfu/ml	1.65×10^{11} cfu/ml
24	1.83×10^{11} cfu/ml	1.67×10^{11} cfu/ml	1.75×10^{11} cfu/ml
32	5.69×10^{11} cfu/ml	5.19×10^{11} cfu/ml	5.44×10^{11} cfu/ml
40	6.11×10^{11} cfu/ml	5.93×10^{11} cfu/ml	6.02×10^{11} cfu/ml
48	5.73×10^{11} cfu/ml	6.77×10^{11} cfu/ml	6.25×10^{11} cfu/ml

Appendix Table B5 Growth of *E. fecalis* N1-33 in Riceberry malt extract

Time (hours)	rep 1	rep 2	Ave.
0	1.84 x 10^8 cfu/ml	1.68 x 10^8 cfu/ml	1.76 x 10^8 cfu/ml
4	2.91 x 10^8 cfu/ml	2.73 x 10^8 cfu/ml	2.82 x 10^8 cfu/ml
8	6.02 x 10^8 cfu/ml	5.94 x 10^8 cfu/ml	5.98 x 10^8 cfu/ml
12	8.69 x 10^8 cfu/ml	8.83 x 10^8 cfu/ml	8.76 x 10^8 cfu/ml
16	1.28 x 10^9 cfu/ml	1.32 x 10^9 cfu/ml	1.30 x 10^9 cfu/ml
20	4.71 x 10^9 cfu/ml	4.63 x 10^9 cfu/ml	4.67 x 10^9 cfu/ml
24	8 x 10^9 cfu/ml	8.26 x 10^9 cfu/ml	8.13 x 10^9 cfu/ml
32	2.33 x 10^{10} cfu/ml	2.17 x 10^{10} cfu/ml	2.25 x 10^{10} cfu/ml
40	2.89 x 10^{10} cfu/ml	2.21 x 10^{10} cfu/ml	2.55 x 10^{10} cfu/ml
48	3.25 x 10^{10} cfu/ml	3.31 x 10^{10} cfu/ml	3.28 x 10^{10} cfu/ml

Appendix Table B6 The change of pH of fermented Riceberry malt extract by five lactic acid bacteria

	pH							
	0 hr				48 hr			
	1	2	3	Ave.	1	2	3	Ave.
L. plantarum TC24	4.88	4.87	4.89	4.88	2.44	2.45	2.46	2.45
L. acidphillus TISTR 450	4.8	4.8	4.8	4.8	2.5	2.5	2.2	2.4
L. reuteri KUB-AC5	4.6	4.7	4.8	4.7	2.78	2.8	2.82	2.8
L. Johnsonii KUNN19-2	4.86	4.88	4.84	4.86	2.3	2.25	2.23	2.26
E. fecalis N1-33	4.87	4.84	4.84	4.85	2.7	2.63	2.77	2.7

Appendix Table B7 The change of total soluble solid of fermented Riceberry malt extract by five lactic acid bacteria

	%Brix							
	0 hr				48 hr			
	1	2	3	Ave.	1	2	3	Ave.
L. plantarum TC24	18	18	18	18	15.9	15.8	16	15.9
L. acidphillus TISTR 450	18	18	18	18	16	16	16	16
L. reuteri KUB-AC5	18	18	18	18	17	17	17	17
L. Johnsonii KUNN19-2	18	18	18	18	15.7	15.5	15.6	15.6
E. fecalis N1-33	18	18	18	18	16.8	16.7	16.6	16.7

Appendix Table B8 The change of acidity of fermented Riceberry malt extract by five lactic acid bacteria

	Acid (ml)							
	0 hr				48 hr			
	1	2	3	Ave.	1	2	3	Ave.
L. plantarum TC24	1.5	1.6	1.4	1.5	5.2	4.9	4.9	5
L. acidphillus TISTR450	1.6	1.4	1.5	1.5	3.9	4.1	4	4
L. reuteri KUB-AC5	1.2	1.3	1.4	1.3	4.4	4.6	4.5	4.5
L. Johnsonii KUNN19-2	1.1	1.1	1.1	1.1	4.2	3.9	3.9	4
E. fecalis N1-33	1.2	1.1	1.3	1.2	4.2	4.2	4.2	4.2

Appendix Table B9 The acid tolerance test of probiotic bacteria

	Acid tolerance (cfu/ml)					
	0 hr			3 hr		
	1	2	Ave.	1	2	Ave.
L. plantarum TC24	98.84×10^9	92.16×10^9	95.5×10^9	59.85×10^9	54.15×10^9	57×10^9
L. acidphillus TISTR450	7.98×10^9	8.82×10^9	8.4×10^9	6.12×10^9	6.88×10^9	6.5×10^9
L. reuteri KUB-AC5	18.05×10^9	18.35×10^9	18.2×10^9	12.06×10^9	10.94×10^9	11.5×10^9
L. Johnsonii KUNN19-2	70.23×10^9	64.77×10^9	67.5×10^9	12.53×10^9	11.97×10^9	12.25×10^9
E. fecalis N1-33	6.91×10^9	5.89×10^9	6.1×10^9	2.58×10^9	2.22×10^9	2.4×10^9

Appendix Table B10 The bile tolerance test of probiotic bacteria

	Bcid tolerance (cfu/ml)					
	0 hr			3 hr		
	1	2	Ave.	1	2	Ave.
L. plantarum TC24	59.87 x10^9	56.13 x10^9	58 x10^9	7.05 x10^9	6.55 x10^9	6.8 x10^9
L. acidphillus TISTR 450	7.35 x10^9	8.65 x10^9	8 x10^9	5.47 x10^8	6.73 x10^8	6.1 x10^8
L. reuteri KUB-AC5	9.67 x10^9	10.33 x10^9	10 x10^9	1.09 x10^9	1.61 x10^9	1.35 x10^9
L. Johnsonii KUNN19-2	14.02 x10^9	11.98 x10^9	13 x10^9	3.12 x10^8	4.88 x10^8	4 x10^8
E. fecalis N1-33	3.49 x10^9	3.11 x10^9	3.3 x10^9	2.11 x10^8	1.97 x10^8	2.04 x10^8

Appendix Table B11 Antibacterial activity of cell free supernatant of *L.plantarum* TC24

	Inhibition zone (mm)		
	1	2	Ave.
Staphylococcus aureus TISTR118	20	20	20
E. coli 010	14.7	15.3	15
Bacillus subtilis TISTR024	14	14	14
Samonella enteritidis DMST17368	15.9	16.1	16

Appendix Table B12 Antibacterial activity of cell free supernatant of *L. acidophilus* TISTR 450

	Inhibition zone (mm)		
	1	2	Ave.
Staphylococcus aureus TISTR118	17	17	17
E. coli 010	15	15	15
Bacillus subtilis TISTR024	10.9	11.1	11
Samonella enteritidis DMST17368	14	14	14

Appendix Table B13 Antibacterial activity of cell free supernatant of *L. reuteri* KUB-AC5

	Inhibition zone (mm)		
	1	2	Ave.
Staphylococcus aureus TISTR118	15.1	14.9	15
E. coli 010	14	14	14
Bacillus subtilis TISTR024	12	12	12
Samonella enteritidis DMST17368	15	15	15

Appendix Table B14 Antibacterial activity of cell free supernatant of *L. johnsonii* KUNN19-2

	Inhibition zone (mm)		
	1	2	Ave.
Staphylococcus aureus TISTR118	16	16	16
E. coli 010	15.1	14.9	15
Bacillus subtilis TISTR024	11	11	11
Samonella enteritidis DMST17368	14.9	15.1	15

Appendix Table B15 Antibacterial activity of cell free supernatant of *E. fecalis* N1-33

	Inhibition zone (mm)		
	1	2	Ave.
Staphylococcus aureus TISTR 118	14	14	14
E. coli 010	14	14	14
Bacillus subtilis TISTR 024	13	11	12
Samonella enteritidis DMST17368	14.1	13.9	14

Appendix Table B16 Sensory test of aroma on five probiotic beverages

comsumer	Score				
	L. plantarum TC24	*L. acidphillus* *TISTR*450	*L. reuteri* KUB-AC5	*L. johnsonii* KUNN192	*E. fecalis* N1-33
1	5	4	1	3	2
2	4	5	1	3	2
3	5	2	1	4	3
4	3	2	4	1	5
5	5	4	1	2	3
6	3	5	1	2	4
7	5	2	1	4	3
8	4	3	1	5	2
9	5	1	2	4	3
10	4	5	1	3	2
Ave.	4.3	3.3	1.4	3.1	2.9

Appendix Table B17 Sensory test of taste on five probiotic beverages

comsumer	Score				
	L. plantarum TC24	*L. acidphillus* *TISTR*450	*L. reuteri* KUB-AC5	*L. johnsonii* KUNN192	*E. fecalis* N1-33
1	5	4	1	3	2
2	4	5	2	1	3
3	4	3	1	2	5
4	2	5	3	1	4
5	4	5	2	1	3
6	4	5	1	2	3
7	5	2	1	4	3
8	5	4	1	2	3
9	4	1	2	5	3
10	4	5	1	3	2
Ave.	4.1	3.9	1.5	2.4	3.1

Appendix Table B18 Sensory test of acceptability on five probiotic beverages

comsumer	Score				
	L. plantarum TC24	*L. acidphillus* *TISTR*450	*L. reuteri* KUB-AC5	*L. johnsonii* KUNN192	*E. fecalis* N1-33
1	5	4	1	3	2
2	3	5	1	4	2
3	5	3	1	2	4
4	2	5	3	1	4
5	5	4	2	1	3
6	4	5	1	2	3
7	5	2	1	4	3
8	5	4	1	2	3
9	5	1	2	4	3
10	5	4	1	3	2
Ave.	4.4	3.7	1.4	2.6	2.9

Appendix Table B19 Probiotic (*Lactobacillus plantarum* TC24) growth in modified MRS + inulin 0.98 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	9.8×10^6 cfu/ml	1.02×10^7 cfu/ml	1×10^7 cfu/ml
24	3.21×10^9 cfu/ml	2.79×10^9 cfu/ml	3×10^9 cfu/ml

Appendix Table B20 Probiotic (*Lactobacillus plantarum* TC24) growth in modified MRS + Glu 0.02 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	1.42×10^7 cfu/ml	1.58×10^7 cfu/ml	1.5×10^7 cfu/ml
24	2.83×10^8 cfu/ml	3.17×10^8 cfu/ml	3×10^8 cfu/ml

Appendix Table B21 Probiotic (*Lactobacillus plantarum* TC24) growth in modified MRS + FOS 0.95 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	1.96×10^7 cfu/ml	2.04×10^7 cfu/ml	2×10^7 cfu/ml
24	1.28×10^9 cfu/ml	1.12×10^9 cfu/ml	1.2×10^9 cfu/ml

Appendix Table B22 Probiotic (*Lactobacillus plantarum* TC24) growth in modified MRS + Glu 0.05 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	3.31 x 10^7 cfu/ml	3.09 x 10^7 cfu/ml	3.2 x 10^7 cfu/ml
24	4.09 x 10^9 cfu/ml	4.11 x 10^9 cfu/ml	4.1 x 10^8 cfu/ml

Appendix Table B23 Pathogen (mixed culture of *E.coli , Staphylococcus aureus* and *salmonella enteritidis*) growth in M9 medium + inulin 0.98 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	3.15 x 10^6 cfu/ml	3.05 x 10^6 cfu/ml	3.1 x 10^6 cfu/ml
24	1.79 x 10^9 cfu/ml	1.89 x 10^9 cfu/ml	1.84 x 10^9 cfu/ml

Appendix Table B24 Pathogen (mixed culture of *E.coli , Staphylococcus aureus* and *salmonella enteritidis*) growth in M9 medium + Glu 0.02 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	2.32 x 10^6 cfu/ml	2.48 x 10^6 cfu/ml	2.4 x 10^6 cfu/ml
24	2.74 x 10^8 cfu/ml	2.06 x 10^8 cfu/ml	2.4 x 10^8 cfu/ml

Appendix Table B25 Pathogen (mixed culture of *E.coli , Staphylococcus aureus* and *salmonella enteritidis*) growth in M9 medium + FOS 0.95 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	2.11 x 10^6cfu/ml	2.09 x 10^6 cfu/ml	2.1 x 10^6 cfu/ml
24	2.02 x 10^9 cfu/ml	1.98 x 10^9 cfu/ml	2x 10^9 cfu/ml

Appendix Table B26 Pathogen (mixed culture of *E.coli , Staphylococcus aureus* and *salmonella enteritidis*) growth in M9 medium + Glu 0.05 g/100ml

Time (hours)	rep		Ave.
	1	2	
0	4.86 x 10^6cfu/ml	5.14 x 10^6 cfu/ml	5 x 10^6 cfu/ml
24	3.09 x 10^8 cfu/ml	3.51 x 10^8 cfu/ml	3.3 x 10^8 cfu/ml

Appendix Table B27 Effect of inulin in growth of *L.plantarum* TC24

Time (hours)	rep		Ave.
	1	2	
0	4.96×10^7 cfu/ml	4.74×10^7 cfu/ml	4.85×10^7 cfu/ml
4	6.98×10^7 cfu/ml	6.38×10^7 cfu/ml	6.68×10^7 cfu/ml
8	4.51×10^8 cfu/ml	3.95×10^8 cfu/ml	4.23×10^8 cfu/ml
12	9.68×10^8 cfu/ml	9.12×10^8 cfu/ml	9.40×10^8 cfu/ml
16	1.20×10^9 cfu/ml	1.16×10^9 cfu/ml	1.18×10^9 cfu/ml
20	1.11×10^9 cfu/ml	1.07×10^9 cfu/ml	1.09×10^9 cfu/ml
24	1.09×10^9 cfu/ml	1.13×10^9 cfu/ml	1.11×10^9 cfu/ml
32	4.98×10^9 cfu/ml	4.86×10^9 cfu/ml	4.92×10^9 cfu/ml
40	5.34×10^9 cfu/ml	4.96×10^9 cfu/ml	5.15×10^9 cfu/ml
48	5.11×10^9 cfu/ml	4.97×10^9 cfu/ml	5.04×10^9 cfu/ml

Appendix Table B28 Effect of FOS in growth of *L.plantarum* TC24

Time (hours)	rep		Ave.
	1	2	
0	5.68×10^7 cfu/ml	5.46×10^7 cfu/ml	5.57×10^7 cfu/ml
4	6.47×10^7 cfu/ml	6.81×10^7 cfu/ml	6.64×10^7 cfu/ml
8	4.05×10^8 cfu/ml	3.953×10^8 cfu/ml	3.99×10^8 cfu/ml
12	9.56×10^8 cfu/ml	8.96×10^8 cfu/ml	9.26×10^8 cfu/ml
16	1.11×10^9 cfu/ml	1.13×10^9 cfu/ml	1.12×10^9 cfu/ml
20	1.04×10^9 cfu/ml	1.02×10^9 cfu/ml	1.03×10^9 cfu/ml
24	1.17×10^9 cfu/ml	1.11×10^9 cfu/ml	1.14×10^9 cfu/ml
32	5.13×10^9 cfu/ml	4.95×10^9 cfu/ml	5.04×10^9 cfu/ml
40	5.22×10^9 cfu/ml	5.12×10^9 cfu/ml	5.17×10^9 cfu/ml
48	5.11×10^9 cfu/ml	5.09×10^9 cfu/ml	5.10×10^9 cfu/ml

Appendix Table B29 Growth of encapsulation *L.plantarum* TC24

Time (hours)	rep 1	rep 2	Ave.
0	9.77×10^7 cfu/ml	9.53×10^7 cfu/ml	9.65×10^7 cfu/ml
16	1.69×10^9 cfu/ml	1.91×10^9 cfu/ml	1.80×10^9 cfu/ml
24	2.22×10^9 cfu/ml	2.06×10^9 cfu/ml	2.14×10^9 cfu/ml
32	2.13×10^9 cfu/ml	2.27×10^9 cfu/ml	2.20×10^9 cfu/ml
40	2.62×10^9 cfu/ml	2.54×10^9 cfu/ml	2.58×10^9 cfu/ml
48	2.23×10^9 cfu/ml	2.61×10^9 cfu/ml	2.42×10^9 cfu/ml

Appendix Table B30 Growth of leak cell *L.plantarum* TC24

Time (hours)	rep 1	rep 2	Ave.
0	3 cfu/ml	1.1 cfu/ml	2.05 cfu/ml
16	1.09×10^1 cfu/ml	1.21×10^1 cfu/ml	1.15×10^1 cfu/ml
24	2.22×10^1 cfu/ml	2.06×10^1 cfu/ml	2.14×10^1 cfu/ml
32	3.41×10^2 cfu/ml	3.21×10^2 cfu/ml	3.31×10^2 cfu/ml
40	5.83×10^2 cfu/ml	5.91×10^2 cfu/ml	5.87×10^2 cfu/ml
48	3.46×10^3 cfu/ml	3.86×10^3 cfu/ml	3.66×10^3 cfu/ml

Appendix Table B31 The survival cell concentration in acid condition (in bead)

Time (hours)	rep 1	rep 2	Ave.
0	9.56×10^9 cfu/ml	8.44×10^9 cfu/ml	9×10^9 cfu/ml
3	6.1×10^9 cfu/ml	5.9×10^9 cfu/ml	6×10^9 cfu/ml

Appendix Table B32 The survival cell concentration in acid condition (leak cell)

Time (hours)	rep 1	rep 2	Ave.
0	23 x 10^7 cfu/ml	21 x 10^7 cfu/ml	22 cfu/ml
3	48.6 x 10^9 cfu/ml	51.4 x 10^9 cfu/ml	50 x 10^3 cfu/ml

Appendix Table B33 The survival cell concentration in bile condition (in bead)

Time (hours)	rep 1	rep 2	Ave.
0	5.24 x 10^9 cfu/ml	4.76 x 10^9 cfu/ml	5 x 10^9 cfu/ml
3	1.87 x 10^9 cfu/ml	1.91 x 10^9 cfu/ml	1.89 x 10^9 cfu/ml

Appendix Table B34 The survival cell concentration in bile condition (leak cell)

Time (hours)	rep 1	rep 2	Ave.
0	13 x 10^9 cfu/ml	11 x 10^9 cfu/ml	12 cfu/ml
3	56.1 x 10^9 cfu/ml	53.9 x 10^9 cfu/ml	55 x 10^3 cfu/ml

Appendix Table B35 Survival of *L.plantarum* TC 24 in gastric condition.

	gastric (cfu/ml)					
	0 hr			3 hr		
	1	2	Ave.	1	2	Ave.
Free cell	2.92×10^9	3.02×10^9	2.97×10^9	1.49×10^9	1.57×10^9	1.53×10^9
Encapsulated cell	3.12×10^9	3×10^9	3.06×10^9	2.29×10^9	2.45×10^9	2.37×10^9
leak cell	14	16	15	90	82	86

Appendix Table B36 Survival of *L.plantarum* TC 24 in bile juice condition.

	bile juice (cfu/ml)					
	0 hr			3 hr		
	1	2	Ave.	1	2	Ave.
Free cell	1.26×10^9	1.04×10^9	1.15×10^9	5.61×10^8	5.15×10^8	5.35×10^8
Encapsulated cell	2.37×10^9	2.71×10^9	2.54×10^9	1.74×10^9	1.58×10^9	1.66×10^9
leak cell	22	24	23	76	78	77

Appendix Table B37 Growth of free cell at 8 °C

Time (days)	Viable cell (cfu/ml)		Ave.
	1	2	
0	3.1×10^{9}	2.9×10^{9}	3×10^{9}
1	1×10^{10}	1.4×10^{10}	1.2×10^{10}
2	8.5×10^{10}	8.9×10^{10}	8.7×10^{10}
3	1.1×10^{11}	1.5×10^{11}	1.30×10^{11}
4	5.8×10^{11}	5×10^{11}	5.4×10^{11}
5	3.9×10^{11}	3.7×10^{11}	3.8×10^{11}
6	1.9×10^{11}	2.1×10^{11}	2×10^{11}
7	1.5×10^{11}	1.1×10^{11}	1.3×10^{11}
8	7.6×10^{10}	7.4×10^{10}	7.5×10^{10}
9	5.9×10^{10}	5.5×10^{10}	5.7×10^{10}
10	8.1×10^{9}	7.26×10^{9}	7.68×10^{9}
11	5.9×10^{9}	5.7×10^{9}	5.8×10^{9}
12	3.6×10^{9}	3.2×10^{9}	3.4×10^{9}
13	1.7×10^{9}	1.9×10^{9}	1.8×10^{9}
14	1.65×10^{9}	1.77×10^{9}	1.71×10^{9}
17	5.66×10^{8}	5.58×10^{8}	5.62×10^{8}
20	1.56×10^{8}	1.14×10^{8}	1.35×10^{8}
23	9.45×10^{6}	9.01×10^{6}	9.23×10^{6}
26	5.59×10^{6}	6.87×10^{6}	6.23×10^{6}
29	7.71×10^{5}	8.53×10^{5}	8.12×10^{5}
31	4.22×10^{4}	4.46×10^{4}	4.34×10^{4}

Appendix Table B38 Growth of free cell at 30 °C

Time (days)	Viable cell (cfu/ml)		Ave.
	1	2	
0	2.9×10^{9}	2.1×10^{9}	2.5×10^{9}
1	1.5×10^{11}	1.3×10^{11}	1.4×10^{11}
2	4.46×10^{11}	4.84×10^{11}	4.65×10^{11}
3	1.98×10^{12}	2.02×10^{12}	2×10^{12}
4	5.62×10^{12}	5.18×10^{12}	5.4×10^{12}
5	1.86×10^{12}	1.94×10^{12}	1.9×10^{12}
6	1.32×10^{12}	1.14×10^{12}	1.23×10^{12}
7	8.96×10^{11}	10.64×10^{11}	9.8×10^{11}
8	8.6×10^{11}	8.8×10^{11}	8.7×10^{11}
9	6.96×10^{11}	7.04×10^{11}	7×10^{11}
10	5.5×10^{10}	6.1×10^{10}	5.8×10^{10}
11	3.4×10^{10}	3.2×10^{10}	3.3×10^{10}
12	4.12×10^{9}	3.88×10^{9}	4×10^{9}
13	1.83×10^{9}	2.65×10^{9}	2.24×10^{9}
14	8.82×10^{8}	9.1×10^{8}	8.96×10^{8}
17	4.77×10^{7}	4.97×10^{7}	4.78×10^{7}
20	6.12×10^{6}	6.02×10^{6}	6.07×10^{6}
23	2.36×10^{5}	2.54×10^{5}	2.45×10^{5}
26	7.76×10^{4}	8.2×10^{4}	7.98×10^{4}
29	10×10^{2}	9.78×10^{2}	9.89×10^{2}
31	1.28×10^{2}	1.46×10^{2}	1.37×10^{2}

Appendix Table B39 Growth of encapsulate cell at 8 ℃

Time (days)	Viable cell (cfu/ml)		Ave.
	1	2	
0	1.3×10^{9}	1.1×10^{9}	1.2×10^{9}
1	1.64×10^{10}	1.52×10^{10}	1.58×10^{10}
2	2.8×10^{10}	2.6×10^{10}	2.7×10^{10}
3	2.8×10^{11}	3×10^{11}	2.9×10^{11}
4	4.4×10^{11}	5.6×10^{11}	5×10^{11}
5	2.6×10^{11}	2.8×10^{11}	2.7×10^{11}
6	2.6×10^{11}	2.26×10^{11}	2.43×10^{11}
7	1×10^{11}	1×10^{11}	1×10^{11}
8	3.24×10^{10}	3.3×10^{10}	3.27×10^{10}
9	2.91×10^{10}	2.89×10^{10}	2.9×10^{10}
10	6.8×10^{9}	7.2×10^{9}	7×10^{9}
11	3.18×10^{9}	3.34×10^{9}	3.26×10^{9}
12	1.8×10^{9}	1.4×10^{9}	1.6×10^{9}
13	1.35×10^{9}	1.25×10^{9}	1.3×10^{9}
14	1.23×10^{9}	1.29×10^{9}	1.26×10^{9}
17	2.54×10^{8}	2.7×10^{8}	2.62×10^{8}
20	2.26×10^{8}	2.44×10^{8}	2.35×10^{8}
23	4.72×10^{6}	3.69×10^{6}	3.98×10^{6}
26	9.64×10^{5}	8.82×10^{5}	9.23×10^{5}
29	1.32×10^{5}	1.56×10^{5}	1.44×10^{5}
31	1.36×10^{4}	1.1×10^{4}	1.23×10^{4}

Appendix Table B40 Growth of encapsulate cell at 30 °C

Time (days)	Viable cell (cfu/ml)		Ave.
	1	2	
0	2.3×10^{9}	2.1×10^{9}	2.2×10^{9}
1	1.9×10^{11}	2.3×10^{11}	2.1×10^{11}
2	2.68×10^{11}	3.12×10^{11}	2.9×10^{11}
3	3.97×10^{11}	4.23×10^{11}	4.1×10^{11}
4	4.9×10^{12}	4.7×10^{12}	4.8×10^{12}
5	2.3×10^{12}	2.36×10^{12}	2.33×10^{12}
6	1.65×10^{12}	1.55×10^{12}	1.6×10^{12}
7	9.23×10^{11}	9.37×10^{11}	9.3×10^{11}
8	4.03×10^{11}	4.65×10^{11}	4.34×10^{11}
9	1.16×10^{11}	1.36×10^{11}	1.26×10^{11}
10	6.53×10^{10}	6.47×10^{10}	6.5×10^{10}
11	2.2×10^{10}	2.4×10^{10}	2.3×10^{10}
12	6.3×10^{9}	5.5×10^{9}	5.9×10^{9}
13	7.98×10^{8}	8.02×10^{8}	8×10^{8}
14	1.65×10^{8}	1.75×10^{8}	1.7×10^{8}
17	2.26×10^{7}	2.46×10^{7}	2.36×10^{7}
20	1.10×10^{6}	1.14×10^{6}	1.12×10^{6}
23	9.69×10^{4}	10.03×10^{4}	9.86×10^{4}
26	1.25×10^{4}	1.21×10^{4}	1.23×10^{4}
29	5.37×10^{2}	5.53×10^{2}	5.54×10^{2}
31	9.75×10^{1}	9.77×10^{1}	9.76×10^{1}

Appendix Table B41 pH of free cell at 8 °C

Time (days)	pH		Ave.
	1	2	
0	4.84	4.8	4.82
1	4.65	4.59	4.62
2	4.50	4.52	4.51
3	4.4	4.38	4.39
4	4.38	3.32	4.35
5	4.2	4.18	4.19
6	4.05	4.09	4.07
7	3.78	3.82	3.8
8	3.65	3.75	3.7
9	3.6	3.8	3.7
10	3.6	3.8	3.7
11	3.6	3.8	3.7
12	3.62	3.6	3.61
13	3.61	3.61	3.61
14	3.51	3.53	3.52
17	3.38	3.4	3.39
20	3.4	3.34	3.37
23	3.38	3.34	3.36
26	3.54	3.52	3.53
29	3.44	3.42	3.43
31	3.4	3.44	3.42

Appendix Table B42 pH of free cell at 30 ℃

Time (days)	pH		Ave.
	1	2	
0	4.8	4.84	4.82
1	3	3	3
2	2.78	2.82	2.8
3	2.7	2.7	2.70
4	2.7	2.68	2.69
5	2.69	2.67	2.68
6	2.66	2.68	2.67
7	2.65	2.66	2.64
8	2.62	2.62	2.62
9	2.58	2.56	2.57
10	2.51	2.51	2.51
11	2.5	2.5	2.5
12	2.5	2.5	2.5
13	2.5	2.5	2.5
14	2.5	2.5	2.5
17	2.5	2.5	2.5
20	2.5	2.5	2.5
23	2.48	2.48	2.48
26	2.48	2.48	2.48
29	2.42	2.42	2.42
31	2.39	2.39	2.39

Appendix Table B43 pH of encapsulate cell at 8 °C

Time (days)	pH		Ave.
	1	2	
0	4.82	4.82	4.82
1	4.82	4.82	4.82
2	4.82	4.82	4.82
3	4.8	4.78	4.79
4	4.74	4.72	4.73
5	4.71	4.73	4.72
6	4.70	4.70	4.70
7	4.65	4.65	4.65
8	4.58	4.58	4.58
9	4.59	4.56	4.57
10	4.54	4.54	4.54
11	4.52	4.52	4.52
12	4.51	4.53	4.52
13	4.50	4.50	4.50
14	4.45	4.45	4.45
17	4.32	4.32	4.32
20	4.21	4.21	4.21
23	4.13	4.13	4.13
26	4.11	4.11	4.11
29	4.06	4.06	4.06
31	4.04	4.04	4.04

Appendix Table B44 pH of encapsulate cell at 30°C

Time (days)	pH		Ave.
	1	2	
0	4.7	4.9	4.82
1	3.33	3.35	3.34
2	3	3	3
3	2.8	2.86	2.83
4	2.8	2.82	2.81
5	2.74	2.74	2.74
6	2.67	2.67	2.67
7	2.61	2.65	2.63
8	2.54	2.6	2.57
9	2.6	2.58	2.59
10	2.59	2.57	2.58
11	2.5	2.52	2.51
12	2.51	2.51	2.51
13	2.5	2.5	2.5
14	2.5	2.5	2.5
17	2.5	2.5	2.5
20	2.5	2.5	2.5
23	2.5	2.5	2.5
26	2.5	2.5	2.5
29	2.49	2.49	2.49
31	2.49	2.49	2.49

Appendix Table B45 Acidity of free cell at 8°C

Time (days)	Acidity		Ave.
	1	2	
0	0.8	0.8	0.8
1	0.8	1	0.9
2	0.9	0.9	0.9
3	0.9	0.9	0.9
4	1	1	1
5	1	1	1
6	1.2	1.2	1.2
7	1.5	1.5	1.5
8	1.5	1.5	1.5
9	1.5	1.5	1.5
10	1.5	1.5	1.5
11	1.4	1.6	1.5
12	1.5	1.5	1.5
13	1.5	1.5	1.5
14	1.5	1.5	1.5
17	1.6	1.6	1.6
20	1.6	1.6	1.6
23	1.6	1.6	1.6
26	1.6	1.6	1.6
29	1.6	1.6	1.6
31	1.6	1.6	1.6

Appendix Table B46 Acidity of free cell at 30 ℃

Time (days)	Acidity		Ave.
	1	2	
0	0.8	0.8	0.8
1	1.9	1.9	1.9
2	3	3	3
3	3.2	3.2	3.2
4	3.5	3.5	3.5
5	3.5	3.5	3.5
6	3.6	3.4	3.5
7	3.6	3.8	3.7
8	3.8	3.8	3.8
9	3.9	3.9	3.9
10	4	4	4
11	3.9	4.1	4
12	4.1	4.1	4.1
13	4.2	4.2	4.2
14	4.2	4.2	4.2
17	4.2	4.2	4.2
20	4.3	4.3	4.3
23	4.4	4.5	4.3
26	4.3	4.3	4.3
29	4.3	4.3	4.3
31	4.3	4.3	4.3

Appendix Table B47 Acidity of encapsulate cell at 8 °C

Time (days)	Acidity		Ave.
	1	2	
0	0.8	0.8	0.8
1	0.9	0.9	0.9
2	0.9	0.9	0.9
3	0.9	0.9	0.9
4	0.9	0.9	0.9
5	0.9	0.9	0.9
6	0.8	1	0.9
7	0.9	1.1	1
8	1	1	1
9	1	1	1
10	1	1.2	1.1
11	1.1	1.1	1.1
12	1.1	1.1	1.1
13	1.1	1.1	1.1
14	1.1	1.1	1.1
17	1.1	1.1	1.1
20	1.1	1.1	1.1
23	1.1	1.1	1.1
26	1.2	1.2	1.2
29	1.2	1.2	1.2
31	1.2	1.2	1.2

Appendix Table B48 Acidity of encapsulate cell at 30 °C

Time (days)	Acidity		Ave.
	1	2	
0	0.7	0.9	0.8
1	1.1	1.1	1.1
2	2	2	2
3	2.3	2.5	2.4
4	2.7	2.7	2.7
5	3	3.2	3.1
6	3.3	3.3	3.3
7	3.8	3.8	3.8
8	3.9	3.9	3.9
9	3.9	3.9	3.9
10	3.9	3.9	3.9
11	3.9	3.9	3.9
12	4.1	3.9	4
13	4.1	4.1	4.1
14	4.2	4.2	4.2
17	4.3	4.3	4.3
20	4.4	4.4	4.4
23	4.5	4.5	4.5
26	4.5	4.5	4.5
29	4.5	4.5	4.5
31	4.5	4.5	4.5

Appendix Table B49 Total soluble solid of free cell at 8 ℃

Time (days)	%brix		Ave.
	1	2	
0	18	18	18
1	17.8	17.8	17.8
2	17	17	17
3	17	17	17
4	17	17	17
5	16.9	16.9	16.9
6	16.5	16.5	16.5
7	16.5	16.5	16.5
8	16.5	16.5	16.5
9	16.5	16.5	16.5
10	16.5	16.5	16.5
11	16.5	16.5	16.5
12	16.4	16.4	16.4
13	16.4	16.4	16.4
14	16.4	16.4	16.4
17	16.4	16.4	16.4
20	16.4	16.4	16.4
23	16.4	16.4	16.4
26	16.3	16.3	16.3
29	16.3	16.3	16.3
31	16.3	16.3	16.3

Appendix Table B50 Total soluble solid of free cell at 30 ℃

Time (days)	%brix		Ave.
	1	2	
0	18	18	18
1	16	16	16
2	15	15	15
3	15	15	15
4	15	15	15
5	15	15	15
6	14.5	14.5	14.5
7	14.5	14.5	14.5
8	14.5	14.5	14.5
9	14.5	14.5	14.5
10	14	14	14
11	14	14	14
12	14	14	14
13	14	14	14
14	14	14	14
17	14	14	14
20	14	14	14
23	14	14	14
26	13.9	13.9	13.9
29	13.9	13.9	13.9
31	13.9	13.9	13.9

Appendix Table B51 Total soluble solid of encapsulate cell at 8 °C

Time (days)	%brix		Ave.
	1	2	
0	18	18	18
1	17.6	17.6	17.6
2	17.6	17.6	17.6
3	17	17	17
4	17	17	17
5	17	17	17
6	16.8	16.8	16.8
7	16.5	16.5	16.5
8	16.5	16.5	16.5
9	16.5	16.5	16.5
10	16.4	16.4	16.4
11	16.4	16.4	16.4
12	16.4	16.4	16.4
13	16.4	16.4	16.4
14	16.3	16.3	16.3
17	16.3	16.3	16.3
20	16.2	16.2	16.2
23	16.2	16.2	16.2
26	16.2	16.2	16.2
29	16.1	16.1	16.1
31	16.1	16.1	16.1

Appendix Table B52 Total soluble solid of encapsulate cell at 30 °C

Time (days)	%brix		Ave.
	1	2	
0	18	18	18
1	16	16	16
2	15.9	15.9	15.9
3	15.5	15.5	15.5
4	15.5	15.5	15.5
5	15	15	15
6	15	15	15
7	14.9	14.9	14.9
8	14.8	14.8	14.8
9	14.8	14.8	14.8
10	14.8	14.8	14.8
11	14.8	14.8	14.8
12	14.7	14.7	14.7
13	14.7	14.7	14.7
14	14.6	14.6	14.6
17	14.6	14.6	14.6
20	14.6	14.6	14.6
23	14.5	14.5	14.5
26	14.5	14.5	14.5
29	14.5	14.5	14.5
31	14.5	14.5	14.5

Appendix Table B53 Growth of probiotic added honey flavor

Time (days)	Viable cell (cfu/ml)		Ave.
	1	2	
0	1.86×10^{9}	1.94×10^{9}	1.9×10^{9}
1	3.64×10^{10}	3.36×10^{10}	3.5×10^{10}
2	7.89×10^{10}	7.51×10^{10}	7.7×10^{10}
3	1.8×10^{11}	2×10^{11}	1.9×10^{11}
4	2.46×10^{11}	2.54×10^{11}	2.5×10^{11}
5	6.98×10^{11}	7.02×10^{11}	7×10^{11}
6	8.3×10^{11}	8.2×10^{11}	8.25×10^{11}
7	9.2×10^{11}	9.4×10^{11}	9.3×10^{11}
10	2.4×10^{9}	2.6×10^{9}	2.5×10^{9}
13	1×10^{9}	1×10^{9}	1×10^{9}
16	9.26×10^{8}	9.46×10^{8}	9.36×10^{8}
19	7.88×10^{8}	8.12×10^{8}	8×10^{8}
21	9.11×10^{6}	8.89×10^{6}	9×10^{6}
25	6.7×10^{5}	6.3×10^{5}	6.5×10^{5}
28	1.8×10^{5}	1.72×10^{5}	1.76×10^{4}
31	1×10^{4}	1×10^{4}	1×10^{4}

Appendix Table B54 pH of probiotic added honey flavor

Time (days)	pH		Ave.
	1	2	
0	3.49	3.47	3.48
1	3.4	3.3	3.35
2	3.31	3.31	3.31
3	3.31	3.31	3.31
4	3.3	3.26	3.28
5	3.27	3.27	3.27
6	3.27	3.27	3.27
7	3.26	3.26	3.26
10	3.26	3.26	3.26
13	3.25	3.25	3.25
16	3.24	3.24	3.24
19	3.23	3.23	3.23
21	3.23	3.23	3.23
25	3.2	3.2	3.2
28	3.18	3.2	3.19
31	2.8	2.8	2.8

Appendix Table B55 Acidity of probiotic added honey flavor

Time (days)	Acidity		Ave.
	1	2	
0	1.6	1.8	1.7
1	3	2.8	2.9
2	3	3	3
3	3	3	3
4	3	3	3
5	3.2	3	3.1
6	3.2	3.2	3.2
7	3.3	3.3	3.3
10	3.3	3.5	3.4
13	3.5	3.5	3.5
16	3	3	3
19	3	3	3
21	3	3	3
25	2.8	2.8	2.8
28	2.8	2.8	2.8
31	2.8	2.8	2.8

Appendix Table B56 Total soluble solid of probiotic added honey flavor

Time (days)	%Brix		Ave.
	1	2	
0	22	22	22
1	20.9	20.9	20.9
2	20.8	20.8	20.8
3	20.8	20.8	20.8
4	20.7	20.7	20.7
5	20.7	20.7	20.7
6	20.5	20.5	20.5
7	20.4	20.4	20.4
10	20.4	20.4	20.4
13	20.3	20.3	20.3
16	20.3	20.3	20.3
19	20.3	20.3	20.3
21	20.1	20.1	20.1
25	20.1	20.1	20.1
28	20.1	20.1	20.1
31	20	20	20

Appendix Table B57 Free amiono nitrogen (mg/l) in probiotic beverage with other product in market

	solution at 570 nm			
	1	2	3	Ave.
blank	0.0315	0.0317	0.0292	0.0308
standard	0.3406	0.3032	0.364	0.335933
probiotic drink	0.1108	0.1022	0.1141	0.109033
scotch	0.3103	0.3074	0.3111	0.3096
brand	0.6345	0.6558	0.6908	0.660367
meiji	0.071	0.0713	0.0751	0.072467
betagen	0.075	0.07	0.0791	0.0747

Appendix Table B58 Sensory test of probiotic beverage at 0 days

consumer	Free cell 8°C			Free cell 30°C			Encapsulation cell 8°C			Encapsulation cell 30°C		
	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall
1	6	5	6	6	5	6	6	5	6	6	5	6
2	7	6	6	7	6	6	7	6	6	7	6	6
3	5	7	7	5	7	7	4	7	6	4	7	6
4	7	7	7	7	7	7	6	8	7	6	8	7
5	7	5	6	7	5	6	5	4	4	5	4	4
6	7	7	7	7	7	7	7	6	6	7	6	6
7	6	6	6	6	6	6	7	4	5	7	4	5
8	6	5	6	6	5	6	7	5	4	7	5	4
9	7	6	7	6	6	6	7	7	5	6	5	4
10	7	7	7	6	6	6	7	7	4	7	5	4
Ave.	6.5	6.1	6.5	6.3	6	6.3	6.3	5.9	5.3	6.2	5.5	5.2

Appendix Table B59 Sensory test of probiotic beverage at 7 days

consumer	Free cell 8°C			Free cell 30°C			Encapsulation cell 8°C			Encapsulation cell 30°C		
	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall
1	6	5	6	6	3	4	6	5	5	6	3	4
2	6	5	5	6	4	4	7	6	6	4	1	2
3	7	7	7	6	8	7	7	8	8	8	6	8
4	7	7	7	5	2	2	5	6	5	5	3	4
5	7	7	7	7	5	6	6	7	7	6	5	5
6	6	7	7	7	7	7	6	7	7	5	6	6
7	7	6	6	8	6	6	7	4	5	6	3	4
8	7	5	5	7	5	5	8	6	7	8	5	6
9	5	6	6	6	5	5	5	4	4	6	4	5
10	5	5	7	6	5	5	5	5	4	7	4	4
Ave.	6.3	6	6.3	6.4	5	5.1	6.2	5.8	5.8	6.1	4	4.8

Appendix Table B60 Sensory test of probiotic beverage at 14 days

consumer	Free cell 8°C			Free cell 30°C			Encapsulation cell 8°C			Encapsulation cell 30°C		
	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall
1	1	2	2	2	2	2	2	1	2	1	1	2
2	4	5	5	4	6	5	4	4	4	4	3	3
3	5	3	3	3	2	2	3	1	1	2	1	1
4	6	6	6	7	3	6	6	6	6	7	3	4
5	6	7	7	7	6	7	6	6	7	6	4	4
6	5	3	4	4	3	4	4	3	4	3	3	3
7	4	3	3	3	3	4	4	3	3	3	3	3
8	5	4	3	3	3	3	3	3	3	3	3	3
9	4	4	3	4	3	3	4	3	3	2	3	2
10	3	4	4	3	3	3	3	2	3	3	2	2
Ave.	4.3	4.1	4	4	3.4	3.9	3.9	3.2	3.6	3.4	2.6	2.7

Appendix Table B61 Sensory test of probiotic beverage at 21 days

consumer	Free cell 8°C			Free cell 30°C			Encapsulation cell 8°C			Encapsulation cell 30°C		
	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall
1	3	5	3	5	6	6	7	2	6	7	2	5
2	4	3	3	3	3	3	2	1	1	2	1	1
3	5	2	2	4	2	2	3	1	1	3	1	1
4	7	7	7	5	4	3	6	7	6	5	2	4
5	7	6	6	7	4	4	6	3	3	7	4	3
6	3	3	3	2	2	2	3	3	1	2	1	1
7	4	2	4	3	5	3	3	1	1	1	2	1
8	3	3	3	2	3	3	2	3	2	2	2	2
9	3	3	3	3	3	2	2	3	2	3	3	2
10	3	3	3	3	2	2	2	2	1	2	1	1
Ave.	4.2	3.7	3.7	3.7	3.4	3	3.6	2.6	2.4	3.4	1.9	2.1

Appendix Table B62 Sensory test of probiotic beverage at 28 days

consumer	Free cell 8°C			Free cell 30°C			Encapsulation cell 8°C			Encapsulation cell 30°C		
	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall	aroma	taste	overall
1	7	5	5	6	4	3	3	2	2	4	1	1
2	6	5	5	5	5	5	5	4	3	5	2	3
3	4	5	5	5	3	3	3	2	3	3	2	4
4	7	5	3	5	7	5	7	3	3	7	3	3
5	2	3	2	1	1	1	1	1	1	2	1	1
6	3	3	2	3	2	2	2	3	2	2	1	2
7	2	2	3	2	3	2	2	2	2	1	1	1
8	3	3	2	3	3	2	2	3	2	2	1	1
9	2	2	2	3	2	3	2	2	2	2	2	2
10	4	3	3	3	2	3	1	2	1	1	2	1
Ave.	4	3.6	3.2	3.6	3.2	2.9	2.8	2.4	2.1	2.9	1.6	1.9

Appendix Table B63 Sensory test of probiotic beverage added honey flavor

No.	Sex	Age	Education	Income	Allergic	Health Prod User	Overall liking	Color	Odor	Taste	Acceptance Before
1	2	4	5	4	2	1	7	7	6	7	1
2	2	1	2	1	1	1	4	7	2	3	2
3	2	1	2	1	1	1	4	7	8	3	2
4	2	1	2	1	1	1	7	8	3	4	2
5	2	1	2	1	1	1	6	7	6	6	1
6	2	2	4	1	1	1	6	4	4	6	1
7	2	2	4	1	1	1	7	4	7	6	1
8	2	2	5	1	1	1	6	7	6	7	1
9	2	2	4	1	1	1	4	4	4	4	1
10	2	1	4	1	1	1	5	8	5	5	2
11	2	2	5	1	1	1	6	6	6	6	1
12	2	2	5	1	1	1	6	6	7	6	1
13	1	4	5	3	1	1	7	6	7	6	1
14	2	2	5	1	1	1	5	6	6	5	2
15	2	2	4	1	1	1	7	7	7	7	1

Appendix Table B63 (continued)

No.	Sex	Age	Education	Income	Allergic	Health Prod User	Overall liking	Color	Odor	Taste	Acceptance Before
16	2	2	4	1	2	1	7	6	9	7	1
17	1	2	5	1	2	1	6	6	7	6	1
18	2	2	5	1	1	1	7	7	6	6	1
19	2	5	3	1	1	1	7	6	6	7	1
20	1	1	4	1	1	1	9	8	9	8	1
21	1	2	4	2	1	1	7	6	4	7	1
22	1	2	2	1	1	1	4	2	6	2	1
23	1	2	4	1	1	1	4	5	5	4	2
24	1	2	4	1	2	1	6	4	8	6	2
25	1	2	4	2	1	1	7	7	7	8	1
26	2	2	5	1	1	1	7	7	6	6	1
27	2	2	5	2	1	1	6	4	3	4	2
28	2	3	4	1	1	1	7	7	7	8	1
29	2	2	4	1	1	1	2	5	3	2	2
30	2	3	2	1	1	1	7	7	8	7	1

Appendix Table B63 (continued)

No.	Sex	Age	Education	Income	Allergic	Health Prod User	Overall liking	Color	Odor	Taste	Acceptance Before
31	2	4	5	3	2	1	4	4	4	4	2
32	1	1	4	1	1	1	5	4	6	7	1
33	1	2	4	1	1	1	7	7	9	6	1
34	2	2	4	1	1	1	7	7	8	4	2
35	1	1	4	1	1	1	7	6	8	6	1
36	1	1	4	1	1	1	4	4	4	4	2
37	1	4	4	1	1	1	6	7	6	6	1
38	1	4	2	1	1	1	7	7	7	7	1
39	2	2	4	1	1	1	6	7	3	4	1
40	2	2	4	1	1	1	5	6	6	5	2
41	2	2	4	1	1	1	6	7	7	6	1
42	2	1	2	1	1	1	4	6	4	4	1
43	2	2	2	1	1	2	5	6	4	5	2
44	2	1	4	1	1	1	6	2	2	4	1
45	2	1	4	1	1	2	7	6	6	6	1

Appendix Table B63 (continued)

No.	Sex	Age	Education	Income	Allergic	Health Prod User	Overall liking	Color	Odor	Taste	Acceptance Before
46	1	2	4	1	1	1	6	7	6	7	1
47	1	2	4	1	1	1	6	7	4	5	1
48	1	2	4	1	1	2	5	6	4	4	1
49	1	4	2	1	1	1	6	7	6	7	1
50	2	2	4	1	1	1	4	4	3	4	2

Appendix Table B63 (continued)

No.	Cost Before	Buy Before	Acceptance After	Cost After	Buy After	Comment
1	20	1	1	20	1	มีกลิ่นหอม มีประโยชน์ ไม่ชอบรสเปรี้ยว
2	30	2	1	30	2	ไม่ชอบกลิ่นฉุนไป รสชาติขม
3	27	2	1	27	2	ไม่ชอบกลิ่นฉุนไป รสชาติขม
4	30	2	2	30	2	ไม่ชอบกลิ่นและรสชาติ
5	30	2	1	30	2	มีประโยชน์ต่อสุขภาพแต่เป็นสินค้าฟุ่มเฟือย
6	30	1	1	30	1	มีประโยชน์กับระบบขับถ่าย
7	30	1	1	40	1	มีประโยชน์ต่อร่างกาย
8	30	1	1	35	1	มีประโยชน์กับระบบขับถ่าย
9	39	2	1	39	1	มีประโยชน์ต่อร่างกายแต่ไม่ชอบรับประทาน
10	25	2	1	25	1	ไม่ชอบกลิ่นและรสชาติ
11	35	1	1	40	1	แปลก น่าสนใจ อร่อย
12	35	1	1	35	1	ดื่มง่าย น่าสนใจ
13	20	1	1	25	1	อยากทดลองของใหม่
14	40	2	1	40	2	

Appendix Table B63 (continued)

No.	Cost Before	Buy Before	Acceptance After	Cost After	Buy After	Comment
15	30	1	1	30	1	มีกลิ่นหอม ทานง่าย มีประโยชน์
16	35	1	1	40	1	มีกลิ่นหอม ทานง่าย มีประโยชน์
17	35	1	1	35	1	มีกลิ่นหอม ทานง่าย มีประโยชน์
18	25	2	1	25	1	ไม่ชอบผลิตภัณฑ์กลุ่มนี้ แต่คุ้มค่าก็ทาน
19	50	1	1	50	1	ไม่คาวเหมือนซุปไก่
20	60	1	1	60	1	กลิ่นรสไม่แรงจนเกินไป
21	45	2	1	30	2	กลิ่นและรสชาติยังไม่ถูกใจ ประโยชน์ไม่ชัดเจน
22	30	2	1	30	2	ไม่ชอบกลิ่นและรสชาติ อยากให้เพิ่มหวาน สีไม่สวย
23	25	1	1	25	1	รสชาติแปลก ดีต่อสุขภาพ
24	40	2	1	40	1	ดูมีประโยชน์กว่าซุปไก่
25	20	1	1	25	1	ดื่มง่าย มีประโยชน์
26	35	1	1	40	1	ดูมีประโยชน์
27	30	2	1	20	2	นมเปรี้ยวดูมีประโยชน์ หาง่าย อร่อยกว่า
28	30	1	1	30	1	

Appendix Table B63 (continued)

No.	Cost Before	Buy Before	Acceptance After	Cost After	Buy After	Comment
29	20	2	1	25	2	ไม่อร่อย
30	50	1	1	50	1	ชอบลองสินค้าใหม่
31	25	2	1	30	2	ไม่ชอบกลิ่นและรสชาติ
32	23	1	1	45	1	ผลิตภัณฑ์ใหม่น่าสนใจ
33	45	1	1	45	1	หอม รสชาติอร่อย
34	30	2	1	30	2	ไม่ชอบรับประทานสินค้ากลุ่มนี้
35	28	1	1	28	1	ผลิตภัณฑ์ใหม่ กลิ่นหอมรสชาติเฉพาะตัว
36	35	2	1	45	2	รสชาติเหมือนของหวานแต่กินแล้วรู้สึกขม
37	30	1	1	33	1	อยากให้ปรับปรุงด้านรสชาติ
38	30	1	1	30	1	อยากทดลองของใหม่
39	40	1	1	40	1	พอทานได้มีประโยชน์
40	50	2	2	50	2	ไม่ค่อยชอบผลิตภัณฑ์กลุ่มนี้
41	35	1	1	35	1	ดื่มง่าย มีประโยชน์

Appendix Table B63 (continued)

No.	Cost Before	Buy Before	Acceptance After	Cost After	Buy After	Comment
42	30	1	1	35	1	มีประโยชน์
43	50	1	2	50	1	กลิ่นแรงไป
44	69	2	1	89	1	รสชาติขมและเปรี้ยว
45	50	1	1	50	1	รสชาติพอรับได้ กลิ่นไม่แรงจนเกินไป
46	29	1	1	29	1	หอม ดูมีประโยชน์
47	20	1	1	30	1	กลิ่นดีกว่าซุปไก่
48	20	1	1	20	2	ไม่ค่อยรับประทาน
49	40	1	1	40	1	หอม หวาน รสชาติเหมาะสม
50	30	1	1	35	1	สี กลิ่น รส ควรปรับปรุง

Appendix C

Questionnaire

แบบทดสอบ กลิ่น รสชาติและความชอบโดยรวม เพื่อคัดเลือกเชื้อจุลินทรีย์ในการนำไปใช้ผลิตเครื่องดื่มโปรไบโอติก

แบบทดสอบนี้มีวัตถุประสงค์เพื่อคัดเลือกเชื้อจุลินทรีย์ที่มีกลิ่น รสชาติ และความชอบโดยรวม ที่ผู้บริโภคชอบมากที่สุดในการนำไปใช้ผลิตเครื่องดื่มโปรไบโอติกส์ ซึ่งเป็นผลิตภัณฑ์ทดลอง ผ่านการตรวจคุณภาพว่าไม่เป็นพิษต่อผู้บริโภค และเป็นส่วนหนึ่งในการทำวิจัยของนิสิตปริญญาโท ภาควิชาเทคโนโลยีชีวภาพ คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์

ส่วนที่ 1 ข้อมูลทั่วไปเกี่ยวกับผู้ตอบแบบสอบถาม

ชื่อ _____ อายุ _____ปี เพศ ( ) ชาย ( ) หญิง

ส่วนที่ 2 แบบทดสอบความชอบของกลิ่น รสชาติและความชอบโดยรวมในเครื่องดื่ม

คำชี้แจง 1) เรียงลำดับความชอบของกลิ่น รสชาติ และความชอบโดยรวมในเครื่องดื่ม โดยกรอกรหัสตัวอย่างที่ชอบมากที่สุดในช่องหมายเลข 1 และกรอกตัวอย่างที่ชอบน้อยที่สุดในช่องหมายเลข5

เรียงลำดับความชอบ **กลิ่น** จากมากที่สุดไปถึงชอบน้อยที่สุด

1.....2.....3.....4.....5.....

เรียงลำดับความชอบ **รสชาติ** จากมากที่สุดไปถึงชอบน้อยที่สุด

1.....2.....3.....4.....5.....

เรียงลำดับความชอบ **โดยรวม** จากมากที่สุดไปถึงชอบน้อยที่สุด

1.....2.....3.....4.....5.....

ข้อเสนอแนะ

.....

.....

.....

แบบทดสอบความพึงพอใจของเครื่องดื่มโปรไบโอติกส์

แบบทดสอบนี้มีวัตถุประสงค์เพื่อศึกษาความพึงพอใจของผู้บริโภคต่อเครื่องดื่มโปรไบโอติกส์ซึ่งเป็นผลิตภัณฑ์ทดลอง ผ่านการตรวจคุณภาพว่าไม่เป็นพิษต่อผู้บริโภค และเป็นส่วนหนึ่งในการทำวิจัยของนิสิตปริญญาโท ภาควิชาเทคโนโลยีชีวภาพ คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์

ส่วนที่ 1 ข้อมูลทั่วไปเกี่ยวกับผู้ตอบแบบสอบถาม

1. ชื่อ _____ อายุ _____ปี เพศ ( ) ชาย ( ) หญิง

ส่วนที่ 2 ความพึงพอใจของผู้บริโภค

คำชี้แจง 1) กรุณาประเมินคุณสมบัติของเครื่องดื่มโปรไบโอติกส์ทีละตัวอย่างตามลำดับการจัดวางที่กำหนดไว้
2) หากท่านต้องการประเมินตัวอย่างใดซ้ำอีกครั้ง สามารถทำได้โดยปรับการรับรสด้วยน้ำเปล่าที่เตรียมไว้
3) ใส่หมายเลขคะแนนความพึงพอใจลงในช่องว่างตามความคิดเห็นของท่านที่มีต่อเครื่องดื่มโปรไบโอติกส์ โดยมีการจัดระดับดังนี้

Aroma, teste และ overall

ระดับคะแนน 9 หมายถึง ชอบมากที่สุด
ระดับคะแนน 8 หมายถึง ชอบมาก
ระดับคะแนน 7 หมายถึง ชอบปานกลาง
ระดับคะแนน 6 หมายถึง ชอบเล็กน้อย
ระดับคะแนน 5 หมายถึง เฉยๆ
ระดับคะแนน 4 หมายถึง ไม่ชอบเล็กน้อย
ระดับคะแนน 3 หมายถึง ไม่ชอบปานกลาง
ระดับคะแนน 2 หมายถึง ไม่ชอบมาก
ระดับคะแนน 1 หมายถึง ไม่ชอบมากที่สุด

ตัวอย่างเครื่องดื่มโปรไบโอติกส์	----------	----------	----------	----------
Aroma				
teste				
Overall				

คำแนะนำสำหรับการปรับปรุงคุณภาพในแต่ละตัวอย่าง

ตัวอย่าง _____ _____
ตัวอย่าง _____ _____
ตัวอย่าง _____ _____
ตัวอย่าง _____ _____

แบบสอบถามสำหรับคัดเลือกผู้ทดสอบ

1. เพศ ☐ ชาย ☐ หญิง

2. อายุ ☐ น้อยกว่า 15 ปี ☐ 15 - 30 ปี

☐ 30 - 50 ปี ☐ มากกว่า 50 ปี

3. รายได้

☐ น้อยกว่า 10,000 บาท ☐ มากกว่า 10,000 บาท

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1. จำนวนผู้ทดสอบทั้งหมดที่ต้องการคือ 50 คน

2. เพศ ต้องการเพศหญิงและเพศชาย

3. อายุ 15 ปีขึ้นไป

4. รายได้ 10,000 บาทขึ้นไป

ข้อมูลที่บอกแก่ผู้ทดสอบ

1. ตัวอย่างที่ทดสอบคือ เครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่ จำนวน 1 ตัวอย่าง

2. ใช้เวลาทดสอบประมาณ 10-15 นาที

3. ขณะทดสอบให้ปฏิบัติตามคำแนะนำในแบบทดสอบ ขอความร่วมมือไม่พูดคุย หรือใช้อุปกรณ์สื่อสารใดๆ ขณะทำการทดสอบ

4. เมื่อทดสอบเสร็จ จะได้รับค่าตอบแทนจำนวน 50 บาท

แบบทดสอบความชอบของผู้บริโภคต่อผลิตภัณฑ์เครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่

วันที่ทดสอบ..... ชื่อ-นามสกุล ผู้ทดสอบ.....

คำชี้แจง: แบบทดสอบชุดนี้มีวัตถุประสงค์เพื่อทดสอบความชอบและการยอมรับของผู้บริโภคที่มีต่อผลิตภัณฑ์ Lifeberry (เครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่ผสมน้ำผึ้ง) **โดยข้อมูลทั้งหมดของท่านจะเป็นประโยชน์อย่างยิ่งสำหรับงานวิจัยและไม่มีผลกระทบใดๆ ต่อท่านทั้งสิ้น** ขอขอบพระคุณทุกท่านที่สละเวลามาเข้าร่วมการทดสอบครั้งนี้

ส่วนที่ 1: ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

คำชี้แจง: กรุณาทำเครื่องหมาย **กากบาท** (X) ลงใน ▪ หน้าคำตอบที่ตรงกับข้อมูลของท่านมากที่สุด

1. เพศ

▪ ชาย ▪ หญิง

2. อายุ

▪ 15-20 ปี ▪ 21-30 ปี ▪ 31-40 ปี

▪ 41-50 ปี ▪ มากกว่า 50 ปี

3. ระดับการศึกษาสูงสุด

▪ ต่ำกว่ามัธยมศึกษา ▪ มัธยมศึกษา ▪ อนุปริญญา

▪ ปริญญาตรี ▪ สูงกว่าปริญญาตรี

4. รายได้โดยเฉลี่ยต่อเดือน

▪ 10,001-20,000 บาท ▪ 20,001-30,000 บาท

▪ 30,001-40,000 บาท ▪ มากกว่า 40,000 บาท

5. ท่านมีประวัติการแพ้อาหารหรือไม่

▪ ไม่แพ้ ▪ แพ้ โปรดระบุ.....

6. ท่านเคยบริโภคผลิตภัณฑ์เพื่อสุขภาพ เช่น ซุปไก่สกัด น้ำพรุนสกัดเข้มข้น หรือ ผลิตภัณฑ์ในกลุ่มเดียวกันนี้หรือไม่

▪ เคย ▪ ไม่เคย

ส่วนที่ 2: การทดสอบความชอบต่อตัวอย่างผลิตภัณฑ์เครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่

คำแนะนำ: ท่านจะได้รับตัวอย่างเครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่ จำนวน 1 ตัวอย่าง กรุณาทดสอบตัวอย่างตามคำแนะนำด้านล่าง **แล้วกากบาท (X) ข้อความในช่องสี่เหลี่ยมด้านล่างที่ตรงกับความรู้สึกของท่าน** ที่มีต่อคุณลักษณะต่างๆ ของตัวอย่าง

รหัสตัวอย่าง.....

กรุณา**ชิมตัวอย่างอย่างน้อย 1ใน 3 ของปริมาณที่ได้รับ**

1. **โดยรวม**แล้วท่านชอบตัวอย่างนี้มากน้อยเพียงใด

ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	บอกไม่ได้ว่าชอบหรือไม่ชอบ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

กรุณา**ดูตัวอย่าง**แล้วประเมินความชอบด้านสีของตัวอย่าง

2. ท่านชอบ**สี**ของตัวอย่างนี้มากน้อยเพียงใด

ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	บอกไม่ได้ว่าชอบหรือไม่ชอบ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

กรุณา**ดมตัวอย่าง** แล้วประเมินความชอบด้านกลิ่นของตัวอย่าง

3. ท่านชอบ**กลิ่นโดยรวม**ของตัวอย่างนี้มากน้อยเพียงใด

ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	บอกไม่ได้ว่าชอบหรือไม่ชอบ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

กรุณา**ชิมตัวอย่างอย่างน้อย 1ใน 3 ของปริมาณที่ได้รับ** อีกครั้งก่อนตอบคำถามต่อไป

4. ท่านชอบ**รสชาติโดยรวม**ของตัวอย่างนี้มากน้อยเพียงใด

ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	บอกไม่ได้ว่าชอบหรือไม่ชอบ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

5. ท่านยอมรับตัวอย่าง**เครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่**นี้หรือไม่

☐ ยอมรับ เพราะ.....

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☐ ไม่ยอมรับ เพราะ.....

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6. ท่านคิดว่าราคาต่อหน่วย (40 มล. หรือปริมาณใกล้เคียงกับผลิตภัณฑ์กลุ่มซุปไก่สกัด/น้ำลูกพรุนสกัดเข้มข้น) ที่เหมาะสม

ของผลิตภัณฑ์นี้คือ ..... บาท

7. หากมีผลิตภัณฑ์นี้ จำหน่ายในท้องตลาด ในราคาที่ท่านระบุในข้อ 6 ท่านจะซื้อหรือไม่

☐ ซื้อ เพราะ.....

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☐ ไม่ซื้อ เพราะ.....

.....

ผลิตภัณฑ์เครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่ ที่ท่านได้ทดสอบไปนั้นเป็นเครื่องดื่มมอลท์สกัดจากข้าวมอลท์ไรซ์เบอร์รี่ที่ **เติมจุลินทรีย์โปรไบโอติกส์และพรีไบโอติกส์**เพื่อส่งเสริมการทำงานของจุลินทรีย์เมื่อผ่านระบบทางเดินอาหารไปสู่ลำไส้ อุดมไปด้วยกรดอะมิโนที่เป็นประโยชน์ วิตามิน และน้ำ ตาลที่ให้พลังงาน **จุลินทรีย์โปรไบโอติกส์**เป็นจุลินทรีย์ที่เป็นประโยชน์ต่อระบบขับถ่าย และสร้างสมดุลของร่างกาย **มีพรีไบโอติกส์** ที่จุลินทรีย์สามารถใช้เพื่อการอยู่รอดในระบบลำไส้ใหญ่

8. ท่านยอมรับตัวอย่าง**เครื่องดื่มโปรไบโอติกส์จากมอลท์ไรซ์เบอร์รี่** นี้หรือไม่

☐ ยอมรับ เพราะ.....

.....

☐ ไม่ยอมรับ เพราะ.....

9. ท่านคิดว่าราคาต่อหน่วย (40 มล. หรือปริมาณใกล้เคียงกับผลิตภัณฑ์กลุ่มซุปไก่สกัด/น้ำลูกพรุนสกัดเข้มข้น) ที่เหมาะสมของผลิตภัณฑ์นี้คือ ..... บาท

10. หากมีผลิตภัณฑ์นี้ จำหน่ายในท้องตลาด ในราคาที่ท่านระบุในข้อ 9 ท่านจะซื้อหรือไม่

☐ ซื้อ เพราะ.....

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☐ ไม่ซื้อ เพราะ.....

.....

CURRICULUM VITAE

NAME : Miss Atchareeya Nakkarat

BIRTH DAY : June 19, 1987

BIRTH PLACE : Lampang, Thailand

EDUCATION :

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CONFERENCE : Poster presentation at the 5th Science Research Conference 2013 Phayao University, Phayao, Thailand