FULL RESEARCH REPORT

PROJECT TITLED

HEALTH-PROMOTING EFFECTS OF MAO JUICE POWDER CONTAINING PROBIOTICS ON THE COLON MICROBIOTA RESIDING IN THE *IN VITRO* AND *IN VIVO* GASTROINTESTINAL ENVIRONMENTS

DR.PITTAYA CHAIKHAM

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Abstract

Project Code : TRG5880002

Project Title : Health-promoting effects of mao juice powder containing probiotics on the colon microbiota residing in the *in vitro* and *in vivo* gastrointestinal environments

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The objectives of this research were; 1) to determine the physicochemical and microbiological qualities of fresh, thermally, ultra-sonicated processed mao juices, 2) to investigate the effect of spray-drying and storage on probiotic survival of probiotic-mao juice powders, 3) to study the survival of immobilized probiotic cells under simulated stomach and small intestinal environments, 4) to study the influence of spray-dried probiotic-mao juice powder on colon microflora and their metabolic activity under *in vitro* colon section, and 5) to study the effect of spray-dried probiotic-mao juice powder intake on colon microbiota of adult rats.

In this study, it was found that no significant changes in total soluble solids, pH, and viscosity of thermally and ultra-sonic treated mao juices could be observed. Ultra-sonication had a noticeable effect on color parameters, but sensorial characteristics of treated juice were no different from fresh juice. Although pasteurization is normally applied to extend shelf-life of fruit juices, this method damages the desired characteristics and antioxidant constituents of fruit juice products. Ultra-sonication could be an alternative mao processing method to obtain a juice with high retention of bioactive compounds and antioxidant capacities, and low residual polyphenol oxidase and peroxidase activities as well as microbial counts.

The probiotic-supplemented mao juice powder in vacuum-sealed package stored at 4ºC could maintain high levels of *Lactobacillus casei* 01 viability and bioactive compounds than those in the other packaging conditions throughout the storage period. Some properties of the powders *viz.* moisture content, water activity (a_w), bulk density and solubility were found to change significantly. Refrigerated storage allowed the powder, especially that in the vacuum sealing package, to have the optimal parameters.

Thus, this indicates that stability of probiotic, powder properties and bioactive compounds in spray dried probiotic-supplemented mao juice could be preserved for a long time when using the appropriate packages and storage conditions.

Accordingly, the probiotic lactobacilli including *L. casei* 01 and *Lactobacillus acidophilus* LA5 in mao juice powder encapsulated with maltodextrin plus *T. triandra* gum and maltodextrin plus inulin similarly showed protective ability on probiotic bacteria against adverse condition of simulated stomach and small intestinal fluids. Spray dried probiotic along with mao juice containing *T. triandra* gum also modulated the microbiome in the colon model, by increasing lactic acid, short-chain fatty acids, lactobacilli and bifidobaccteria. Significant decreases of toxic ammonia, clostridia, fecal coliforms and total anaerobes were also observed. Therefore, this concluded that *T. triandra* gum can be used as an effective co-encapsulating material for spray drying probiotic. The benefit impacts of this probiotic product for improving of health status were confirmed by the *in vivo* models.

Keywords : Mao juice power, Probiotic bacteria, Spray drying, Physicochemical qualities, Gut microbiota

บทคัดย่อ

รหัสโครงการ : TRG5880002

ชื่อโครงการ : ผลดีต่อการส่งเสริมสุขภาพของเม่าผงผสมโปรไบโอติกต่อกิจกรรมของจุลินทรีย์ในลำไส้ใหญ่โดยทดสอบในสิ่งแวดล้อมของทางเดินอาหารจำลองและสิ่งมีชีวิต

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จุดประสงค์ของงานวิจัยนี้ คือ 1) เพื่อวิเคราะห์คุณภาพด้านเคมีกายภาพ และด้านจุลชีววิทยาของน้ำเม่าสด น้ำเม่าที่ผ่านการแปรรูปด้วยความร้อน และผ่านการแปรรูปด้วยคลื่นเสียงความถี่สูง, 2) เพื่อศึกษาผลของการทำแห้งแบบพ่นฝอย และการเก็บรักษาต่อการรอดชีวิตของโปรไบโอติกในน้ำเม่าผงผสมโปรไบโอติก, 3) เพื่อศึกษาการรอดชีวิตของโปรไบโอติกในน้ำเม่าผงภายใต้สภาวะของกระเพาะ และลำไส้เล็กจำลอง, 4) เพื่อศึกษาผลของน้ำเม่าผงผสมโปรไบโอติกต่อกิจกรรมของจุลินทรีย์ในลำไส้ใหญ่ภายใต้สภาวะจำลอง และ 5) เพื่อศึกษาผลของน้ำเม่าผงผสมโปรไบโอติกกิจกรรมของจุลินทรีย์ในลำไส้ใหญ่ในหนูทดลอง

ในการศึกษานี้ พบว่า ไม่มีการเปลี่ยนแปลงของปริมาณของแข็งที่ละลายในน้ำได้ทั้งหมด ค่าความเป็นกรด-ด่าง และค่าความหนืดของตัวอย่างน้ำเม่าที่ผ่านการแปรรูปด้วยความร้อน และการแปรรูปด้วยคลื่นเสียงความถี่สูง การแปรรูปด้วยคลื่นเสียงความถี่สูงมีผลต่อค่าสีของตัวอย่าง แต่ไม่มีผลต่อการยอมรับของผู้บริโภคเมื่อเปรียบเทียบกับน้ำเม่าสด ถึงแม้ว่าการแปรรูปด้วยการพาสเจอไรซ์จะเป็นวิธีการที่สามารถยืดอายุการเก็บรักษาผลิตภัณฑ์ได้ แต่วิธีการนี้จะไปทำลายคุณลักษณะที่พึงปรารถนา และปริมาณสารแอนติออกซิแด๊นท์ของผลิตภัณฑ์น้ำผลไม้ การแปรรูปด้วยคลื่นเสียงความถี่สูงจึงเป็นวิธีที่มีศักยภาพในการแปรรูปน้ำเม่า เนื่องจากมีปริมาณคงเหลือของสารสำคัญต่างๆ และประสิทธิภาพในการต้านอนุมูลอิสระอยู่สูง และพบว่ากิจกรรมคงเหลือของเอนไซม์พอลิฟีนอลออกซิเดส และเปอร์ออกซิเดส รวมทั้งจำนวนจุลินทรีย์ที่พบมีค่าค่อนข้างต่ำ

น้ำเม่าผงผสมโปรไบโอติกถูกบรรจุในบรรจุภัณฑ์ภายใต้สภาวะสุญญากาศ และเก็บรักษาที่อุณหภูมิ 4 องศาเซลเซียส สามารถคงรักษาระดับของการรอดชีวิตของ *Lactobacillus casei* 01 และสารสำคัญต่างๆ ได้ดีกว่าการเก็บรักษาที่สภาวะอื่นๆ จากการวิเคราะห์คุณสมบัติบางอย่างของตัวอย่าง ได้แก่ ปริมาณความชื้น ค่ากิจกรรมของน้ำ (a_w) ค่า bulk density และอัตราการลำลาย พบว่า มีการเปลี่ยนแปลงอย่างมีนัยสำคัญทางสถิติในระหว่างการเก็บรักษาการแช่เย็น และบรรจุแบบสุญญากาศจึงเป็นวิธีที่เหมาะสมในการเก็บตัวอย่าง ดังนั้นจึงสรุปได้

ว่าการคงรักษาโปรไบโอติก คุณสมบัติด้านต่างๆ ของผง และสารสำคัญต่างๆ ในน้ำเม่าผงผสมโปรไบโอติก สามารถป้องกันได้โดยการบรรจุในบรรจุภัณฑ์ที่เหมาะสม และสภาวะในการเก็บรักษา

การทดลองลำดับต่อไป คือ เมื่อนำน้ำเม่าผงผสมกับโปรไบโอติก *L. casei* 01 และ *Lactobacillus acidophilus* LA5 มอลโตเดกซ์ตริน และกัมจากใบย่านาง หรืออินูลิน พบว่าส่วนผสมดังกล่าวช่วยป้องกันโปรไบโอติกจากสภาวะที่เป็นอันตรายกับเซลล์จากสภาวะจำลองของกระเพาะและลำไส้เล็ก น้ำเม่าผงผสมโปรไบโอติก และกัมจากใบย่านาง มีผลต่อการเพิ่มขึ้นของกรดแลคติก กรดไขมันสายสั้น แลคโตบาซิลไล และไบฟิโดแบคทีเรีย และพบว่ามีการลดลงของแอมโมเนีย คลอสตริเดีย ฟีคอลคอลิฟอร์ม และจุลินทรีย์ที่ไม่ใช้อากาศทั้งหมด ดังนั้นจึงสรุปได้ว่ากัมจากใบย่านางสามารถใช้เป็นสารเคลือบสำหรับการทำแห้งโปรไบโอติกแบบพ่นฝอยได้ ซึ่งประโยชน์ต่อสุขภาพของผลิตภัณฑ์นี้ยืนยันได้จากผลการทดลองในสัตว์ทดลอง

คำหลัก : น้ำเม่าผง แบคทีเรียโปรไบโอติก การทำแห้งแบบพ่นฝอย คุณภาพทางเคมีกายภาพ กิจกรรมของจุลินทรีย์ในลำไส้ใหญ่

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International publications

1. **Chaikham, P.**, Prangthip, P. and Seesuriyachan, P. 2016. Ultra-sonication effects on quality attributes of maoberry (*Antidesma bunius* L.) juice. Food Science and Technology Research, 22(5), 647-654. (ISI impact factor = 0.357)

2. **Chaikham, P.**, Kemsawasd, V. and Seesuriyachan, P. 2017. Spray drying probiotics along with maoluang juice plus *Tiliacora triandra* gum for exposure to the *in vitro* gastrointestinal environments. LWT-Food Science and Technology, 78, 31–40. (ISI impact factor = 2.711)

Note: Chaikham, P is Pittaya Chaikham.

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CHAPTER 1
INTRODUCTION

1.1 Rationale

Mao (*Antidesma thwaitesianum* Müell. Arg.) juice, commercially available for human health benefits, have become more popular in Thailand. Its fruit is classified in the family *Stilaginaceae*, genus *Antidesma* and commonly grown in the warm climate of tropical Asia, Africa and Australia. Mao fruits contain high amounts of several bioactive components such as ascorbic acid, phenols, tannin, anthocyanins and flavonoids, and have been recognized to possess a numeral of health-promoting effects including antioxidant, anti-carcinogenic, anti-apoptotic and anti-inflammatory activities. These beneficial physiological effects also have preventive applications in a variety of pathologies. Mao seeds and skin-pulp residues also contained high amounts of phenolic acids and high antioxidant activity, the methanolic extracts showed inhibitory activities against some pathogenic and spoilage bacteria. Thus, to add value to the juice by fortification with probiotics is a challenging implementation for this research area.

Probiotics are live microbial food supplements, which benefit health of consumers by maintaining, or improving their intestinal microbial balance. Most probiotics are associated with dairy products. Thus application of these cultures in non-dairy products, such as baby foods, beverages and confectionery, represents a great challenge. Since, it is important that the formulation maintains the activity and viability of the probiotic for extended periods of time. Factors like water activity, oxygen tension and temperature become important when dealing with these kinds of products. There are various techniques to preserve these microbes, encapsulation via spray drying is one of the feasible method used in dairy industry. For fruit juice including Mao juice needs specific environment to maintain their survivability during encapsulating these live microorganisms through spray drying. At presence, only little research has been done in this respect, therefore, this research is considered to be a novel technology and worth investigation. Moreover, there is also an evaluation of microbial survival and their metabolic products in the *in vitro* and *in vivo* system which commences from stomach, small intestine and colon. The number of survival probiotics and other colon bacterial communities will be assessed by the standard plating counts.

1.2 Research objectives

1.2.1 To determine the physicochemical and microbiological qualities of fresh and processed mao juices.

1.2.2 To investigate the effect of spray-drying on probiotic survival of probiotic-mao juice powders.

1.2.3 To study the effect of storage conditions on qualities of probiotic-mao juice powders.

1.2.4 To study the survival of immobilized probiotic cells under simulated stomach and small intestinal environments.

1.2.5 To study the influence of spray-dried probiotic-mao juice powder on colon microflora and their metabolic activity under *in vitro* colon section.

1.2.6 To study the effect of spray-dried probiotic-mao juice powder intake on colon microbiota of adult rats.

CHAPTER 2

LITERATURE REVIEW

5.1 Mao and its health benefits

Mao (*Antidesma bunius* L. Spreng) is classified in the family of *Euphorbiaceae* and the genus *Antidesma*. Mao is a tropical evergreen fruit tree with more branches and up to 25 m tall, annually flowering in April to June and fruit ripe in August to September. Its fruits can be cultivated successfully in many regions particularly in Thailand. The fruit is an economically important crop. They display an attractive bright red color before full ripeness and are red-black when fully ripe (Figure 2.1). They have a special sweet-sour taste and a distinctive flavor, and are popular with local people. Mao fruits are rich in nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals, vitamins, anthocyanins, flavonoids and phenolic acids (Butkhup and Samappito, 2008).

Figure 2.1 Mao fruits

In Thailand, mao fruits have been used as a raw material for making tasty jelly jam, drinking juices, juice concentrate and even wine. Samappito and Butkhup (2008) reported that many villagers in Northeast Thailand used Mao juice to heal their health problems on diabetes, dysentery, indigestion and constipation. In Cambodia, where the plant is called "*Choimoi*", a decoction of the bark is used as a drink to treat diarrhea, to promote menses, to recover from childbirth, and to invigorate. The leaves are used externally to assuage headaches in children, In Malaysia, a paste of the leaves is

applied externally to relieve headache, skin diseases and abdominal swelling. The leaves are also used to make a water bath to reduce fever. In the Philippines, the leaves are used to promote the haling of wounds.

The pharmacological property of this plant unexplored. Dechsupa et al. (2006) studied on apoptosis mechanism on breast cancer cell (MDA-MA-435) which transferred at the neck of nude mice whom were cut Thymus gland of the polyphenol isolated from the red wine Siammour extract (SRPE) and the wood Mao (MWE) 1:1 ratio. They found that polyphenol from both extracts inhibited growth of tumor cells of nude mices significantly. The results were also displayed that their extracts exhibited in vitro cytotoxic assay against breast cancer cells (MDA-MA-435), erythrocyte cancer cells (K562, K562/adr) and small lung cell carcinoma (GLC4). Haripyaree et al. (2010) report that methanolic extract of *A. bunius* fruit which is grown in Manipur, India, showed higher antioxidant activity with an average IC50 value of 100.08 μg/ml, when compared to other fruits. In Indonesia, the use of *A. bunius* as a medicinal plant has been practiced long before recorded history. The study by Eun-Mi and Jae-Kwan (2005), investigated several medicinal plant extracts traditionally and commonly used in Indonesia. The study focused on their inhibitory action against NO release from macrophages and antioxidant activity. These scientists found that leaf crude extract of *A. bunius* showed high inhibitory effect on NO release in RAW264.7 cells and high reducing power. In this study the leaf crude extracts had a high concentration of total phenolic compounds. Moreover, Butkhup and Samappito (2011) also found that Mao seeds and skin–pulp residue possess considerable amounts of phenolic compounds and significant radical-scavenging activity on stable 2, 2-diphenyl-1-picrylhydrazyl radicals and methanol extracts exhibited inhibitory activities against some of the microorganisms. In addition, mao seeds and skin-pulp residue could afford health benefits by preventing unwanted free radical-induced oxidative reactions. In view of the fact that most of the bioactive components including anthocyanins and phenolic compounds remained in the seeds and skin-pulp residues, our interest is focused on the Mao seeds and skin-pulp residue which obtained from by-product of juice as a potential source of bioactive components, which can be useful as beneficial food constituents, food flavors, antioxidants, pharmaceutical and cosmetic industry.

2.2 Ultrasonication and food technology

From the past many years, food industry demand for minimal processed food leads to significant alterations in the processing methods as some processing techniques applied under critical conditions lower their nutrient level and bioavailability by inducing physical and chemical changes, thereby reducing organoleptic acceptability. Thus, in lieu of such techniques, newer mild processing methods in food industry have been devised in order to retain nutrient, non-nutrient (bioactive) and sensory characteristics (Czechowska-Biskup et al., 2005). Ultrasonic method is one among those rapidly emerging techniques that were devised to minimize processing, enhance quality, and safeguard the safety of food products (Knorr et al., 2011). Ultrasound technology as a key area of research and development in the food industry (Ercan and Soysal, 2011) is based on mechanical waves at a frequency above the threshold of human hearing (> 16 kHz), and can be categorized into two frequency ranges: low and high energy. Low-energy (low power, low intensity) ultrasound has frequencies higher than 100 kHz at intensities below 1 W/cm^2 while high-energy (high power, high-intensity) ultrasound uses intensities higher than 1 W/cm^2 at frequencies between 20 and 500 kHz (Mason et al., 2011). The representative range for the frequency that is commonly applied in ultrasonic technology lies between 20 kHz and 500 MHz (Yusaf and Al-Juboori, 2014). High-frequency ultrasound as an analytical technique is used to obtain information on the physicochemical properties of food such as acidity, firmness, sugar content, ripeness, etc. While as, low-frequency ultrasound is used to change physical and chemical properties of food (Soria and Villamiel, 2010) by inducing pressure, shear, and temperature difference in the medium through which they propagate (Dolatowski et al., 2007) and is capable of producing cavitations in order to inactivate microorganisms in foods (Piyasena et al., 2003). The typical limit for the frequency that is usually used in ultrasound applications ranges between 20 kHz and 500 MHz (Yusaf and Al-Juboori, 2014). Ultrasonication finds its application in quality control of fresh vegetables and fruits in both pre-harvest and post-harvest, cheese processing, commercial cooking oils, bread and cereal products, bulk and emulsified fat-based food products, food gels, aerated, and frozen foods. Other applications include the detection of honey adulteration and assessment of the aggregation state, size, and type of protein. Low power ultrasound (LPU): The frequency range of LPU along with spectroscopy and nuclear magnetic resonance (NMR) are currently the most popular, practical, and widely used nondestructive analytical methods. For many years, LPU has

been successfully utilized for studying the physicochemical and structural properties of fluid foods (McClements, 1997).

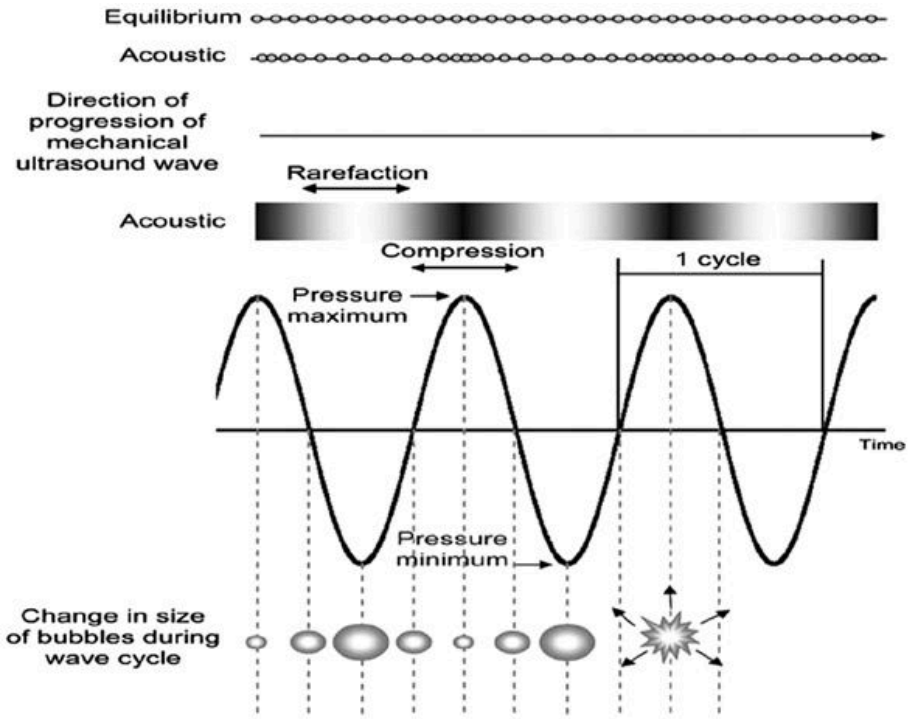

Figure 2.2 Cavitation caused by ultrasonication (Soria and Villamiel, 2010)

2.2.1 Mechanism of action

Application of ultrasound to liquid systems causes acoustic cavitation which is the phenomenon of generation, growing and eventual collapse of the bubbles (Figure 2.2). As ultrasound waves propagate, the bubbles oscillate and collapse which causes the thermal, mechanical, and chemical effects. Mechanical effects include collapse pressure, turbulences, and shear stresses (Yusaf and Al-Juboori, 2014), while the chemical effects include generation of free radicals (Lateef et al., 2007). The effects in the cavitation zone generate extremely high temperatures (5,000 K) and pressures (1,000 atm) (Soria and Villamiel, 2010). Depending on the frequency of the ultrasound, locally produced alternating positive and negative pressures cause expansion or compression of the material, resulting in cell rupture. Ultrasound causes hydrolysis of water inside the oscillating bubbles leading to formation of H^+ and OH^- free radicals that can be captured in some chemical reactions e.g. free radicals can be scavenged by amino acids of the enzymes involved in structure stability, substrate binding, or catalytic functions. This disruption effect of sonication is significantly resisted by

homogenous liquids (Ercan and Soysal, 2011). During sonication treatment, bubbles produced are divided into two types on the basis of their structure:

1) Non-linear, forming large bubble clouds with equilibrium size during pressure cycles are known as stable cavitations bubbles.

2) Non-stable, rapidly collapsing and disintegrating into smaller bubbles are known as internal (transient) cavitations bubbles. These small bubbles quickly dissolve, but during bubble stretching, the mass-transfer boundary layer is thinner and the interfacial area is greater than during bubble collapse which implies that more air transfers into the bubble during the stretching phase than leaks out during the collapse phase (Tiwari and Mason, 2012).

2.2.2 Application

Presently, ultrasound technology has gained wider applications in almost all fields including medical scanning ultrasonic therapy, mineral processing, nanotechnology, food and beverage technology, non-destructive testing, industrial welding, surface cleaning, and environmental decontamination applications (Nithila et al., 2014) and in food industry, it has gained enormous attention (Jambrak et al., 2009). Wide spread applicability of ultrasonication as a non-thermal technology in heat-sensitive foods is because it retains sensory, nutritional, and functional characteristics along with enhanced shelf life, microbial safety (Alegria et al., 2009), and carrying away of bacterial biofilms (Baumann et al., 2009). Over the past few decades, ultrasonic applications were optimized for processing or testing with the result ultrasonic applications for emulsification, defoaming, decontamination, extraction, wastewater treatment, extrusion, and tenderization of meat existed commercially (Anonymous, 2012). In addition, ultrasonic radiation, a type of low frequency energy (20 kHz–1 MHz), has been enormously utilized for enhancing pretreatment processes like, degassing, crystallization, precipitation, leaching, cleaning, extraction, digestion sample preparation (Jiao and Zuo, 2009), changing functional characteristics of food proteins, textural properties of fat products (sonocrystallization), and promoting the extraction of bioactive constituents (Gallego-Juárez et al., 2010). Favorable effects of ultrasound in food processing involves enhancement in food preservation, aid in thermal treatments, improved mass transfer, and alteration of food texture and analysis (Knorr et al., 2011). Ultrasound technology has achieved significant importance due to advancement of novel ultrasound-based and ultrasound-aided detection systems assisted by modern

developments in ultrasound electronic/transducer designs (Jerman Klen and Mozetič Vodopivec, 2012). Ultrasound is applied by three different methods; (1) applying directly to the product, (2) coupling with the device and (3) submerging in an ultrasonic bath (Majid et al., 2015).

1) Effect of ultrasonication on protein

Application of ultrasound in protein modification has received ample attention in recent years either as pretreatment in order to enhance modification or chemical reaction of protein by changing its physical and functional attributes such as, gelation, foamability, emulsification, and solubility. Ultrasonication has proved as an efficient method in producing protein conjugates and to improve the hydrolysis of proteins enzymatically (Chen et al., 2011).

2) Effect of ultrasonication on microbial inactivation

Combined effect of power ultrasound and heat (thermosonication) has proved to be more efficient method of microbial in cativation than either of the two methods alone (Raviyan et al., 2005). Microbial inactivation of ultrasound treatment accounts for generation of acoustic cavitations, resulting in increased permeability of membranes, selectivity loss, cell membrane thinning (Sams and Feria, 1991), confined heating (Suslick, 1998), singlet electron transfer in cooling phase (Lee and Feng, 2011), and hydroxyl radical formation (Kadkhodaee and Povey, 2008) (Figure 2.3). High-frequency ultrasound method, patented as sonoxide, has more than 600 applications and provided best results in inhibiting bacterial and algal growth in industrial waters (Broekman et al., 2010). Ultrasonic-treated cells were found to lack internal content when viewed under transmission electron microscopy, but disintegration was not affirmed to be main reason of cell death (Cameron et al., 2008). Ultrasonication has achieved the FDA requirement of a 5-log reduction in microbial population (Salleh-Mack and Roberts, 2007). Earlier, ultrasound as disinfection treatment was used by the electronics industry but now is used as substitute sanitization process in food industry (Sagong et al., 2011). Exploitation of ultrasound as means of inhibiting and killing microorganisms came from the observation that sonar used for anti-submarine warfare resulted in killing of fishes (Scherba et al., 1991). Ultrasound frequency of 20 kHz and power of 12.8 W was used on 50 cm^3 water contaminated with *Streptococcus mutans* for a period of 15 min and 97% microbial reduction was achieved (Koda et al., 2009). Ultrasonic power of around 100 W was found to be optimal for maximum microbial inactivation (Yusaf and Al-Juboori, 2014) and ultrasonication has been found to be effective method for microbial

inactivation in *Escherichia coli* (Furuta et al., 2004), *Listeria monocytogenes*, and other pathogens. Efficiency of ultrasonic treatment as antimicrobial tool depends on the physical (size, hydrophobicity) and biological (Gram-status, growth phase) characteristics of the microorganisms. It has been demonstrated that microorganisms with “soft” and thicker capsule are extremely resistant to ultrasonic treatment (Gao et al., 2014).

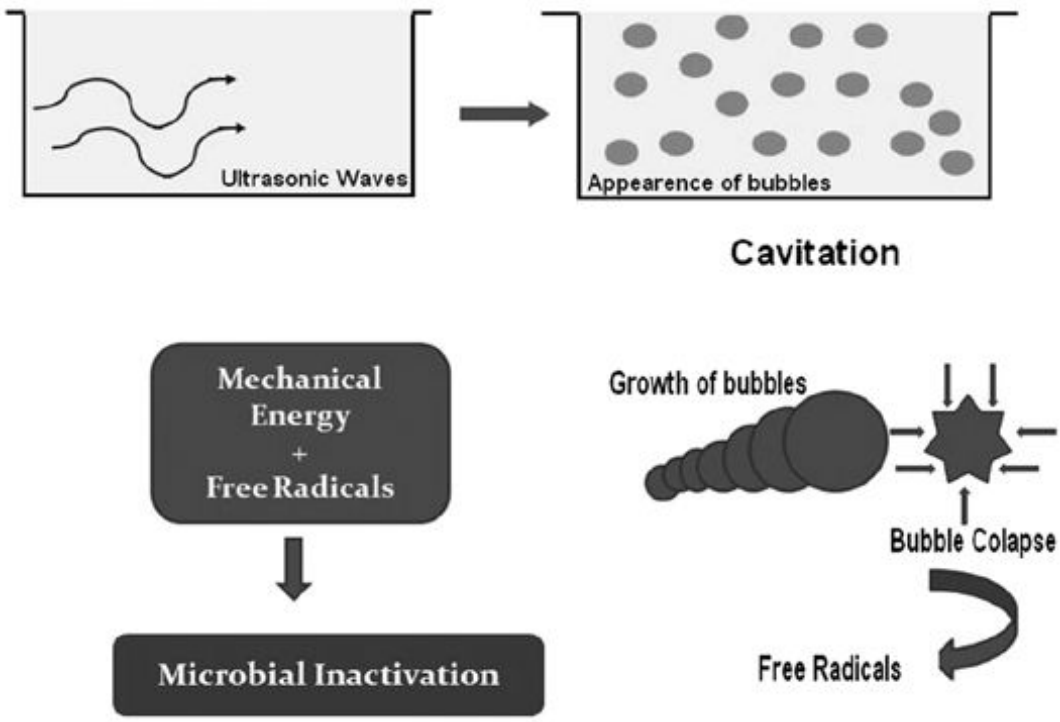

Figure 2.3 Cavitation phenomenon and microbial inactivation by ultrasonic waves (de Sao Jose et al., 2014)

3) Ultrasonication in meat technology

A large number of applications of ultrasonic treatment are reported in meat technology like, reduction of meat toughness due to large proportion of connective tissue (Jayasooriya et al., 2007), examining the composition of fish, poultry, raw, and fermented meat products by supporting genetic enhancement programs in case of livestock (Gallego-Juárez et al., 2010) and in the tenderization of meat products.

4) Ultrasonication in fruit and vegetable processing

Ultrasonication is used to maintain both pre- and post-harvest quality attributes in fresh fruits and vegetables (Gallego-Juárez et al., 2010) and is considered a substitute for washing of fruit and vegetable in food industry (Alexandre et al., 2013). In

an attempt to meet the consumers needs of not only maintaining but also improving the nutritional value of fruit juices (Bhat et al., 2011a, b), ultrasonication has proved to be one such technique (Abid et al., 2013) and is reported to retain fresh quality, nutritional value, and microbiological safety in guava juice (Cheng et al., 2007), orange juice (Valero et al., 2007), and tomato juice (Wu et al., 2008). Ultrasound treatment can also be used to recover the nutrient loss occurred during blanching, resulting in achieving the collaborative benefit of both the techniques (Jabbar et al., 2014). Ultrasonication cleaners (20–400 kHz) have been efficiently used to produce fruits and vegetables free of contamination (Lin and Erel, 1992) and at 40 kHz, it has been applied on strawberry fruits in which decay and infection was considerably reduced along with quality maintenance (Cao et al., 2010).

5) Ultrasonication in dairy technology

Ultrasound treatment is applied in dairy industry for removal of fat from dairy wastewater using enzyme (Lipase z) as a catalyst (Adulkar and Rathod, 2014), improvement in whey ultrafiltration, cutting of cheese blocks, crystallization of ice and lactose, alter the functionality of dairy proteins (Ashokkumar et al., 2010), cleaning of equipment, pasteurization, and homogenization which involve minimum loss of flavor, and increased homogeneity and considerable savings in energy (Chouliara et al., 2010).

6) Ultrasonication in extraction of plant materials or ultrasound-assisted hydrolysis

Extraction of plant components using ultrasound with its lower operating temperatures successfully dodged the limitations of degradation and loss of thermolabile constituents in conventional extraction methods (Jadhav et al., 2009). Ultrasound extraction involves lower running cost, considerable reduction in time and temperature of extraction with almost same yields (Yang et al., 2008), and has been employed in extracting various intracellular components such as soybean oil (Hu et al., 2006; Li et al., 2006), isoflavones from oregano (Rostagno et al., 2007), xyloglucan (Caili et al.,2005), and cellulose nanofibers from wood. Ultrasonication is reported to induce some secondary plant metabolites such as ginsenoside saponins by 75% in ginseng cell (Lin et al., 2001), taxol by three times in *Taxus baccata* cell culture (Rezaei et al., 2011), and resveratrol by 8–143 times in whole or sliced peanut kernel (Rudolf and Resurreccion, 2005). Ultrasound hydrolysis with higher polyphenol amounts in extracts (Teh and Birch, 2014) has gained much popularity in phenolic compound analysis in various plant matrices because of its faster extraction, efficiency, and low consumption

of solvent in strawberries, red raspberries, grape seeds, olive fruits, and leaves (JermanKlen and Mozetič Vodopivec, 2012) and it was reported that to extract naringenin, ellagic acid, naringin, rutin, quercetin, and kaempferol in three cycles of 30 s compared to 2–20 h of traditional methods (maceration/stirring) in case of strawberries and conjugated phenolics of cranberry in less than 1.5 has compared to 16 h by traditional hydrolysis methods. Ultrasonication is used to extract lycopene (Eh and Teoh, 2012), to improve the separation of protein-starch in the wet-milling industry (Zhang et al., 2005), and to reduce particle size of milled corn for sugar release in corn dry-milling (Khanal et al., 2007).

7) Ultrasonication in equipment design and analytical operations

Application of ultrasound in food science and technology for improving food quality has widened due to the probable recent advancement in electronics that designed ultrasound instruments and probes with greater convenience and resolution either as sensors (LPU) or as modifiers (high power ultrasound). However, ultrasound equipment are designed for use in a particular application as they can't be postulated to suit all different applications e.g. in studying functional and physicochemical characteristics of a particular food item selection of suitable processing or sensing system (probe design, frequency, geometry) and operation variables that give optimum outputs in a particular application should be considered (Knorr et al., 2011). LPU in conjugation with spectroscopy and NMR are extensively used in non-destructive analytical techniques for studying the characteristics of fluid foods (McClements, 1997) and any deviation in ultrasound characteristics helps to evaluate the properties of fluids and to assess foreign gents in foods through container walls thus, allowing measurements using relatively cheap and robust instrument in the lab as well as online (Coupland, 2004).

8) Ultrasonication in emulsification

Ultrasonication is relatively cheaper technique for emulsion formation with significant effect on emulsion droplet size and structure. In ultrasonic emulsification application of high energy reported viscosity decrease and lesser particle size distribution in sub-micron oil-droplets emulsions. However, change in sonication parameters caused remarkable change in stability and oil droplet size of the emulsion formed (Kaltsa et al., 2013). Ultrasonically produced W/O emulsions are used by emulsion liquid membrane for the separation and recapture of cationic dyes, and the stability is governed by operating variables such as emulsification time, carrier,

ultrasonic power, surfactant and internal phase concentrations, volume ratios of internal phase to organic phase and of external phase to W/O emulsions, stirring speed, contact time, and diluents (Djenouhat et al., 2008).

9) Ultrasonication in oil technology

Ultrasonication stimulates the mixing and required reaction for conversion of soya bean oil to biodiesel, and can achieve optimum yield using 9:1 oil to methanol ratio (Santos et al., 2009). Ultrasonic irradiation is also used to increase the rate of trans-esterification (Deshmane et al., 2009).

10) Ultrasonication in water treatment

Ultrasound treatment in combination with other water treatment methods (chlorination, ozonation) is considered efficient and economically feasible technique as in ultrasound equipment, energy requirement is huge (Nithila et al., 2014). Ultrasonication is reported to remove all impurities such as worms, sludge, mold, fungi, bacteria, and agrochemicals (Cao et al., 2010). Ultrasonication does not use chemicals for mineralization and destruction of recalcitrant organic compounds in water (Gogate, 2007). In anaerobic digestion process, ultrasonication is used to increase the process efficiency, leading to more methane production and significant decrease in digestion time. Anaerobic digestion process uses ultrasound treatment either as high or as low strength depending on the irradiation location. High-strength ultrasonication (HS-ultrasonication) is irradiated as a pretreatment to feedstock and low-strength ultrasonication (LS-ultrasonication) is irradiated in the aerobic digestion process to the microorganisms involved (Cho et al., 2013).

11) Ultrasonication in enzyme technology

Ultrasonication has been used to influence enzyme activity (Fahmi et al., 2011) and to obtain intracellular enzymes from microbial cells. Ultrasound treatment helps in the release of glucose-oxidase from *Aspergillus niger*, galactosidases from *Lactobacillus* strains and *E. coli*, and invertase from *A. niger*. Despite positive implications on enzymatic activity, high-intensity ultrasonication leads to denaturation and hence making ultrasound treatment enzyme-specific and sonication parameter-specific (Lateef et al., 2007). Thermosonication a combination treatment of incorporating high static pressure in an ultrasound treatment chamber is used as a means for enzyme inactivation such as lipoxygenase, peroxidase, lipase, and protease, and tomato or orange pectin methylesterase (Raviyan et al., 2005). In cellulose preparation, the cellulolytic activity was found to increase with the ultrasonic intensity because of some minor changes in

spatial structure of enzyme molecules that helped in the formation of enzyme–substrate complex and increased the adsorption of cellulase on insoluble cellulose (Nguyen and Le, 2013).

12) Applications in membrane filtration

Use of ultrasound in conventional membrane filtration has proved to improve process efficiency and utilized in membrane cleaning (Masselin et al., 2001). Both cross-flow (Li et al., 2002) and dead-end filtration (Simon et al., 2000) uses online ultrasonication. Most commonly ultrasonic water baths are used as ultrasound devices which are associated with high loss of acoustic power of about 90% (Cai et al., 2009). In an attempt to improve ultrasound equipment, several workers (Juang and Lin, 2004; Mirzaie and Mohammadi, 2012; Simon et al., 2000) have developed an ultrasonic probe system that in a dead-end filtration process passes ultrasonic waves directly to the feed medium. Also a membrane module fitted with many packed in type ultrasonic transducers are used to apply cross-flow filtration and involve minor loss of ultrasonic energy (Kyllönen et al., 2006). Filtration performance is measured as the rate of release of permeate flux but is not correlated with processes involved in irreversible fouling and reversible concentration polarization layer in the feed. However, mass-transfer coefficients and concentration of filtrate at the membrane surface have been predicted by modeling and hypothetical methods (Muthukumaran et al., 2005).

13) Ultrasonication in honey

Ultrasound applications in honey include use of velocity of ultrasonic wave propagation as a means to differentiate between different types of honey determination of adulteration in honey and evaluation of the type of protein, aggregation state, and size (Gallego-Juárez et al., 2010).

14) Other applications

Ultrasonication singly or in combination with other preservation methods have been used to decrease the required processing temperature and time, or both, in pasteurization of liquid foods like milk, wine, and juices. It is used as a substitute or additional process to traditional thermal methods (Valero et al., 2007). Numerous other applications of ultrasound are reported in several foods including, cooking oils, bread, cereal products, and emulsified fat-based food products, food gels, aerated foods, and frozen foods (Gallego-Juárez et al., 2010). Ultrasound has also been used to determine the interaction of powder with solvent in order to evaluate the reconstitution of powders (Richard et al., 2012) and is dependent on product porosity (García-Pérez et al., 2011).

2.2.3 Advantages and limitations of ultrasonication

Ultrasound applications offer numerous advantages in the food industry some of which are enlisted as follows (Majid et al., 2015):

1) Ultrasound waves are non-toxic, safe, and environmentally friendly.

2) Ultrasonication in combination with other non-thermal methods is considered an effective means of microbial inactivation.

3) Ultrasonication involves lower running cost, ease of operation, and efficient power output.

4) Ultrasonication does not need sophisticated machinery and wide range of technologies.

5) Use of ultrasound provides more yield and rate of extraction as compared to other conventional methods of extraction.

6) Ultrasonication involves minimum loss in flavor, superior consistency (viscosity, homogenization), and significant savings in energy expenditure.

7) Ultrasound has gained huge applications in the food industry such as processing, extraction, emulsification, preservation, homogenization, etc.

Despite having lot of advantages, use of ultrasonication has also many disadvantages such as (Majid et al., 2015):

1) Ultrasound due to shear stress developed by swirls from the shock waves (mechanical effects) cause inactivation of the released products.

2) Ultrasound application needs more input of energy which makes industrialists to think over while using this technique on commercial scale.

3) Ultrasound induces physicochemical effects which may be responsible for quality impairment of food products by development of off-flavors, alterations in physical properties, and degradation of components.

4) Ultrasonication leads to the formation of radicals as a result of critical temperature and pressure conditions that are responsible for changes in food compounds. The radicals (OH and H) produced in the medium deposit at the surface of cavitation bubble that stimulates the radical chain reactions which involve formation of degradation products and thus lead to considerable quality defects in product.

5) Frequency of ultrasound waves can impose resistance to mass transfer.

6) Ultrasonic power is considered to be responsible for change in materials based on characteristics of medium. So, this power needs to be minimized in food industry in order to achieve maximum results.

2.3 Application use of probiotics in non-dairy beverage

Probiotics are dietary supplements containing potentially beneficial bacteria or yeasts. According to the currently adopted definition by FAO/WHO (2001), probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Some strains of lactic acid bacteria (LAB) have been used in the food industry for many years, because they are able to convert sugars (including lactose) and other carbohydrates into lactic acid. This not only provides the sours taste of fermented foods, but also by lowering the pH may create fewer opportunities for spoilage organisms to grow, hence creating possible health benefits on preventing gastrointestinal infections (Nichols, 2007). Strains of the genera *Lactobacillus* and *Bifidobacterium*, are the most widely used probiotic bacteria (Tannock, 2005). The lactobacilli currently being used as probiotics comprise: *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus delbreuckii* subsp. *bulgaricus*, *Lactobacillus brevis*, *Lactobacillus cellibiosus*, *Lactobacillus lactis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacillus rhamnosus*. The desirable characteristics of a good probiotic strain are listed below (Kalantzopoulos, 1997);

1) Normal habitant of the human intestinal tract,

2) Easy proliferation *in vitro* (production),

3) Remaining viable during processing, storage and mixing in food/feed,

4) Survive the upper gastrointestinal tract (to reach in the intestine e.g. acid and bile resistant),

5) Colonization and/or proliferation in the gastrointestinal tract e.g. by the production of bacteriocins, lactic acid,

6) Non-pathogenic and nontoxic/carcinogenic not absorbed in digestive tract/not invasive, no tissue residues,

7) Generically stable, no mutations and lack of potential for making with pathogenic bacteria,

8) Improvement of growth and efficiency in animals,

9) Prevention of intestinal infection, compete with pathogenic microorganisms,

10) (Pre) digestion of lactose (lactose intolerance),

11) Anticholesterolaemic effects,

12) Antitumor activity.

Probiotics may be perceived as “protective” intestinal bacteria. They are involved in the production of essential mucosal nutrients, such as short chain fatty acids (SCFAs), as well as the amino acids arginine, cysteine and glutamine. Probiotics can prevent the overgrowth of potentially pathogenic organisms and stimulate the intestinal immune defense system. They have been shown to eliminate toxins and unnecessary substance from cholesterol reduces the overall amount of circulating cholesterol. Probiotics may also participate in the regulation of intestinal functions, such as mucous secretion and utilization, nutrient absorption, gastrointestinal motility and splanchnic blood flow (Bengmark, 1996). To provide health and clinical benefits, probiotic bacteria must possess a number of properties, the most important of which are that they ought to be of human origin, have the ability to adhere to human intestinal cells, and should be able to colonize the gut (Lee and Salminen, 1995). Isolauri et al. (1993) has argued that the ability of lactobacilli to adhere to mucosa is of crucial importance for clinical effect. Interestingly, most of the bacteria currently used in commercial production of dairy products do not colonize, rendering them essentially useless in promoting gastrointestinal health and preventing disease (Chauvire et al., 1992).

Lactobacilli are tolerate a lower pH than do other bacteria. This common property makes them especially suitable for functional food products, as it contributes to a much longer shelf-life compared with other bacteria (Lee and Salminen, 1995). Probiotic products are usually marketed in the form of fermented milks and yoghurts; however, with an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. Furthermore, lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products (Heenan et al., 2004). There are a wide variety of traditional non-dairy fermented beverages produced around the world. Much of them are non-alcoholic beverages manufactured with cereals as principal raw material (Prado et al., 2008). Yoon et al. (2004) determined the suitability of the tomato juice as a raw material for the production of probiotic juice by *L. acidophilus* LA39, *L. plantarum* C3, *L. casei* A4 and *L. delbrueckii* D7. The tomato juice was inoculated with a 24 h-old culture and incubated at 30°C. The lactic acid bacteria cultures reduced the pH to 4.1 and the

viable cell counts reached nearly (1.0–9.0) x 10^9 CFU/ml after 72 h ranged fermentation. The viable cell counts of the four lactic acid bacteria in the fermented tomato juice ranged from 10^6 to 10^8 CFU/ml after 4 weeks of cold storage at 4°C. Yoon et al. (2005) also evaluated the potential of red beets as the substrate for the production of probiotic beet juice by the above four species of lactic acid bacteria. All the lactic acid bacteria cultures were capable of rapidly utilizing the beet juice for the cell synthesis and lactic acid production. *L. acidophilus* and *L. plantarum* produced higher amount of lactic acid than other cultures and reduced the pH of the fermented beet juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30°C. Although the lactic acid bacteria cultures in fermented beet juice gradually lost their viability during the cold storage, the viable cell counts of these bacteria, except for *L. acidophilus*, in the fermented beet juice still remained at 10^6 – 10^8 CFU/ml after 4 weeks of cold storage at 4°C. Moreover, Yoon et al. (2006) also developed a probiotic cabbage juice using lactic acid bacteria. Cabbage juice was inoculated with a 24 h-old lactic culture and incubated at 30°C. The cultures (*L. plantarum* C3, *L. casei* A4 and *L. delbrueckii* D7) grew well on cabbage juice and reached about 1 × 10^9 CFU/ml after 48 h of the fermentation. *L. casei* produced a lower amount of titratable acidity, expressed as lactic acid, than *L. delbrueckii* or *L. plantarum*. After 4 weeks of the cold storage at 4°C, the viable cell counts of *L. plantarum* and *L. delbrueckii* were 4.1 × 10^7 and 4.5 × 10^5 CFU/ml, respectively. *L. casei* did not survive at low pH and lost cell viability completely after 2 weeks of the cold storage. The fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers. Kun et al. (2008) determined the suitability of carrot juice as a raw material for the production of probiotic food with *Bifidobacterium* strains (*B. lactis* Bb-12, *B. bifidum* B7.1 and B3.2). Pasteurization of freshly prepared carrot juice at 80°C for 20 min decreased the microbial population to below the detection limit (10 CFU/ml). All tested bifidobacteria strains were capable of growing well on pure carrot juice without nutrient supplementation. Moreover, 10^7 CFU/ml initial cell concentrations resulted in 10^8 CFU/ml after 6 h of incubation, and were kept viable up to the end of fermentation (24 h). Volumetric productivities of *B. lactis* Bb-12, *B. bifidum* B7.1 and *B. bifidum* B3.2 were 2.16 × 10^{10}, 4.65 × 10^{10} and 3.85 × 10^{10} CFU/L h, respectively. Due to intense metabolism of the bacteria strains, carrot juice media were acidified to a pH level of less than 4.5. During the fermentation, the amounts of glucose and sucrose decreased significantly. Meanwhile the fructose concentration did not change. Degradation of

carotenoids (α-carotene and β-carotene) was between 15-45% depending on the strain used. Production of lactic and acetic acids was in the range of 14.8 - 16.7 and 3.3 - 5.3 mg/ml, respectively.

2.4 Applications of prebiotics in health and nutrition

Prebiotics are non-digestible carbohydrate substrates in the diet that are the preferred foods for bifidobacteria and lactobacilli and result in their increased number in the large intestine (Gibson and Roberfroid, 1995). The definition of prebiotics overlaps significantly with the dietary fiber definition; with the exception of its selectivity for certain species of the gut bacteria. According to Gibson et al. (2004), in order to being considered as an effective prebiotic, any food ingredient must demonstrate the following characteristics:

1) Non-digestibility and non-absorption in the gastrointestinal tract (GIT)

2) Fermentability by the gut microflora

3) Selective stimulation of the growth and activity of one or a limited number of colonic bacteria

4) An ability to increase the number of saccharolytic species and decrease putrefactive microorganisms such as *Clostridia* in order to alter the colonic microflora balance towards a healthier composition.

The concept of prebiotic is to improve the gut microflora through dietary means. According to Saxelin et al. (2003), while the large intestine contains several hundred strains of anaerobic bacteria, the prebiotic concept assumes that there is already favorable microflora in the GIT and therefore, the prebiotics only need to stimulate the growth and metabolic activities of those bacteria. The most important functional effects of prebiotics on the gut microflora include (i) gut microflora modification, (ii) maintaining the intestinal mucosa with the capacity to prevent pathogen activation, (iii) modification of dietary proteins by the intestinal microflora, (iv) reducing the risk of tumor induction by improving the bacterial enzyme activity and (v) improving gut mucosal permeability (Salminen et al., 1998).

Using prebiotics in food formulation process has some advantages over the probiotic strategy as they could reduce the problem of keeping the organisms alive during transit through upper gastro-intestinal tract as well as during storage (Crittenden, 1999). Thus, the prebiotic approach involves the interaction of a non-digestible food

ingredient and beneficial micro-organisms such as bifidobacteria and lactobacilli in the human colon. The most researched prebiotics are non-digestible oligosaccharide molecules, containing 3 to 10 monosaccharide residues connected by glycosidic linkages (Niness, 1999). Most of them occur naturally as native components in plants e.g. raffinose and stachyose in beans and peas, oligofructose and inulin in chicory, garlic, artichoke, onion and leek (Van Loo et al., 1995). Inulin is a prebiotic for which sufficient data has been generated to allow an evaluation of its classification as a functional food ingredient. Inulin includes native inulin, enzymatically hydrolyzed inulin or oligofructose and synthetic fructooligosaccharides (De Leenheer and Hoebregs, 1994).

2.5 Application of spray drying for preservation of probiotics

Spray drying is considered a good long-term preservation method for lactic acid and probiotic cultures (Riveros et al., 2009). The spray drying of microorganisms dates back to 1914 to the study of Rogers on dried lactic acid cultures. The speed of drying and continuous production capability is very useful for drying large amounts of starter cultures. Since then, much research has been reported on the spray drying of bacteria without loss of cell activity in order to overcome the difficulties involved in handling and maintaining liquid stock cultures (Boza et al., 1987; Teixeira et al., 1995). The low production cost of spray drying makes it more energy efficient compared with freeze drying. Nevertheless, compared with other drying methods (e.g., freeze drying), spray drying of microbial cultures has been less developed commercially. The reasons for this are mainly low survival rates during drying of the cultures, low stability under storage and the difficulty in rehydrating the product (Ananta et al., 2005; Boza et al., 2004; Chavez and Ledeboer, 2007; Mauriello et al., 1999; Teixeira et al., 1995).

However, spray drying is an economical process for production of industrial scale quantities of viable microorganisms. Its application to generate preparations of lactic acid bacteria has recently received considerable interest. Therefore, in the present article we provide a survey of the state of knowledge of starter culture production at high levels of viability using spray drying. The spray drying parameters that affect the survival of lactic acid starter cultures are discussed. The results from several researchers on different aspects of spray drying of lactic acid starter cultures are summarized. The features, advantages, and disadvantages of drying lactic acid cultures by spray drying are described.

Spray drying is a common industrial and economic process for the preservation of microorganisms and for the preparation of starter cultures that are used to prepare lactic fermented products (Lian et al., 2002; To and Etzel, 1997). The survival of lactic acid bacteria is an important issue when spray drying is used for the preparation of microbial cultures. However, biological activity of a lactic acid starter, which includes cell viability and physiological state, is a criterion for evaluating starter quality. Biological activity is defined as the ability of a lactic acid starter to acidify a certain medium. Activity tests for lactic acid bacteria are normally based on measurements of the increase in titratable acidity or the decrease in pH during incubation of inoculated milk for 3-5 h. These tests are long and laborious. Therefore, other methods such as impedimetric methods have been developed. Carvalho et al. (2003) analyzed the residual activity of *L. delbrueckii* ssp. *bulgaricus* cultures using pH and various impedimetric methods to quantify the loss in activity following freeze drying. They reported that the measurement of capacitance can be used as an alternative method for estimating the residual activity of freeze dried preparations of *L. bulgaricus*. Preparation of dried starter cultures is a long process beginning with cell cultivation to storage of the dried powder. Many factors are involved in the viability of the cultures during spray drying (Boza et al., 2004; Corcoran et al., 2004; Fu and Etzel, 1995; Johnson and Etzel, 1995; Santivarangkna et al., 2007) which are summarized in the following sections.

1) Process parameters (inlet and outlet temperature, drying time, nozzle pressure)

Although it is reported that an increase in inlet air temperature decreases cell viability (Mauriello et al., 1999), the higher temperature is not directly correlated to the inactivation and has only a slight effect (Kim and Bhowmik, 1990). This may be because the extent of inactivation of bacteria during spray drying depends on the temperature-time combination. Various investigators have also reported that increasing outlet air temperature reduces the survival of microorganisms after spray drying (Kim and Bhowmik, 1990; Lian et al., 2002; To and Etzel, 1997).

The temperature-time history of the particles can be divided into two periods. At the constant drying rate period, the temperature of the product and heat inactivation is limited to the wet bulb temperature. As a result of the evaporative cooling effect during this period, the survival of bacterial cells is strongly correlated to the outlet temperature. The inlet temperature of the dryer indirectly influences microbial survival. Thus, thermal inactivation is limited during the constant rate period, because the high evaporation rate

and the resulting wet bulb temperature protect the cells from the higher air temperature in the dryer. During the falling rate period, the particle surface becomes dry and the temperature of the product increases depending on dryer configuration (Boza et al., 2004). During this stage, the extent of thermal inactivation depends on the drying parameters such as outlet temperature, residence time, and feed rate (Santivarangkna et al., 2008). The falling rate period is important for heat inactivation, and therefore the optimum residence time is the time for complete removal of moisture with minimum increase in the temperature of the dried products. When low drying air temperatures are required for heat-sensitive products, the residence time may be very long. This can require drying towers of great height and/or the need for dehumidified air (Masters, 1985).

The kinetics of temperature evolution is as important as that of moisture evaporation. The mass transfer kinetics and final moisture content also depend on temperature-time composition (Konavalov et al., 2010). Water content and temperature are controlling parameters for inactivation of microorganisms. In spray drying, removal of moisture takes place quickly and hence the temperature change, and moisture concentration change, and inactivation processes are also fast. The inactivation rate reduces as moisture content decreases. It is shown that the rate-dependence of microbial inactivation is greater at higher drying rate conditions and also during the early stage of the drying (Chen and Patel, 2007).

The outlet air temperature is believed to be the major drying parameter affecting the viability of spray-dried starter cultures. This parameter depends on the inlet air temperature, air flow rate, product feed rate, medium composition, and atomized droplet size (Boza et al., 2004; Santivarangkna et al., 2007, 2008). However, the proper settings for these variables are difficult to calculate in advance, and this may lead to great variation in the viability of the dried culture (Roelans and Taeymans, 1990). Many researchers have obtained higher viability of microbial cells at lower outlet air temperatures (Ananta et al., 2005; Desmond et al., 2002a, b; Kim and Bhowmik, 1990; Prajapati et al., 1987). It is believed that two principal mechanisms are responsible for viability deterioration during convective drying of bacterial cultures: inactivation due to dehydration and inactivation due to temperature (Janning and in'T Veld, 1994). It is not clear which of these mechanisms is more damaging, as they generally occur simultaneously. In spray drying, lactic acid starter cultures are sprayed into a flow of hot air. Therefore, inactivation of cells due to high temperature can occur besides the

dehydration inactivation. A theory for heat inactivation is that heat is assumed to inactivate a critical component, while at the same time destroys many other components.

Loss of the less critical components does not cause death until their numbers are reduced or the cell is subjected to additional stress. Although, some macromolecules (e.g. DNA, RNA and proteins), membranes and ribosomes damage from the action of heat, but ribosomes have been identified to be the critical component for heat inactivation (Lee and Kaletung, 2002; Santivarangkna et al., 2007). In addition to ribosomes, critical components can also be cell envelopes, DNA, and RNA polymerase. It is not necessary that the same critical component is lost in all cells, and death may be due to destruction of more than one critical component. Dehydration inactivation in lactic acid starter cultures may occur either solely during the drying of cells at their physiological temperatures or in concert with thermal- or cryo-inactivation during the drying of cells at an extreme low or high temperature. Since water molecules contribute to the stabilities of proteins, DNA and lipids as well as conferring structural order upon cells, the removal water imposes physiological constraints to cells. When cells are dried to a low water content, a number of cellular components will be affected (Santivarangkna et al., 2007). Cytoplasmic membrane is considered to be the most sensitive component in the dehydration process, because there is a loss of several intracellular components when the membrane is damaged (Li et al., 2006; Riveros et al., 2009). Membrane lipid bilayer structures are thermodynamically unstable. Therefore, the lipid membrane is a primary target for dehydration induced damage. Besides that, during spray drying, cells come into contact with a large volume of air and consequently lipid oxidation is likely to occur (Santivarangkna et al., 2007; Teixeira et al., 1996).

The spray dryer configuration can influence on survival of lactic acid bacteria. Chavez and Ledeboer (2007) applied different dryer configurations to a given set of inlet and outlet temperatures for spray drying of bifidobacteria. Three elements of the spray dryer configuration were modified: nozzle, direction of air flow and feed atomization, and chamber volume. Small improvements in microbial survival were observed when a two-phase nozzle was used instead of a rotary atomizer. Extending the chamber volume did not cause a significant reduction in bacteria survival when using a co-current configuration with either atomization device. For a given set of inlet and outlet temperatures, it was reported that the spray dryer configuration had a minor effect, compared with the type of carrier materials, on the survival of bifidobacteria

during storage. Chavez and Ledeboer (2007) also showed that co-current flow was preferable. According to Fu and Etzel (1995), exposure of *L. lactis* cells to high temperatures in the atomizer and during droplet drying in spray dryer resulted in cellular injury. Lievense and van't Riet (1994) stated the effect of the atomization pressure on bacteria viability, provoking in some cases a negative stress effect on cells, resulting in cell injury. Riveros et al. (2009) reported an increase in *L. acidophilus* viability with a decrease in spray pressure from 100 to 50 kPa. The viability of cells after drying was 8.62 and 9.48 log CFU/g at a nozzle pressure of 100 and 50 kPa, respectively. Lievense and van't Riet (1994) reported an increase of 0.8-1.5% in the survival of *L. bulgaricus* when the spray pressure was decreased from 200 to 100 kPa. This result is explained by the diminished stress experienced by the bacteria when submitted to the low shear force produced in the nozzle at low pressures (Riveros et al., 2009).

2) Product parameters (carrier medium, concentration)

Lian et al. (2002) spray-dried bifidobacteria cultures with various carrier media. Comparison of different carrier concentrations revealed that using 10% (w/w) gelatin, Arabic gum or soluble starch led to the highest survival of bacterial cultures. It was concluded that the viability of bifidobacteria is highly dependent on the type of carriers and varies with strains. The concentration of carrier medium can also affect the survival of bacteria after spray drying. In the study of Lian et al. (2002), increasing the concentration of gelatin, Arabic gum or soluble starch from 10 to 20% (w/w) or more caused reduced survival of bifidobacteria. Espina and Packard (1979) also reported higher viability of *L. acidophilus* spray dried with 25% non-fat milk solids compared with 40% non-fat milk solids. However, higher solid content of carrier medium would result in larger particles that require longer drying times. Thus, microorganisms entrapped in the particles would be subjected to more heat damage, leading to less viability of bacterial cultures (Santivarangkna et al., 2007). According to Lievense and van't Riet (1994), an increase in solid content of carrier medium leads to an increase in particle size, resulting in an increase in contact time between the hot air and the material, and consequently in the thermal inactivation. Ananta et al. (2005) used reconstituted skim milk (RSM) at a concentration of 20% (w/w) for spray drying of *L. rhamnosus* GG. It was shown that RSM leads to a microbial survival rate of 60% at an outlet temperature of 80°C in a spray drier. The partial incorporation of commercial prebiotics such as raftilose (P95) and polydextrose in the skim milk powder resulted in a high level of survival. Nevertheless, the storage stability of the dried powder was reduced as the

amount of skim milk solids in the carrier was lowered. RSM at a concentration of 20% (w/w) was regarded as the optimal solid content to ensure high residual viability of different strains of lactic acid bacteria (Ananta et al., 2005).

3) Biological parameters (species, growth media, growth phase, intrinsic stress tolerance)

The viability of distinct species of a given genus or even distinct strains of a given species differs under the same drying or storage conditions (Simpson et al., 2005). It has been reported that the reduction in survival rate of some starter cultures is dependent on the species and method of preservation (To and Etzel, 1997). The survival of microorganisms after spray drying was greatest for *Streptococcus thermophilus* followed by *L. paracasei* ssp. *paracasei*; *L. lactis* ssp. *cremoris* was the least tolerant microorganism. Corcoran et al. (2004) compared the thermal tolerance of three strains of probiotic lactobacilli in reconstituted skim milk (RSM, 20% w/v) in a temperature range of 55-61°C. *L. rhamnosus* E800 was the most heat resistant, followed by *L. salivarius* UCC 500; *L. rhamnosus* GG was the least heat resistant. Although *L. rhamnosus* GG was the most thermally sensitive of the three *Lactobacillus* strains studied, it was the best survivor during spray drying. This indicates that thermal tolerance alone is not an accurate predictor of performance during spray drying; other phenomena, such as dehydration affect cell viability during drying. Thus, sensitivity to dehydration differs among lactic acid starter cultures and it is an individual trait (Santivarangkna et al., 2007).

Growth conditions and media can affect the viability of lactic acid starter cultures during spray drying or the subsequent storage period. For instance, it has been shown that compatible solutes such as amino acids, quaternary amines (e.g., glycine, betaine, carnitine), and sugars in the medium increase the viability of lactic acid starter cultures during the drying process (Kets et al., 1996). During drying, microorganisms face decreasing water activity. Under these conditions, some microorganisms accumulate compatible solutes in order to maintain osmotic balance with the highly concentrated extracellular environment (Morgan et al., 2006; Santivarangkna et al., 2007). Lactic acid bacteria do not synthesize compatible solutes and therefore are dependent on the environment to take up these solutes. Thus, these solutes can help microbial cultures to stabilize proteins and the cell membrane during osmotic stress conditions brought on by low water activity during the drying process (Morgan et al., 2006). Carvalho et al. (2004) studied the role of various sugar substrates in the growth medium upon both thermo-

tolerance and survival during storage after freeze drying of *L. bulgaricus*. They concluded that the growth medium supplemented with lactose yielded cells bearing the highest heat resistance. Presence of glucose, fructose, lactose, mannose or sorbitol in the drying medium mostly led in the enhancement of protection during storage, to a degree that was growth medium-dependent. The results of Carvalho et al. (2003b) showed that the influences of growth conditions and probably drying medium composition on the survival of lactic acid bacteria in the dried state were strain specific. The study of relationship between death of freeze-dried *L. bulgaricus* and solubility of freeze-dried skim milk during storage showed that the degree of survival was proportional to the square root of the absorbance of the supernatant. A darker skim milk powder resulted when most of the bacteria had died (Carvalho et al., 2007). The optimal growth phase of cells for starter culture production depends on the specific microorganism (Morgan et al., 2006). Generally, lactic acid bacteria are harvested either in the late log phase or early stationary phase. It is shown that harvesting bacteria cells at the stationary phase leads to enhanced viability after spray drying (Corcoran et al., 2004; Teixeira et al., 1995; Van de Guchte et al., 2002). The depletion of nutrients and glucose starvation in bacterial cells that occurs in the stationary phase of growth provide conditions that cause the cells to be resistant to many stresses such as osmotic and heat stress (Van de Guchte et al., 2002). In a study by Corcoran et al. (2004), *L. rhamnosus* GG was spray dried in the lag, early log and stationary phases of growth in different prebiotic substances. Over 50% survival was obtained when the cells were harvested during the stationary phase, whereas harvesting bacterial cells at the early log phase led to 14% survival. Other researchers have also reported greatest loss of viability of spray-dried *L. bulgaricus* when they were harvested at the log phase of growth. Stationary phase cultures exhibited more resistance in the spray drying process (Teixeira et al., 1995).

4) Pre-treatments (stress response, protective substances)

Adverse conditions or stresses during microbial growth can lead to tolerance responses. Various stresses such as heat and acid shock make lactic acid bacteria more tolerant to spray drying. Teixeira et al. (1995) showed that heat shock at the exponential growth phase can increase the survival rate of *L. bulgaricus* during spray drying. In the stationary phase of cell growth such a tolerance response was not seen. In the early stationary phase, the unstressed controls exhibited superior survival compared with heat-stressed cells. Acid treatment has been reported to make bacterial

cultures resistant to various other stresses (Lemay et al., 2000; Lorca and De Valdez, 2001). In a study by Silva et al. (2002), *L. delbrueckii* ssp. *bulgaricus* cells were grown without or with pH controlled to 6.5. The cultures grown under non-controlled pH showed greater survival during heating and drying, but not during storage in the dried state. This higher resistance is reported to be related to the enhanced production of heat shock proteins. Salt adaptation stress can also enhance the survival of bacterial cultures during spray drying. It is reported that when exponentially growing cells of *L. paracasei* NFBC 338 undergo pre-adaptation with either heat or NaCl (incubation in 0.3 M NaCl for 30 min), the technological performance of the culture during spray drying is improved (Desmond et al., 2002a, b). The duration of stress sometimes influences the tolerance response by bacterial cultures. For example, in the case of *Enterococcus faecalis*, the duration of glucose starvation led to a progressive increase in heat and oxidative tolerances in bacterial cells during spray drying (Santivarangkna et al., 2007). The addition of protective agents is considered as a common method for protecting starter cultures during drying and storage. Protective agents may be simple or complex components. Different sugars (e.g., glucose, fructose, lactose, mannose, sucrose, sorbitol, adonitol, trehalose) and compounds such as skim milk, acacia gum, monosodium glutamate, starch and oligosaccharides have been investigated for their protective properties on bacterial cells during drying (Desmond et al., 2002a, b; Leslie et al., 1995; Santivarangkna et al., 2007, 2008; Sunny Roberts and Knorr, 2009).

Sugars are preferable as protective agents because of their relatively low price, chemically innocuous nature, and common use in the food industry. The presence of different fermentable sugars in the growth medium leads to the formation of metabolites such as mannitol, which can enhance the viability of bacterial cultures during drying. Non-fermentable sugars exert a hyperosmotic stress on cells. This can induce accumulation of compatible solutes, which make cells resistant the osmotic stress during drying (Santivarangkna et al., 2008). According to Chavez and Ledeboer (2007), sugars, especially disaccharides, can replace water molecules and preserve membrane structures. They also retard protein denaturation (preserve cell structure) by forming hydrogen bonds with proteins. Two hypotheses, the so-called water replacement and vitrification, were proposed to explain the mechanism of membrane stabilization by sugars (Santivarangkna et al., 2008). Vitrification hypothesis is based on glass formation in a dry state by sugars (Crowe et al., 1998; Roos, 1995). According to water replacement hypothesis, specific and particular interactions between phospholipids and

sugars are required for the protective effect. The interactions occur via the hydrogen bond between hydroxyl groups of the sugars and the phosphate group at the surface of the bilayer (Santivarangkna et al., 2008; Sum et al., 2003).

Sorbitol and monosodium glutamate (MSG) have been reported to be effective in maintaining high degrees of viability during storage of freeze-dried *L. plantarum*, *L. bulgaricus*, *L. rhamnosus*, *En. faecalis* and *En. durans*, even though no significant differences has been observed in survival during freeze drying after addition of sorbitol or monosodium glutamate (Carvalho et al., 2002, 2003). Other studies have shown that addition of sucrose and NaCl to drying and growth media can increase survival of freeze-dried *L. bulgaricus*. However, suitable selection of composition of both the growth and the drying media is necessary to afford protection during storage of freeze-dried cells (Carvalho et al., 2003). A combination of different protectants can be used to improve the survival of spray-dried probiotics. According to Chavez and Ledeboer (2007), using a combination of soy protein and maltodextrin or skim milk and Arabic gum resulted in the best survival rate of spray-dried *B. lactis* BB12. Desmond et al. (2002a) used acacia gum to protect probiotic cultures of *L. paracasei* NFBC 338 during spray drying, storage and gastric transit. They showed that acacia containing cultures (10% w/w) survived 10-fold greater than control cells during spray drying. In a study by Corcoran et al. (2004), the influence of various combinations of reconstituted skim milk (RSM), polydextrose (PD) and inulin substances on the viability of probiotic lactobacilli during spray drying was investigated. It was found that PD and inulin do not enhance the viability of cultures during drying or storage. However, RSM and PD showed superior protection to probiotic lactobacilli during storage compared with RSM/inulin combinations. It is important to be noticed that the best protection medium for the drying process may not be the optimum for protection of microbial cells during storage (Santivarangkna et al., 2007).

5) Post drying conditions (rehydration, packaging and storage)

Rehydration is considered to be a critical step in the recovery of spray-dried lactic acid starter cultures. The solution used for rehydration and the rehydration conditions may affect the survival rate of dried microbial cultures. Teixeira et al. (1995) tested four different rehydration media (at 20°C) to revive *L. bulgaricus* cells. Skim milk, MRS broth, deionized water or phosphate buffer were found to have no significant differences on the survival of cells. The temperature of rehydration can also affect cell recovery after spray drying. A temperature increase between 4 and 50°C was found to

linearly increase the viability of *L. bulgaricus*. This finding is similar to a result from Wang et al. (2004) in *S. thermophilus* and *B. longum* dried by spray drying. The results of Mille et al. (2004) showed that the influence of rehydration temperature differs for *L. plantarum* and *L. bulgaricus* dried by fluidized bed drying. In the case of *L. plantarum*, the rehydration temperature (at 30 or 37°C) had no significant effect on the final bacterial concentration. The final concentration of *L. bulgaricus* was greatly influenced by temperature; the higher the temperature, the greater the viability. Another factor that must be taken into account is the rate of rehydration. It was shown that rapid (2 min) or slow (30 min) rehydration lead to a significant difference in the viability of *L. bulgaricus*. Slow rehydration (soaking) led to higher cell viability, possibly because the soaking method limits the amount of osmotic shock (Teixeira et al., 1995). However, a decrease in cell membrane fluidity at a given temperature after rehydration caused by an increased proportion of saturated fatty acids enhances membrane leakage, leading to the loss of culture viability (Santivarangkna et al., 2008).

Storage and packaging conditions affect cell viability after drying. Different studies have shown that temperature is an important parameter for microbial survival during storage. As can be expected, the stability of spray-dried samples decreases during storage, and low storage temperatures lead to higher microbial survival rates (Boza et al., 2004; Corcoran et al., 2004; Desmond et al., 2002a, b; Silva et al., 2002). Lipid oxidation of membrane fatty acid is reported to be a possible mechanism for cell death during storage (Ananta et al., 2005). According to Teixeira et al. (1996) an increase in lipid oxidation during storage changes the lipid composition of the cell membrane. The addition of antioxidant materials such as ascorbic acid and monosodium glutamate are reported to protect *L. delbrueckii* ssp. *bulgaricus* cells during storage at 4°C. The death rate of the culture (at a storage temperature of 20°C) was higher in the presence of these compounds than in the control (Morgan et al., 2006), as a result of the pro-oxidant property of ascorbic acid at higher temperatures (Santivarangkna et al., 2007). Some sugars (sorbitol, maltose and mannitol) are also effective protectants for starter cultures against oxidation damages. The possible mechanisms behind this protective and anti-oxidative role by sugars are reported to be free-radical scavenging, chelating of metal, formation of complexes with hydrogen peroxide, and viscosity restriction of oxygen diffusion (Santivarangkna et al., 2008). Proper packaging for storage of the cultures is also important. Packages under vacuum or nitrogen replacement are suitable for storing anaerobic probiotics such as

bifidobacteria. Vacuum storage was shown to be better than nitrogen and air (Chavez and Ledeboer, 2007). There is little information about the effect of the packaging material on cell viability. It is shown that both spray-dried *S. thermophilus* and *B. longum* can survive better in laminated pouches, followed by glass bottles and polyethylene terphthalate bottles (Wang et al., 2004). Water content is an important parameter for the stability of dried cultures (Wang et al., 2004; Santivarangkna et al., 2007). The optimum residual moisture content depends on the composition of the fluid in which the bacteria are dried, the storage atmosphere, and the species of bacteria (Wang et al., 2004). The moisture content and water activity of dried probiotic cultures must be kept constant in order to achieve long-term storage stability (Chavez and Ledeboer, 2007). In this case, water activity and moisture content should be below 0.25 and 5%, respectively. To better preserve microbial cells in dried dairy products, a residual moisture content of 4% or water activity of 0.2 is reported (Kearney et al., 2009; Masters, 1985). Powder composition, oxygen content and glass transition temperature also have significant influences on the survival of probiotics in dried powders. Meng et al. (2008) reported that high viability of freeze-dried *L. rhamnosus* GG powders in trehalose, lactose/trehalose and lactose/maltose related to their high transition temperature. Mass transfer rates are slower in a glassy state matrix, which is more stable to detrimental processes. Therefore, dried powders must be in the glassy state. As mentioned, relative humidity greatly affects survival of dried starter cultures during storage. High relative humidity causes caking phenomenon in dried powders. Caking is one of the most undesired conditions for the survival of probiotics. Therefore, it is essential that the surrounding relative humidity is kept under the critical equilibrium value that corresponds to the glass/rubber transition. Since vacuum packaging also removes air humidity, packaging of dried probiotics under vacuum is suggested (Chavez and Ledeboer, 2007).

CHAPTER 3

ULTRA-SONICATION EFFECTS ON QUALITY ATTRIBUTES OF MAO (*ANTIDESMA BUNIUS* L.) JUIC

3.1 Introduction

In Thailand, mao, maoluang or maoberry (*Antidesma bunius* L.) products are popularly consumed, particularly as pasteurized juice. Mao juice is commercially recommended as a functional beverage because of its high levels of antioxidants, particularly anthocyanins, phenolic acids, flavonoids, and ascorbic acid (Butkhup and Samappito, 2008). These compounds are well known to help prevent cancer, diabetes, and cardiovascular and inflammatory diseases (Jorjong et al., 2015). The beneficial effects of mao by-products have also been reported in several studies. Puangpronpitag et al. (2011) found that the extracts of mao seed and skin-pulp residue showed anti-apoptotic and anti-inflammatory effects in human breast epithelial cells, and also displayed inhibitory effects against some pathogenic and spoilage bacteria (Butkhup and Samappito, 2011). Kukongviriyapan et al. (2013) illustrated that mao pomace supplementation reduced blood pressure and improved the hemodynamic status of induced hypertensive rats.

Consumers today favor natural fresh foods with high nutritional values. Although pasteurization is one of the most popular methods used to successfully extend the shelf life of numerous fruit juices, this method generally damages the original qualities (i.e. color, flavor, taste etc.) and also causes losses in the antioxidant constituents of the products (Aadil et al., 2013). Therefore, a non-thermal technique such as ultra-sonication is an alternative to pasteurize processed fruit juices, without impairing their health benefits and consumer acceptance (Zafra-Rojas et al., 2013).

The effects of ultra-sonication on the characteristics of various berry juices such as blackberry, strawberry, and mulberry have been investigated by Engmann et al. (2015), Tiwari et al. (2009a) and Wong et al. (2010). The frequency of ultra-sonication is applied at 20 kHz to maintain the fruit's quality and reduce the incidence of decay and infection of microorganisms. However, no studies have investigated the effect of this novel processing technique on the qualities of mao juice. Hence, this study aimed to determine the alteration of physicochemical, antioxidative and microbiological

properties, as well as sensory attributes of mao juice after ultra-sonic treatments at a frequency of 20 kHz and different amplitude levels of 20–80% for 30 min.

3.2 Materials and methods

3.2.1 Mao juice preparation

Mao fruits were harvested from an orchard in Sakon Nakhon province, Northeastern Thailand, from August to September of 2014. The fruits were washed and preserved at -20°C until further experiments. Juice was extracted using a fruit juice extractor. The extract was mixed with distilled water at a ratio of 1:1 (w/w) before adjusting to the total soluble solids of 12°Brix with fructose sugar. A 100-ml of mao juice was treated in a 150-ml glass bottle using a high intensity ultra-sonic processor (VCX 130 PB 130 W, Sonics and Materials Inc., Newtown, CT). The ultra-sonic probe was immersed into the juice to half the depth of the sample which produced a 20 kHz wave frequency. The mao juice was exposed to different amplitude levels of 20–80 % for 30 min (Santhirasegaram et al., 2013). At the final stage of processing, the temperatures of the samples increased from the baseline of 10.79±0.53°C to 41.63±2.36, 51.71±1.50, 58.70±1.23, and 75.46±1.63°C for ultra-sonication with wave amplitudes of 20%, 40%, 60%, and 80%, respectively. For thermal treatment, a 100-mL of fresh juice was filled in a 150-mL glass bottle and heated at 75.34±2.48°C for 30 min. Afterward, all the processed juices were cooled down to 10.27±1.40°C using an ice-water bath prior to analyzing.

3.2.2 Color measurement

Color parameters of fresh, ultra-sonic and thermally treated juices were measured using a colorimeter (Minolta Chroma Meter CR-300, Kyoto, Japan). Analytical data were expressed as *L* (lightness), *a** (redness) and *b** (yellowness) parameters. In addition, chroma value (*C**) and total different colors (ΔE) were calculated using equations (1) and (2).

$$\text{Chroma value } (C^*) = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$\text{Total different colors } (\Delta E) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

3.2.3 Measurements of total soluble solids, pH, and viscosity

Total soluble solids and pH values of all the samples were determined using a refractometer (N-10E, Atago, Japan) and a Sartorius PB-20 pH meter (Sartorius, Gottingen, Germany). Dynamic viscosity of fresh and treated samples was measured using a control stress AR 2000 rheometer (TA Instruments, Inc., New Castle, DE) combined with commercial computer software (Rheology Advantage Analysis software Version 4.1). A concentric cylinder geometry (stator inner radius 15 mm, rotor outer radius 14 mm, cylinder immersed height 42 mm, gap 5,920 μm) was used. Juice (19.6 ml) was poured into the stationary cup and allowed to equilibrate to 25±2°C, which was controlled by a circulating water system. Viscosity was calculated from the average of five points of the flow curves obtained in the shear rate range between 1 and 10 s^{-1}.

3.2.4 Crude enzyme extraction

To extract the crude enzymes, 10 mL of samples were stirred with a mixture of 40 ml of 50 mM potassium phosphate, 1 M potassium chloride and 2% polyvinylpolypyrrolidone at 150 rpm for 20 min. The mixed solution was centrifuged at 4,200 rpm for 20 min before filtering through Whatman paper No. 1 (Apichartsrangkoon et al., 2013).

3.2.5 Polyphenol oxidase (PPO) activity

PPO activity was determined according to the procedure described by Apichartsrangkoon et al. (2013). Briefly, 0.05 ml crude enzyme extract was poured into a mixture of 2.2 ml of 0.1 M potassium phosphate buffer (pH 6.5) and 0.25 ml of 0.2 M pyrocatechol. The absorbance of the mixed solution was recorded every 1 min for 5 min using a UV-Vis spectrophotometer (Perkin Elmer series Lambda 35, USA). One unit of enzymatic activity was defined as an increase of 0.1 unit of absorbance per min at 420 nm.

3.2.6 Peroxidase (POD) activity

POD activity was measured following a modified method of Apichartsrangkoon et al. (2013) using a spectrophotometer at 470 nm. A 0.1-ml supernatant of crude enzyme extract was added into a mixture of 2.15 ml of 0.01 M sodium acetate buffer (pH 6), 0.25 ml of 0.1% hydrogen peroxide and 0.5% guaiacol. Subsequently, the

increase in absorbance was recorded at every 1 min for 5 min. One unit of POD activity was defined as an increase of 0.1 unit of absorbance per min.

3.2.7 Determination of total anthocyanins

Total anthocyanins were analyzed according to the pH differential method (Lee et al., 2005). First, 0.2-ml of the juice was added into 1.8 ml of 0.03 M potassium chloride buffer (pH 1.0) or into 1.8 ml sodium acetate buffer (pH 4.5). Consequently, the absorbance of the well-mixed solution was measured at 520 and 700 nm using a spectrophotometer. Total anthocyanins were expressed as cyanidin 3-glucoside equivalent (mg CE/100 ml).

3.2.8 Determination of total phenolic compounds

Total phenolic compounds were determined following the modified method of Chaikham and Apichartsrangkoon (2012). Accordingly, 2 ml of the juice were mixed with 8 ml of 100% cooled ethanol for 20 min before centrifuging at 4,500 rpm for 10 min. After that, 0.5 ml of supernatant was poured into 2.5 ml of 10 % Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) and allowed to react for 5 min. A 2-ml of saturated sodium carbonate solution was then added to the mixture and held for 2 h at room temperature. The apparent blue complex solution was measured at 765 nm using a spectrophotometer. Total phenolic contents were expressed as mg gallic acid equivalent per 100 ml sample (mg GAE/100 ml).

3.2.9 Determination of ascorbic acid

The concentrations of ascorbic acid in fresh and ultra-sonic treated mao juices were determined using a HPLC system (Chaikham and Apicartsrangkoon 2012). Before injection, 2 ml of the juice were mixed with 18 ml of diluted sulphuric acid (pH 2.2; Merck, Munich, Germany) by stirring at 150 rpm for 15 min, and centrifuged at 4,500 rpm at 4°C for 10 min. Next, the supernatant was filtered through a 0.20-μm nylon membrane (Vertical, Bangkok, Thailand). The HPLC system (Shimadzu LC-10AD; Shimadzu, Kyoto, Japan) consisted of a low-pressure pump and a photodiode array detector (SPD-M20A; Shimadzu) adjusted to a λ_{max} 250 nm. Chromatographic separation was performed with a C18 column (YMC-Pack ODS-AM, 5 μm, 4.6 mm ID × 250 mm; YMC, Kyoto Japan). The isocratic system used 0.1 M acetic acid (Merck) in deionized water (RCI Lab-Scan, Bangkok, Thailand) as a mobile phase with a flow rate

of 1.5 ml/min at 30°C. A 20-μl filtrate was injected into the column. The peak area of each component was determined and converted to concentration.

3.2.10 Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was determined according to the procedure of Chaikham and Apicartsrangkoon (2012). In brief, 2 ml of mao juice were poured into 8 ml of 100 % methanol for 10 min before mixing for 10 min, and then was centrifuged at 4,500 rpm for 10 min. Afterward, 1.6 ml supernatant or methanol (control) was well-mixed with 0.4 ml of 1.5 μM DPPH radical in methanol, and allowed to stand for 30 min at room temperature before measuring the absorbance at 517 nm. DPPH radical scavenging activity (% inhibition) was calculated using equation (3), where A_0 = absorbance of the control and A_1 = absorbance of the sample.

$$\text{DPPH radical scavenging activity} = [1 - (A_1/A_0)] \times 100 \quad (3)$$

3.2.11 Ferric-reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was analyzed using the method of Benzie and Strain (1996). Accordingly, 1 ml of the sample was mixed with 9 ml deionized water and filtered through a Whatman paper No. 1. After that, 3 ml of FRAP reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-triazine solution and 20 mM $FeCl_3.6H_2O$ solution) was added into the filtrate before incubating at 37°C for 30 min. The absorbance of the mixture was measured at 593 nm. The antioxidant capacity based on the ability to reduce ferric ions was expressed as mM $FeSO_4$ per 100 ml sample (mM $FeSO_4$/100 ml).

3.2.12 Microbiological assessments

Total plate counts, yeasts and molds, and fecal coliforms in fresh and processed mao juices were determined following the Bacteriological Analytical Manual (US Food and Drug Administration, 2001).

3.2.13 Sensory evaluation

Sensory evaluation was carried out by consumers recruited within a 5-km radius of the research center. Thirty trained volunteers were enrolled and asked to rate

the degree of preference between the samples. A 9-point hedonic scale test; 9 = like extremely much, 5 = neither like nor dislike and 1 = dislike extremely much was applied. Triplicate sets of 20 ml of fresh, heated and ultra-sonicated mao juices were served at 4°C during the evaluation. Before starting the evaluation, participants were instructed to rinse their mouths with water after tasting the sample, as this could influence the result.

3.2.14 Statistical analysis

Data consist of the means of six replications with standard deviations. Analysis of variance (ANOVA) was carried out using a SPSS Version 11.5. Differences among treatment means were compared by Duncan's multiple range tests with a level of significance of $P < 0.05$.

3.3 Results and discussion

3.3.1 Physicochemical qualities

Color is one of the most important visual criteria to which consumers refer with regards to the overall fruit juice quality. Table 3.1 depicts the effects of ultra-sonication and heating on color parameters in mao juice. For ultra-sonicated samples, it was found that the lightness (*L* parameter) in the juice tended to decrease with the rising wave amplitudes, while an increase in other parameters were observed. In overall, mao juices treated at 80% amplitude and at 75ºC were significantly lower in *L* value than the others, but showed the highest values of *a** (reddish), *b** (yellowish), *C** (chroma) and ΔE (total different colors) parameters. As indicated by the reduction of the lightness parameter, the increasing darkness and yellowish color in the juices could be caused by enzymatic browning involving PPO and POD, because the residual activities of both enzymes still remained in the products (Table 3.2). In addition, the decreased lightness and increased redness values in the ultra-sound treated juices might be because of the better extraction of anthocyanin pigments. Anthocyanins are the predominant polyphenolic compounds present in mao fruits and are responsible for the redness and blueness of this fruit (Butkhup and Samappito, 2008). These results were confirmed by the levels of total anthocyanins in the samples which were enhanced by ultra-sonic conditions (Table 3.3). Therefore, anthocyanins may also protect the darkness effects from PPO. The ultra-sonication effects on color parameters of other fruit juices were previously reported by several researchers. For instance, Abid et al. (2013) and Bhat et

al. (2011) respectively illustrated that the *L* and *a** parameters of ultra-sonicated apple and kasturi lime juices were lower than the fresh juices, whereas the *b** parameters increased noticeably. The significant decreases of those parameters in both grapefruit and Chokanan mango juices (Aadil et al., 2013; Santhirasegaram et al., 2013) were also observed. Moreover, Engmann et al. (2015) discovered that the *C** and ΔE values for most of the ultra-sonic treated mulberry juices were significantly higher than the control.

Table 3.1 Color parameters of fresh, heated and ultra-sonicated mao juices

Samples	Color parameters				
	L	*a**	*b**	*C**	ΔE
Fresh juice	16.59±0.30^{a}	2.53±0.06^{c}	2.31±0.10^{c}	3.43±0.05^{cd}	-
HT 75ºC	13.92±0.27^{d}	2.77±0.06^{b}	2.50±0.03^{b}	3.73±0.03^{b}	2.83±0.26^{a}
US 20%	16.55±0.06^{a}	2.49±0.04^{c}	2.27±0.04^{cd}	3.37±0.05^{d}	0.24±0.05^{d}
US 40%	16.03±0.11^{b}	2.79±0.07^{b}	2.19±0.04^{d}	3.54±0.08^{c}	0.80±0.11^{c}
US 60%	15.90±0.07^{b}	2.63±0.09^{bc}	2.25±0.08^{cd}	3.47±0.10^{cd}	0.87±0.07^{c}
US 80%	14.48±0.15^{c}	3.11±0.10^{a}	2.68±0.03^{a}	4.11±0.05^{a}	2.35±0.15^{b}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). HT is heat treatment and US is ultra-sonication.

The influences of heat and ultra-sonic treatments on total soluble solids, pH and viscosity of mao juice are displayed in Table 3.2. The results indicated that thermal and ultra-sound processes did not induce any changes in the total soluble solids, pH and viscosity of the samples ($P > 0.05$). These values remained stable when the wave amplitudes increased. Our findings are supported by several other studies. For instance, Zafra-Rojas et al. (2013) reported that ultra-sonication treatments had no effect on the pH of purple cactus juice. In addition, Tiwari et al. (2008a) and Bhat et al. (2011) respectively found that orange and kasturi lime juices showed no significant changes in total soluble solids and pH after ultra-sonic processing. Cruz-Cansino et al. (2013) illustrated that the ultra-sonic process induced slight changes in green cactus pear juice pH and total soluble solids. In this study, the dynamic viscosity of processed mao juices did not change when compared to the control. This outcome was different to a report of Santhirasegaram et al. (2013), which revealed that the application of ultra-sonic processing decreased the viscosity of Chokanan mango juice. They suggested that

ultra-sonic cavitation caused the breakdown of large macromolecules and particles such as pectin in the juice, and decreased the viscosity of the juice. Ultra-sonication for 30 min reported to improve the quality of juice compared to control by inactivating spoilage microorganisms and increasing in extractability of bioactive compounds. Thus, 30 min treatment was also chosen in our study.

3.3.2 PPO and POD activities

PPO and POD are the principal enzymes involved in the browning reactions of non-thermally processed fruit juices. Browning is an important aspect that influences consumer acceptance. The residual PPO and POD activities in fresh, thermally and ultra-sonic treated mao juices are described in Table 3.2. It was found that increasing the wave amplitudes reduced the activities of both enzymes, especially the enzymatic inactivation of POD. Inactivation levels of PPO and POD by thermal and ultra-sonic processing at 80% amplitude were markedly higher than those of the other juices ($P <$ 0.05). The application of ultra-sonication with different amplitudes of 20%, 40%, 60% and 80% inhibited PPO by 1.14%, 5.66%, 18.62% and 47.82%, and POD by 10.83%, 15.41%, 34.20% and 73.02%, respectively. In this case, we noted that wave amplitudes had inhibitory effects on both of PPO and POD, but these were more pronounced for POD activity. Likewise, Rithmanee and Intipunya (2012) found that ultrasonic treatment at 100% amplitude and a frequency of 20 kHz for 30 min could inhibit PPO and POD activities in longan pulp by 70.68% and 94.06%, respectively, compared to an untreated sample. Costa et al. (2013) treated pineapple juice at 75% amplitude for 10 min, and they discovered that the PPO activity diminished by 20%. Moreover, Ercan and Soysal (2011) reported that POD activity in tomato extracts noticeably declined with increased ultrasonic amplitude power, and the reduction of POD activity was achieved at 50% and 75% amplitudes for 2.5 and 1.5 min, respectively. They revealed that physical stress because of bubble collapse can contribute toward enzymatic inactivation. Enzymatic inhibition can be affected by high shear generated by the interaction of cavitating bubbles with the acoustic field. da Rocha Cordeiro Dias et al. (2015) also explained that the extreme agitation created by microstreaming can disrupt Van der Waals interactions and hydrogen bonds in the polypeptide, resulting in protein denaturation. Temperatures above 60°C can also denature the protein structures of the enzymes, but may negatively affect consumer acceptance and nutritional values of the products.

Table 3.2 Physicochemical properties and enzymatic browning activities of fresh, heated and ultra-sonicated mao juices

Samples	Physicochemical properties			Enzymatic browning activity			
	TSS (°Brix)	pH	Viscosity (mPa.s)	PPO activity Unit/m/ml)	Relative activity (%)	POD activity (Unit/m/ml)	Relative activity (%)
Fresh juice	12.77±0.21^{a}	3.46±0.03^{a}	5.18±0.42^{a}	89.23±3.71^{a}	100.00±0.00^{a}	125.41±6.32^{a}	100.00±0.00^{a}
HT 75ºC	12.79±0.05^{a}	3.46±0.02^{a}	5.25±0.51^{a}	50.99±2.79^{c}	57.30±5.59^{e}	34.27±2.82^{d}	27.35±0.73^{e}
US 20%	12.73±0.12^{a}	3.44±0.02^{a}	5.55±0.34^{a}	88.16±1.95^{a}	98.86±2.08^{b}	111.72±3.89^{b}	89.17±3.65^{b}
US 40%	12.80±0.08^{a}	3.45±0.02^{a}	5.29±0.64^{a}	84.17±3.66^{a}	94.34±2.45^{c}	106.06±4.40^{b}	84.59±0.91^{c}
US 60%	12.84±0.10^{a}	3.45±0.01^{a}	5.43±0.22^{a}	72.48±2.59^{b}	81.38±6.01^{d}	82.29±2.84^{c}	65.80±5.49^{d}
US 80%	12.81±0.05^{a}	3.47±0.04^{a}	5.61±0.56^{a}	46.49±1.62^{d}	52.18±3.17^{f}	33.78±4.07^{d}	26.98±3.44^{e}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). TSS is total soluble solids, HT is heat treatment and US is ultra-sonication.

Table 3.3 Bioactive components and antioxidant capacities of fresh, heated and ultra-sonicated mao juices

Samples	Total anthocyanins (mg CE/100 ml)	Total phenolics (mg GAE/100 ml)	Ascorbic acid (mg/100 ml)	DPPH inhibition (%)	FRAP value (mM $FeSO_4$/100 ml)
Fresh juice	44.32±5.02^{a}	274.65±9.16^{b}	26.14±2.12^{a}	56.52±5.45^{a}	24.18±1.27^{a}
HT 75ºC	44.65±5.68^{a}	280.04±6.37^{b}	16.09±1.38^{c}	50.47±6.02^{b}	18.68±2.42^{c}
US 20%	45.80±3.61^{a}	290.16±5.62^{ab}	25.35±1.05^{a}	58.91±2.11^{a}	25.60±2.00^{a}
US 40%	48.12±4.30^{a}	288.59±8.12^{ab}	24.05±3.16^{a}	60.43±4.07^{a}	26.08±3.49^{a}
US 60%	50.63±3.18^{a}	300.12±6.89^{a}	25.07±2.08^{a}	62.14±1.92^{a}	23.95±2.68^{ab}
US 80%	49.01±6.75^{a}	292.88±5.21^{a}	18.19±2.33^{b}	59.12±2.85^{a}	22.32±0.86^{b}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). HT is heat treatment and US is ultra-sonication.

3.3.3 Bioactive components and antioxidant activity

We investigated the effect of heat and ultra-sonic treatments on the concentrations of some phytochemicals *viz.* total anthocyanins, total phenolic compounds and ascorbic acid in mao juice. The results in Table 3.3 depicted that the processing conditions had no significant influence on the total anthocyanins in mao juice ($P > 0.05$). Alighourchi et al. (2013) found that the contents of anthocyanins in ultra-sonicated pomegranate juices did not decrease substantially; in fact they increased slightly at some amplitude levels and times. However, Engmann et al. (2015) and Tiwari et al. (2008b) respectively reported the reduction of anthocyanins after ultra-sonic treatments in mulberry and strawberry juices. They explained that the degradation of these compounds might be related to oxidation reactions promoted by the interaction of free radicals formed during ultra-sonication.

Moreover, our results also demonstrated that an increase ($P < 0.05$) of total phenolic compounds occurred in the juices treated at 60% and 80% amplitudes compared with the other samples (Table 3.3). These components were also found to increase significantly in kasturi lime juice (Bhat et al., 2011), grapefruit juice (Aadil et al., 2013) and purple cactus pear juice (Zafra-Rojas et al., 2013) after ultra-sonic processing, compared with the fresh juices. The increased phenolic compounds in the products might be a result of the higher intensities of wave amplitudes which could enhance the interruption of plant-cell walls to facilitate the release of their contents (Chaikham and Prangthip, 2015).

Besides anthocyanins and phenolics, the results in Table 3.3 illustrated that the contents of ascorbic acid in mao juices treated at 20–60% amplitudes did not show any significant change ($P > 0.05$). At a higher amplitude however, it decreased significantly ($P < 0.05$). In this case, after processing at 80% amplitude for 30 min, the temperature of the sample markedly increased to roughly 75.46°C. The declining level of this component was also observed in the heated juice. Therefore, the reduction of ascorbic acid could be primarily because of degradation from the heat. Previously, Abid et al. (2013) found no significant change in ascorbic acid concentration in apple juice after ultra-sonication at a 70% amplitude, frequency of 25 kHz and a controlled temperature of 20°C for 30 min. This finding was similar to a report of Bhat et al. (2011) with kasturi lime juice. Under the same conditions, Aadil et al. (2013) revealed that sonication significantly improved ascorbic acid levels in grape juice by ~ 14.30% compared with the control. In contrast, Adekunte et al. (2010) found a significant decrease of this

component in tomato juice. Similarly, Lee and Feng (2011) depicted that ascorbic acid in orange juice was also reduced by processing, possibly because of the generation of free radicals. This suggests that heat and oxidation reactions are mainly responsible for ascorbic acid degradation during ultra-sonication treatments (de São José et al., 2014). Furthermore, several researchers compared the effects of ultra-sonic and thermal processing on the retention of ascorbic acid in fruit juices, such as tomato juice (Ercan and Soysal, 2011) and orange juice (Tiwari et al., 2009b). They concluded that thermal treatments on juices resulted in a significantly lower level of ascorbic acid than ultra-sonication treatment.

Antioxidants are reducing agents which possess the ability to protect humans and organisms from cell damage and homeostatic disruption caused by free radical-induced oxidative stress. The reducing properties of plant phytochemicals are related to the presence of phenolic constituents and some vitamins which exert their action by breaking the free-radical chain by donating a hydrogen atom (Jorjong et al., 2015). In this study, the antioxidant capacities including DPPH radical inhibition and FRAP values of fresh, thermally and ultra-sonic treated mao juices were investigated. Table 3.3 shows that the DPPH radical scavenging activities of ultra-sonicated mao juices were not significantly different ($P > 0.05$) from the fresh sample, and the percentages of radical inhibition ranged from 59.12±2.85% to 62.14±1.92%; whereas this value was apparently lower in heated sample ($P < 0.05$). In this case, the lowest FRAP value was found in the juices treated at 80% amplitude and at 75ºC respectively, which had significantly lower reducing power than the other batches ($P < 0.05$). This phenomena was interpreted by Namiesnik et al. (2013), who revealed that the DPPH method is generally employed with aqueous-organic extracts containing hydrophilic and lipophilic compounds, while FRAP assay is appropriate only for hydrophilic compounds. However, the processed juice still retained a high level of antioxidant activity. Identical results were found with ultra-sonicated kasturi lime juice (Bhat et al., 2011) and purple cactus pear juices (Zafra-Rojas et al., 2013).

Table 3.4 Microbiological qualities of fresh, heated and ultra-sonicated mao juices

Samples	Total plate counts (CFU/ml)	Yeasts & molds (CFU/ml)	Fecal coliforms (CFU/ml)
Fresh juice	$5.15\pm1.23\times10^{6\ a}$	$6.42\pm1.40\times10^{4\ a}$	$3.50\pm0.80\times10^{2\ a}$
HT 75ºC	nd ^{c}	nd ^{e}	nd ^{c}
US 20%	$3.83\pm2.01\times10^{4\ a}$	$2.18\pm0.57\times10^{2\ b}$	$0.80\pm0.50\times10^{\ b}$
US 40%	$8.24\pm1.52\times10^{2\ b}$	$0.98\pm0.15\times10^{2\ c}$	nd ^{c}
US 60%	$2.49\pm0.74\times10^{2\ d}$	$0.52\pm0.23\times10^{2\ d}$	nd ^{c}
US 80%	nd ^{c}	nd ^{e}	nd ^{c}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). HT is heat treatment, US is ultra-sonication and nd is not detected.

3.3.4 Microbiological assessments

The microbiological count results are exhibited in Table 3.4, where the initial amounts of microorganisms present in fresh mao juice were 6.71, 4.81, and 2.54 log CFU/mL for total plate counts, yeasts and molds, and fecal coliforms, respectively. After ultra-sonication at 80% amplitude and heating at 75ºC, all the indicator microbes in the treated juices were acceptably eliminated by the processing and complied with the limits of the Thai Community Product Standard (TCPS No. 486/2004) for ready-to-drink mao juice (Thai Industrial Standard Institute, 2004). Our results were similar to that observed by Abid et al. (2013) with apple juice. The microbial reduction was because of the enhancement of biocides by cavitation. The formation of free radicals and hydrogen peroxide during ultra-sonication treatment can lead to microbial elimination. In addition, cavitation also creates shock waves that ultimately cause damage to the living microbes, in particular vegetative cells (Abid et al., 2013; Bhat et al., 2011).

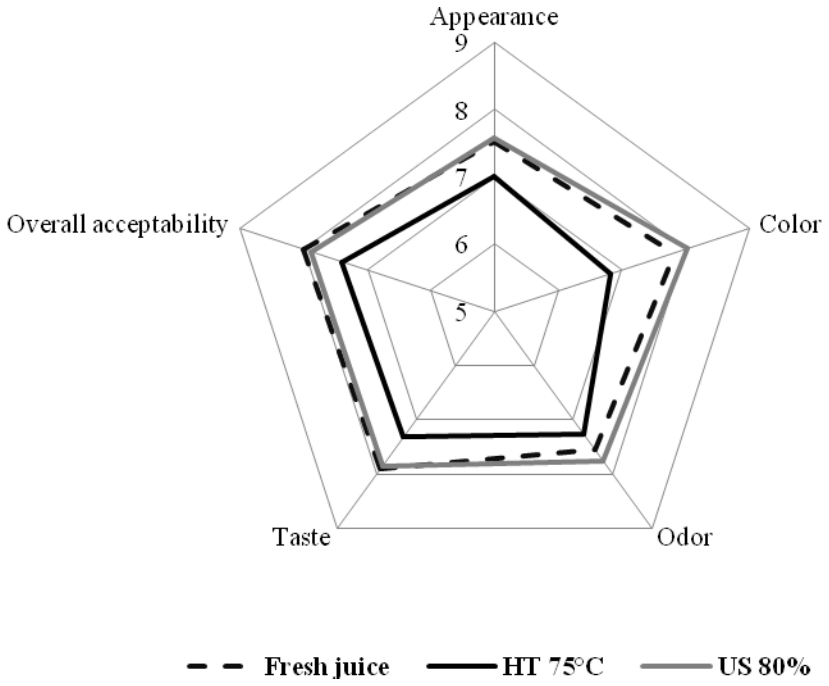

Figure 3.1 Sensory evaluation of fresh, heated and ultra-sonicated mao juices. HT is heat treatment and US is ultra-sonication

3.3.5 Sensory evaluation

With regards to the microbiological assessments, it was found that ultra-sonication at 80% amplitude and heating completely eliminated the general microorganisms in mao juice (Table 3.4). Therefore, these samples were selected for sensory evaluation compared to control (fresh juice). Figure 3.1 elucidates the sensorial attributes of fresh and processed juices which were evaluated by 30 trained panelists. The data showed that the liking scores of appearance, color, odor, taste, and overall acceptability of ultra-sonic treated juice were not significantly different ($P > 0.05$) compared to the fresh sample (appearance = 7.53–7.58, color = 7.82–8.03, odor = 7.54–7.76, taste = 7.85–7.90, and overall acceptability = 7.88–8.01), and were significantly higher ($P < 0.05$) than those from the heated product (appearance = 7.02, color = 6.83, odor = 7.25, taste = 7.32, and overall acceptability = 7.39). Although an alteration of color parameters was observed after ultra-sonication (Table 3.1), there were no major changes in the appearance of the juice, as indicated by the sensorial scores. Similar outcomes were observed between fresh, pasteurized and ultra-sonicated apple juice, where ultra-sonic treated juice was also more accepted than thermally treated juice (Ertugay and Başlar, 2014).

3.4 Conclusion

The results from this experiment revealed that no significant changes in total soluble solids, pH, and viscosity of thermally and ultra-sonic treated mao juices could be observed. Ultra-sonication had a noticeable effect on color parameters, but sensorial characteristics of treated juice were no different from fresh juice. However, variations in the ripeness of mao used in this study could be a limitation on the parameters investigated in this study. Although pasteurization is normally applied to extend shelf-life of fruit juices, this method damages the desired characteristics and antioxidant constituents of fruit juice products. Ultra-sonication could be an alternative mao processing method to obtain a juice with high retention of bioactive compounds and antioxidant capacities, and low residual PPO and POD activities as well as microbial counts.

CHAPTER 4

SURVIVAL OF *LACTOBACILLUS CASEI* 01 IN PROBIOTIC-SUPPLEMENTED MAO JUICE POWDER DURING STORAGE

4.1 Introduction

The products that are supplemented with probiotics, particularly in the genera *Lactobacillus* and *Bifidobacterium*, are playing an important role in the healthy food industry. This may be because the probiotics have been proved to provide health benefits for the host when administered alive and in the appropriate quantities (Chaikham, 2015a; Huang et al., 2017). The minimum recommended dose for live probitoics was between 6 and 7 log CFUs per gram or milliliter of the products at the time of consumption (Nualkaekul et al., 2013). In recent years, the manufacturing of such probiotic-supplemented food products essentially requires the production of cell cultures for direct inoculation and the dehydration of probiotic cells (Salar-Behzadi et al., 2013). In addition, spray drying is an important approach for the production of probiotic powders. This method offers the benefit of minimal time- and cost-consuming production of the cultures in large-scales as well as the easy and inexpensive shipping and storage of the dried cultures (Silva et al., 2011; Heidebachm et al., 2012). Moreover, this immobilization method can be successfully applied for protecting the probiotic cells during processing and moving through the harsh environments of digestive system (Chaikham et al., 2017). However, application of high temperature during the process of spray drying can cause critical damage to the bacterial cells. Cell injury and loss of viability have been reported as common negative side effects due to drying process (Silva et al., 2011). In addition, Salar-Behzadi et al. (2013) revealed that the production yield of probiotic powder depends on the microbial species and strains, the protective carriers and the processing temperatures. Silva et al. (2011) reported that heat stress during spray drying may also impact the stability and the survival rate of probiotics during storage.

Currently, there are only few studies on the fruit juices that containing probiotic bacteria and being powdered by spray drying. Indeed, probiotic-fruit juice powders can retain most of the natural bioactivity found in the original fresh fruit with the additional benefit of a longer storage life (Barbosa and Teixeira, 2017; Huang et al., 2017). They

also offer a great benefit to the consumers who are lactose intolerant or following vegetarian diet (Chaikham et al., 2017). In Thailand, a novel, high-quality functional drink from mao or maoluang fruits (*Antidesma bunius* (Linn) Spreng) that are rich in phytochemical antioxidants is currently becoming popular among the consumers of all ages (Chaikham, 2015b; Chaikham and Baipong, 2016). Jorjong et al. (2015) reported that mao fruits contained large amounts of anthocyanins (69 g/100 g DW), flavonoids (715 mg/100 g DW), phenolic acids (398 mg/100 g DW), and also high levels of antioxidant capacity as shown by FRAP, TEAC ($ABTS^{\bullet+}$ radical cation assay) and VCEAC (DPPH assay) values at approximately 24 mmol Fe(II)/g DW, 30 mmol TE/g DW and 68 mmol VCEAC/g DW, respectively. The other parts of mao have also been evaluated. Choi and Hwang (2005) reported that *A. bunius* leaf extract could inhibit NO release in RAW264.7 cells and exhibit high level of antioxidant activity. Also, Butkhup and Samappito (2011) found that mao residues contained significant amounts of phenolic compounds and also antimicrobial activities against some pathogenic bacteria. Therefore, it is interesting to supplement mao juice with potential probiotics for production of the functional beverage.

The objective of this research was to monitor the viability of *Lactobacillus casei* 01 cells in probiotic-supplemented mao juice powder with different packaging conditions during storage at 4 and 37ºC for 12 weeks. Changes of physicochemical qualities and some bioactive compounds in the product were also evaluated.

4.2 Materials and methods

4.2.1 Probiotic strain and cultivation

Freeze dried *L. casei* 01 culture was purchased from Chr. Hansen (Hørsholm, Denmark). Dried cells (0.5 g) was rehydrated in MRS broth (50 ml) (Hi-Media, Mumbai, India), shaken for 5 min, and incubated at 37ºC for 20 h in an anaerobic jar. After that, the bacterial culture was activated by mixing with MRS broth at 1% (v/v) and incubating anaerobically at 37ºC for 14 h. For separation of cell pellet, the activated culture was centrifuged at 4000 rpm for 20 min and washed twice with 0.85% (w/v) sterile saline water (Hi-Media, Mumbai, India) before being diluted using sterile saline water to be approximately 10^{12} CFU/ml (Chaikham, 2015a).

4.2.2 Preparation of mao juice

Mao fruits were harvested from the mao germplasm collection in Rajamangala University of Technology Isan, Sakon Nakhon province, Thailand, then washed twice and extracted using a fruit juice extractor. Subsequently, the juice was pasteurized using thermostatic water bath at 90°C for 1 min, then cooled down to around 20°C, and finally stored in a refrigerator until required (Chaikham and Baipong, 2016). This thermal pasteurization was found to effectively eliminate the indicator microbes in mao juice such as total aerobic bacteria, *Escherichia coli*, yeasts and moulds (undetectable).

4.2.3 Spray drying of probiotic-supplemented mao juice

To produce the powder, pasteurized mao juice was blended with 15% (w/v) maltodextrin (10 DE) and 1% (v/v) probiotic culture (of which cell counts were approximately 10^{10} CFU/ml). The mixture was then fed into a spray dryer (JCM Engineering concept, Bangkok, Thailand) which was equipped with a fluid atomizer that had internal diameter of 5 mm. The drying conditions were 30°C feeding temperature, 1 L/h feeding rate, 15 psi atomizing pressure and 160°C hot-air-inlet temperature to generate 80°C outlet temperature (Chaikham et al., 2017).

4.2.4 Storage conditions

Spray dried sample powder was packed into laminated bags with or without vacuum condition, and then stored at 4 and 37ºC for 12 weeks. The powders were sampled every 2 weeks for evaluating the number of viable cells and the changes of both physicochemical qualities and bioactive compound contents.

4.2.5 Quantification of viable probiotic cells during storage

To release the probiotic cells, 1 g of sample powder was mixed with 9 ml of 0.1 M sterile phosphate buffer (pH 7) (Merck, Munich, Germany) for 10-15 min using a stomacher (IUL Instruments, Barcelona, Spain). After that, the mixture was 10-fold diluted using 0.1% (w/v) sterile peptone water (Hi-Media, Mumbai, India) before spreading onto MRS agar (Hi-Media, Mumbai, India) and incubating at 37°C for 48 h in anaerobic jar. The colonies were counted and calculated as CFU/g sample.

4.2.6 Determinations of bulk density and solubility

Bulk density of the samples was determined according to the modified method of Goula and Adamopoulos (2005). In brief, 5 g of sample powder were added to a 10 ml cylinder and shaken on a vortex vibrator for 2 min. The ratio between the mass of the powder and the volume occupied in the cylinder was indicated as the bulk density value (g/ml).

To determine the solubility, 1 g of sample powder was mixed with 100 ml of distilled water using a magnetic stirrer at a medium speed for 10 min. Then, the mixture was centrifuged at 4000 rpm for 5 min, and 25 ml of supernatant were transferred to each Petri dish, which was dried at 100°C for 12 h using a hot air oven. The solubility (%) was calculated as the weight difference (Cano-Chaucab et al., 2005).

4.2.7 Measurements of moisture content and water activity

The measurement of moisture content was carried out by placing 5 g of sample powder in a hot air oven at 100°C until reaching a constant weight (AOAC, 2000). Water activity (a_w) of the powder was measured using a water activity meter (AquaLab Series 3, Decagon Devices, Inc., Pullman, WA, USA).

4.2.8 Determination of total phenolic contents

According to the modified method of Chaikham and Baipong (2016), 5 g of sample powder were stirred with 25 ml deionized water for 30 min using a magnetic stirrer. After that, 5 ml-aliquot of the mixture were well-mixed with 15 ml of absolute ethanol for 10 min before centrifugation at 4,000 rpm for 10 min. One ml of the supernatant was added to 5 ml of 10% Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), mixed with 4 ml of saturated sodium carbonate solution (Ajax, Sydney, Australia), kept in the dark for 2 h, and then measured for the absorbance at a 765 nm using a Perkin Elmer UV WINLAB spectrophotometer (Perkin Elmer, Inc., Waltham, MA, USA). Total phenolic contents were expressed as mg GAE (gallic acid equivalent)/g powder.

4.2.9 Determination of ascorbic acid

Briefly, 5 ml-aliquot of the mixture (Section 4.2.8) was added to 20 ml of diluted sulphuric acid (pH 2.2; Merck, Munich, Germany) for 10 min and filtered through 0.20-μm nylon membrane (Vertical, Bangkok, Thailand). Subsequently, 20 μl of the filtrate

were injected to HPLC system (Shimadzu LC-10AD; Shimadzu, Kyoto, Japan), following the modified protocol of Chaikham and Baipong (2016). The isocratic system used 0.1 M acetic acid (a mobile phase) with a flow rate of 1.5 ml/min at 30°C. The peak area of *L*-ascorbic acid was identified and expressed as mg/g concentration.

4.2.10 Determination of total anthocyanins

Two ml-aliquot of the mixture (Section 4.2.8) were well-mixed with 8 ml of 0.03 M potassium chloride buffer (a mixture of 1.9 g of KCl and 980 ml of deionized water; pH 1.0) or 8 ml of 0.4 M sodium acetate buffer (a mixture of 54.4 g of $CH_3CO_2Na.3H_2O$ and 960 ml of deionized water; pH 4.5). The absorbance values of both mixtures were measured at 520 and 700 nm, respectively. Total anthocyanin contents (mg CE/100 g) were calculated [total anthocyanins = $(A \times MW \times DF \times 10^3)/\varepsilon \times 1$], where, CE is cyanidin 3-glucoside equivalent, A is the absorbance $[(A_{\lambda vis\text{-}max})_{pH\ 1.0} - (A_{\lambda vis\text{-}max})_{pH\ 4.5}]$, MW is the molecular weight of CE (449.2 g/mol), DF is the dilution factor and ε is an extinction coefficient of CE (26,900 L × cm^{-1} × mol^{-1}) (Lee et al., 2005).

4.2.11 Statistical analysis

The experiments were performed at least in triplicate and the results were expressed as means ± standard deviations. Analysis of variance (ANOVA) was carried out using IBM SPSS Version 23 (SPSS Inc., Chicago, IL, USA). Also, Duncan's multiple range tests were used to compare the significant differences among the treatment means at $\alpha = 0.05$.

4.3 Results and discussion

4.3.1 Viable cells of *Lactobacillus casei* 01 in probiotic-supplemented mao juice powder

Survival of the probiotic cultures during storage is highly crucial. As shown in Figure 4.1, after storage at 4ºC for 12 weeks, the viable cells of *L. casei* 01 apparently decreased ($P < 0.05$) around 5.5 and 4 log for normally and vacuum-sealed powders, respectively. For the samples kept at 37ºC, there were no probiotic cells under both sealing conditions after 4 weeks of storage. It was noticed that higher survival rates of *L. casei* 01 could be obtained at lower storage temperature and oxygen content. Anekella and Orsat (2014) found that the shelf-life of the probiotics in spray dried raspberry powder was reduced when storage temperature increased. Storage at lower

temperature ensured longer shelf-life and higher cell count retention at the end of 30 days. This result was similar to the previous report of Corcoran et al. (2004), who examined the spray-dried *Lactobacillus rhamnosus* GG in synbiotic powder products. Jonhson and Etzel (1993) and Chaikham (2015a) suggested that the levels of temperature and oxygen content in the package are critical for probiotics during storage. Oxygen may affect the probiotics by three mechanisms: (i) oxygen may directly be toxic to certain cells, (ii) some microbial cultures may produce toxic peroxides in the presence of oxygen and (iii) free radicals produced from the oxidation process may be toxic to the probiotic cells (Korbekandi et al., 2011). During storage, lipid oxidation of the probiotics' cell walls, which resulting in permanent damage, is considered as the major cause of short shelf-life of the spray dried cultures (Meng et al., 2008).

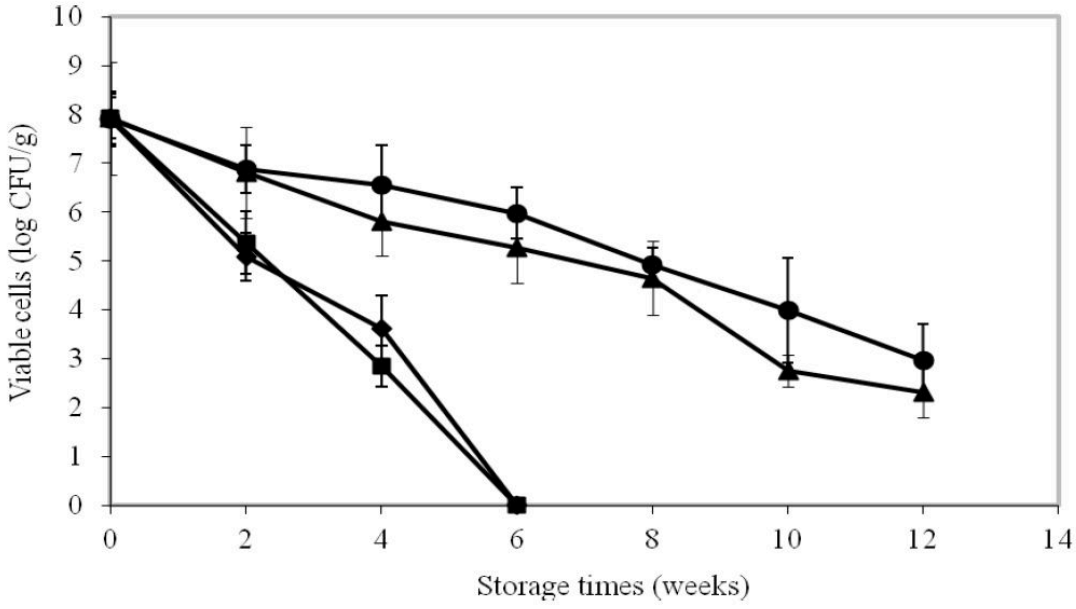

Figure 4.1 Survival of *L. casei* 01 cells in probiotic-supplemented mao juice powder during storage; (▲) normally sealed sample stored at 4°C, (●) vacuum-sealed sample stored at 4°C, (■) normally sealed sample stored at 37°C and (◆) vacuum-sealed sample stored at 37°C

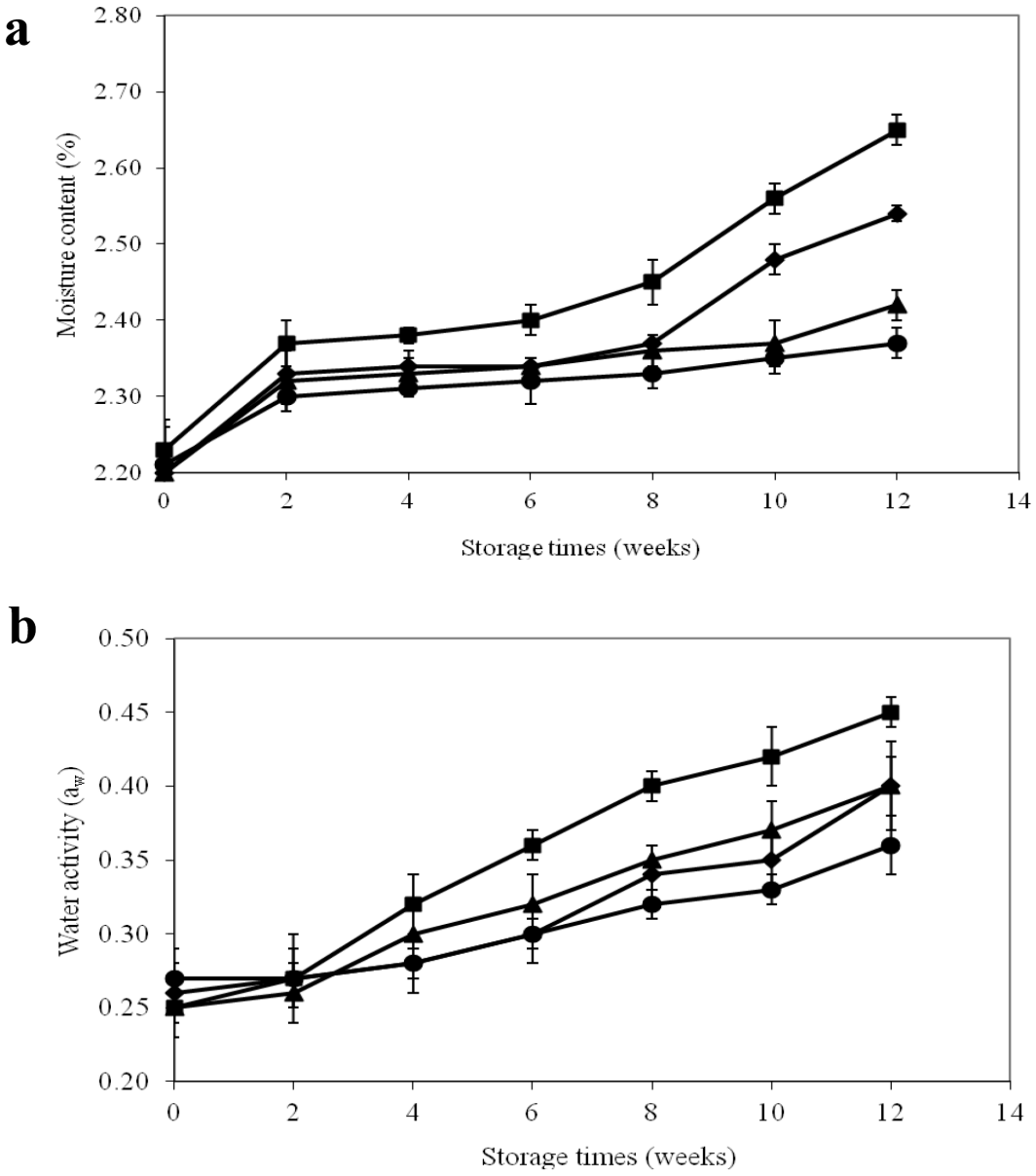

Figure 4.2 Changes of (a) moisture content and (b) water activity of probiotic-supplemented mao juice powder during storage; (▲) normally sealed sample stored at 4°C, (●) vacuum-sealed sample stored at 4°C, (■) normally sealed sample stored at 37°C and (◆) vacuum-sealed sample stored at 37°C

4.3.2 Changes of moisture content and water activity

Different storage temperatures and sealing conditions had significant effect ($P < 0.05$) on moisture content and a_w of probiotic-supplemented mao juice powder (Figure 4.2). The moisture content and a_w of the powder were found to significantly increase ($P < 0.05$) throughout the storage period. During the final stage of storage, the maximal increases of these values were found in the sample packed under normal-sealing

condition and stored at 37°C, while vacuum-sealed sample kept at 4°C was found to have the noticeably lower moisture content and water activity than the other treatments ($P < 0.05$). Relative humidity (RH) inside the package has been shown to influence the glass transition temperature (T_g) of the dried powders, as there was a report of high RH that induced caking in dried powders and subsequently losing of probiotic cells' viability (Barbosa and Teixeira, 2017). Chavez and Ledeboer (2007) and Ying et al. (2012) reported that increased a_w triggered a faster death rate of probiotics during storage as it stimulated the growth of other microorganisms as well as the occurrence of undesirable chemical reactions. Additionally, Peighambardoust et al. (2011) revealed that maintaining of the residual water was essential for spray dried probiotics to preserve the protein conformations for enzymatic activities, cell wall-lipid membrane structural stability, ribosomal functions, and any other organelles. However, a_w of the spray-dried powder was shown to be dependent on the cell suspension media, carriers, additives, and spray drying conditions (Wang et al., 2004). Furthermore, water activity and presence of oxygen were found to be important factors which affecting the survival of probiotics during storage (Weinbreck et al., 2010). Chavez and Ledeboer (2007) and Koc et al. (2010) suggested that $a_w < 0.3$ is essential for the survival of the probiotics during storage and the ideal a_w for most of *Lactobacillus* species should be between 0.11 and 0.23.

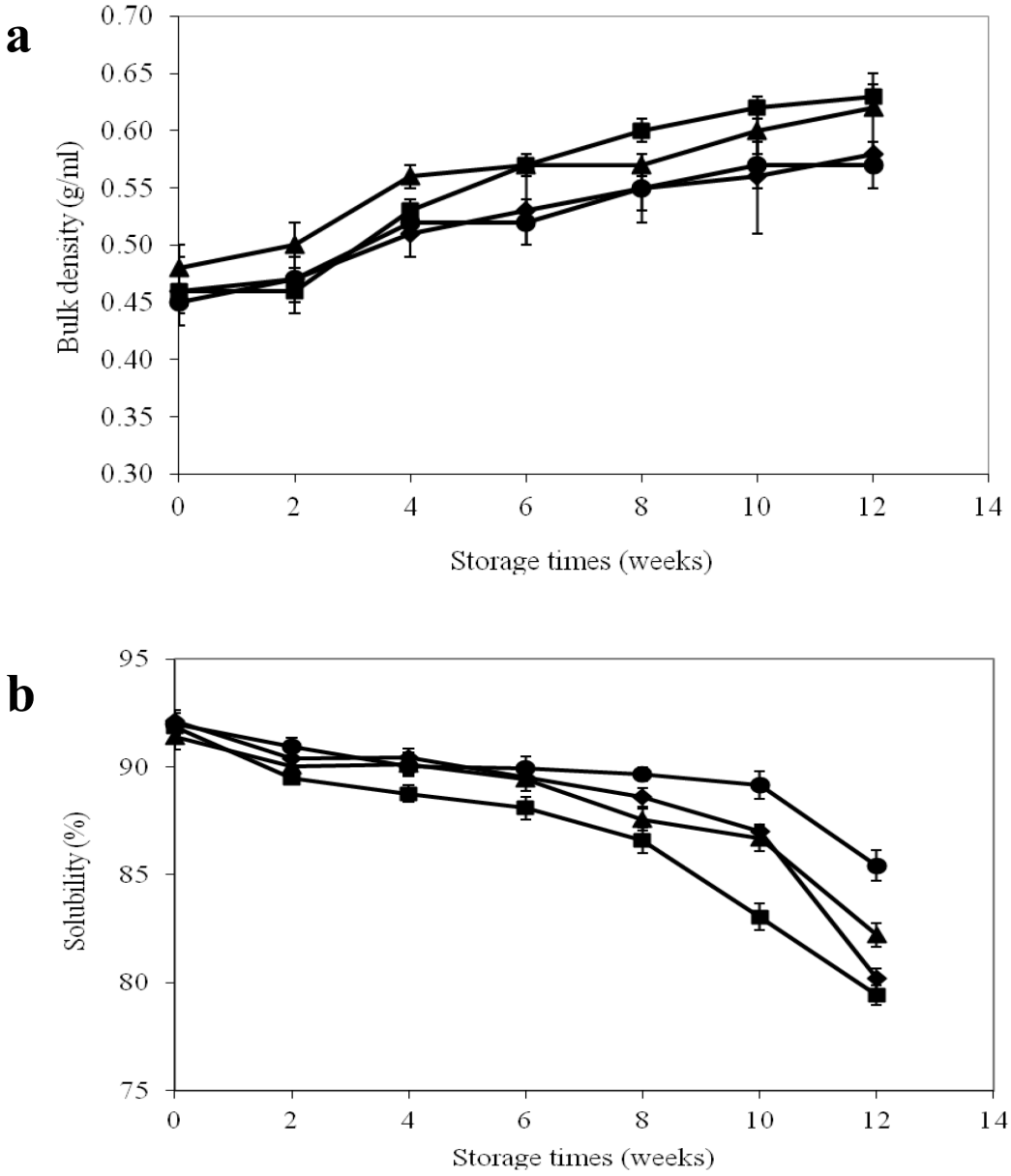

Figure 4.3 Changes of (a) bulk density and (b) solubility of probiotic-supplemented mao juice powder during storage; (▲) normally sealed sample stored at 4°C, (●) vacuum-sealed sample stored at 4°C, (■) normally sealed sample stored at 37°C and (◆) vacuum-sealed sample stored at 37°C

4.3.3 Changes of bulk density and solubility

Figure 4.3a shows that the bulk density of probiotic-supplemented mao juice powder slightly increased during the storage. At the last stage of storage, the levels of bulk density of powders stored under normal-sealing at 4 and 37°C and vacuum-sealing at 37°C were significantly higher ($P < 0.05$) than that of vacuum-sealed powder kept at

4°C. This result could be affected by the increase of moisture content in the samples (Figure 4.2a). Moreover, the results also exhibited that storage temperatures and sealing conditions could significantly affect ($P < 0.05$) the solubility of the samples (Figure 4.3b). The highest solubility values were achieved at the initial stage of storage; however, the solubility tended to decline afterwards, particularly for normally sealed powder stored at 37°C. In this case, moisture contents and a_w of the powders stored at week 12 were significantly higher ($P < 0.05$) than that of the other stages of storage (Figure 4.2), which suggesting that, at the end, the dried powders could have lower solubility. The similar trends of both parameters were reported by Wirjantoro and Phianmongkhol (2009) that investigating *B. bifidum* in yoghurt powder. This may be because that heterogeneous particle distribution could allow the rearrangement of the individual particles and consequently a more compact powder during storage (Fuchs et al., 2006; Flores-Belmont et al., 2015). For this reason, bulk density and solubility for the powders stored at week 12 were found to be significant lower ($P < 0.05$) than that of the rest. This problem may be resolved using different compositions of coating materials for probiotic-fruit juice powders during storage (Barbosa and Teixeira, 2017).

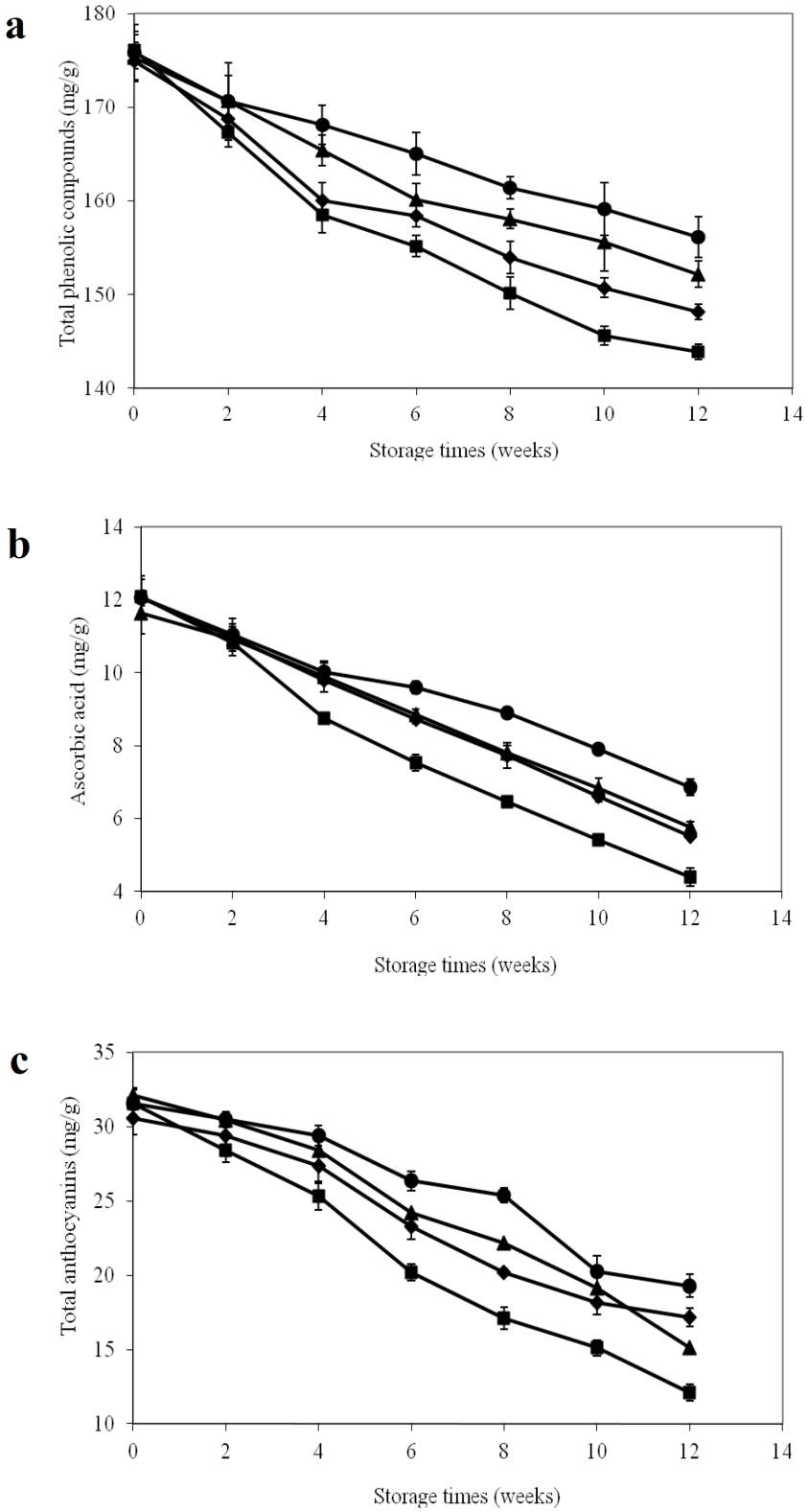

Figure 4.4 Levels of (a) total phenolic compounds, (b) ascorbic acid and (c) total anthocyanins of probiotic-supplemented mao juice powder during storage; (▲) normally sealed sample stored at 4°C, (●) vacuum-sealed sample stored at 4°C, (■) normally sealed sample stored at 37°C and (◆) vacuum-sealed sample stored at 37°C

4.3.4 Changes of bioactive compounds

Mao juice has been known as a great source of total phenolics, ascorbic acid and total anthocyanins (Chaikham, 2015b; Jorjong et al., 2015). As shown in Figure 4.4, total phenolics, ascorbic acid and total anthocyanins in all powders significantly diminished ($P < 0.05$) when the storage time rose. However, most of these bioactive compounds in the vacuum-sealed powders stored at 4ºC still remained at high levels throughout the storage period. Bakowska-Barczak and Kolodziejczyk (2011) found that the total phenolic acids and anthocyanins in immobilized black currant powders stored at 8 and 25ºC for 12 months were approximately 80% and 70%, respectively. The similar results were obtained by Fang and Bhandari (2011) and Ersus and Yurdagel (2007) that evaluating the spray dried bayberry and black carrot powders, respectively. Barbosa et al. (2015) reported that the contents of ascorbic acid in probiotic-orange powder were reduced by about 40% after storage 4ºC for 180 days. In this study, the levels of total phenolic compounds, ascorbic acid and total anthocyanins in normally sealed samples (high oxygen content) stored at 37ºC were also found to greatly reduced. Zorić et al. (2017) reported that sour cherry powder stored at lower temperatures were shown to have greater stability of phenolic compounds and antioxidant capacity (DPPH method), whereas the packaging type did not have significant effect on the retention of phenols and antioxidant capacity during the storage for 6 months. Zerdin et al. (2003) revealed that oxygen could diffuse into the matrix of the products during storage and oxidize the antioxidant compounds and degrade various bioactive compounds over the period of storage time. Santos and Silva (2008) suggested that ascorbic acid may be extremely degraded when being exposed to high oxygen content, temperature and light.

4.4 Conclusion

In this study, the probiotic-supplemented Mao juice powder in vacuum-sealed package stored at 4ºC could maintain high levels of *L. casei* viability and bioactive compounds than those in the other packaging conditions throughout the storage period. Some properties of the powders *viz.* moisture content, a_w, bulk density and solubility were found to change significantly. Refrigerated storage allowed the powder, especially that in the vacuum sealing package, to have the optimal parameters. Thus, this indicates that stability of probiotic, powder properties and bioactive compounds in spray

dried probiotic-supplemented mao juice could be preserved for a long time when using the appropriate packages and storage conditions.

CHAPTER 5

SPRAY DRYING PROBIOTICS ALONG WITH MAO JUICE PLUS *TILIACORA TRIANDRA* GUM FOR EXPOSURE TO THE *IN VITRO* AND *IN VIVO* GASTROINTESTINAL ENVIRONMENTS

5.1 Introduction

Probiotic foods and beverages are dietary supplements containing potentially beneficial bacteria or yeasts. According to the definition of FAO/WHO (2001), probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*". Probiotics consider as "protective" intestinal bacteria which show several health benefits, viz. decreasing the lactose intolerance symptoms, reducing plasmatic cholesterol levels, controlling intestinal infections, stimulation of the immune system and anti-carcinogenic activity (Sanders et al., 2013). Furthermore, these microbes could also enhance the formation of essential mucosal nutrients including short-chain fatty acids (SCFA) and amino acids (i.e. arginine, cysteine and glutamine) (Bengmark, 1996). However, to provide health and clinical benefits, probiotic bacteria must possess a number of properties (> 10^6–10^7 CFU/g or CFU/ml) when implanted in the human digestive tract (Chaikham, 2015a). There are various techniques to preserve these microbes such as immobilization via spray drying (Kingwatee et al., 2015; Riveros et al., 2009).

Spray drying is one of the effective processes to entrap probiotic cells because of it is relatively inexpensive. In drying process, probiotic cell loses viability due to physical injury to microencapsule and heat generation. However, the loss of probiotic cells can be reduced by using the proper protectants such as prebiotics (i.e. inulin, xylose, fructo-oligosacccharides, etc.) and hydrocolloids (i.e. xanthan gum, guar gum, locust bean gum, etc.) for spray drying (Avila-Reyes, Garcia-Suarez et al., 2014; Solanki et al., 2013). Several findings exhibited that spray dried probiotics with optimal forms can protect the living cells from the adverse conditions of storage and digestion (Corcoran et al., 2004; Lian et al., 2002). The findings of Kingwatee et al. (2015) illustrated that maltodextrin and inulin have potentially increased the survival rate of *Lactobacillus casei* 01 during spray drying, storage and under *in vitro* gastrointestinal

environments. They also suggested that these materials could reduce the porous matrix of the microcapsule and improved the stability of immobilized probiotics.

Tiliacora triandra, namely "*Yanang*", is an indigenous vegetable used in many Thai cuisines. It leaves contain high levels of minerals (i.e. calcium and iron), beta-carotene, vitamin E and phenolic compounds (Chaveerach et al., 2016; Singthong et al., 2014). Hydrocolloid/gum in *T. triandra* leaves could be another source of natural polysaccharides and are interesting additives for food industry. The chemical structure of viscous component in *T. triandra* gum was similar to xylan. The predominant monosaccharide of *T. triandra* gum was xylose, together with substantial amounts of other neutral sugars (Singthong et al., 2009). Thus, the addition of *T. triandra* gum to maltodextrin may expect to provide an excellent material for protection the probiotics from heat and others adverse conditions.

Functional synbiotic soft-drinks or dehydrated fruit juices including maoluang (*Antidesma bunius* Linn.) juice containing probiotics and prebiotics are the challenging implementation for this research area. Maoluang juice has become more popular consumption in Thailand because it contains high amounts of bioactive constituents including ascorbic acid and phenols (i.e. anthocyanins and flavonoids), with antioxidant properties (Chaikham and Baipong, 2016; Jorjong et al., 2015). Puangpronpitag et al. (2011) and Butkhup and Samappito (2011) found that maoluang seed and skin-pulp extracts exhibited anti-apoptotic and anti-inflammatory effects in human breast epithelial cells as well as inhibitory effects against several pathogenic microorganisms. In addition, induced hypertensive rats fed with maolaung pomace found to be have lowering blood pressure and improving hemodynamic status (Kukongviriyapan et al., 2013).

Up to date, there is no information on the impact of *T. triandra* gum as carrier material for enhancing the survival of probiotics in mao juice after spray drying and with the *in vitro* gut models. Therefore, this research aimed to monitor the survivability of *L. casei* 01 and *L. acidophilus* LA5 after spray drying with maoluang juice plus maltodextrin, *T. triandra* gum and/or inulin, and under simulated gastrointestinal fluids. Changes of colon microbiome due to probiotic-mao juice powders in the *in vitro* and *in vivo* colon environments were also studied.

5.2 Materials and methods

5.2.1 Preparation of *Tiliacora triandra* gum

T. triandra leaves were freshly harvested from an orchard in Phranakhon Si Ayutthaya province, Thailand. Upon arrival at the laboratory, the leaves were washed with running tap water before dehydration at 65°C for 4 h using a convective hot-air oven (FED series, Binder, Hauppauge, NY, USA). The dehydrated leaves were blended using a blender (MX-20G 250 W, National, Bangkok, Thailand) at high speed for 5 min and then extracted with distill water (a ratio of 1:6.6, w/v) at 85°C for 100 min. After that, the extract was filtered through a vacuum filter and concentrated to half of its volume using a rotary vacuum evaporator (AV 10, IKA® Works, Inc., Wilmington, NC, USA) before being precipitated with three volumes of absolute ethanol (Merck, Darmstadt, Germany). Later, the precipitate was dried using a vacuum oven (XF050, France Etuves, Chelles, France) and powdered using a National blender (Singthong et al., 2009).

5.2.2 Activation of probiotic cultures

Freeze dried probiotics including *L. casei* 01 and *L. acidophilus* LA5 were purchased from Chr. Hansen (Hørsholm, Denmark). The cell pellets of both probiotics were prepared following the procedures of Chaikham et al. (2012). In brief, freeze dried cultures were rehydrated, activated and anaerobically incubated in de Man Rogosa and Sharp (MRS) broth (Hi-media, Mumbai, India) at 37°C for 16 and 18 h respectively to reach their stationary phases. Both activated cultures were harvested by centrifugation at 4,500 rpm and 4ºC for 20 min (Rotina 46 R, Hettich, Tuttlingen, Germany) and then washed twice with 0.85% (w/v) sterile saline water (Merck, Darmstadt, Germany). The cell pellets were then diluted to provide the concentration of 10^{12} CFU/ml using sterile saline water before use.

5.2.3 Spray drying procedure

Maoluang fruits (*A. bunius* Linn.) from an orchard in Sakon Nakhon province, Thailand were cleaned and extracted using a juice extractor (HR1871/10 Collection Juicer, Philips, Singapore). The extracted juice was separately blended with either 20% (w/v) maltodextrin (10.5 dextrose equivalent, DE: Maltrin®, Grain Processing Corporation, Muscatine, IA, USA) or mixture of 10% (w/w) maltodextrin containing 10% (w/w) *T. triandra* gum and/or 10% (w/w) inulin (Sigma-Aldrich, St. Louis, MO, USA)

before pasteurization at 90°C for 1 min (Chaikham, 2015b). After cooling down to 25ºC, the probiotic cultures were individually inoculated into the pasteurized juices to obtain a bacterial concentration of roughly 10^{10} CFU/ml before spray drying. A spray dryer (JCM Engineering concept, Bangkok, Thailand) equipped with a fluid atomizer (inside diameter of 5 mm) was operated in a co-current manner. Drying condition for the entire experiment was adjusted as follows: 25°C feeding temperature, 0.6–1 L/h feeding rate, 15 psi atomizing pressure and 160°C hot-air-inlet temperature to generate 80°C outlet temperature (Kingwatee et al., 2015). Later, the collected powders were vacuum sealed in laminated bags (polyethylene tetraphthalate/polypropylene/aluminum: Siam Pack, Chiang Mai, Thailand) and kept in a refrigerator for further analysis.

5.2.4 Enumeration of viable probiotics after spray drying

To release the entrapped cells, 1 g powder was mixed with 99 ml sterile saline water using a stomacher (IUL Instruments, Barcelona, Spain) for 10 min at room temperature. After that, serial dilutions were made with sterile saline water and they were subsequently plated on MRS agar and anaerobically incubated at 37°C for 48 and 72 h prior to determination the survivors (CFU/g) of *L. casei* 01 and *L. acidophilus* LA5, respectively.

5.2.5 Antagonistic activity assay

A modified version of the agar spot test described by Chaikham et al. (2013) was carried out by spotting 2 µl aliquots of probiotic cultures in stationary phase at on MRS agar. The spotted plateswere incubated anaerobically at 37°C for 24 h to develop colonies. A 7 ml aliquot of Tryptic Soy agar (Labscan, Bangkok, Thailand) containing 100 µl of one of five selected pathogens (*Bacillus cereus* TISTR687, *Enterococcus faecalis* TISTR1482, *Escherichia coli* TISTR780, *Micrococcus luteus* TISTR884 or *Staphylococcus aureus* TISTR118) was poured over the discernible colonies. The inoculated plates were then incubated at 37 ˚C for 48 h. Clear zones surrounding positive probiotic spots appeared after the incubation period.

Table 5.1 Compositions of synthetic gastric and small intestinal fluids of the *in vitro* digestive experiment (Oomen et al., 2003)

Reagents	Gastric fluid (pH 1.4)		Duodenal fluid (pH 8.1)		Bile fluid (pH 8.1)	
Inorganic substances	NaCl	2,752 mg	NaCl	7,012 mg	NaCl	5,259 mg
	NaH_2PO_4	266 mg	$NaHCO_3$	5,607 mg	$NaHCO_3$	5,785 mg
	KCl	824 mg	NaH_2PO_4	80 mg	KCl	376 mg
	$CaCl_2$	400 mg	KCl	564 mg	HCl (conc.)	180 µl
	NH_4Cl	306 mg	$MgCl_2$	50 mg		
	HCl (conc.)	6.5 ml	HCl (conc.)	180 µl		
Organic substances	Glucose	650 mg	Urea	100 mg	Urea	250 mg
	Glucuronic acid	20 mg				
	Urea	85 mg				
	Glucosamine hydrochloride	330 mg				
Other accessories	Bovine serum albumin	1,000 mg	$CaCl_2$	200 mg	$CaCl_2$	222 mg
	Mucin	3,000 mg	Bovine serum albumin	1,000 mg	Bovine serum albumin	1,800 mg
	Pepsin	2,500 mg	Pancreatin	9,000 mg	Bile	30,000 mg
			Lipase	1,500 mg		

5.2.6 Microstructure analysis of spry dried probiotic-mao juice powders

A scanning electron microscope (SEM) was used to examine external microstructure of spry dried probiotic-mao juice powders. The powders were coated with 10 nm of gold using a Hitachi E1010 ion sputter (Hitachi Science Systems Co., Ltd, Tokyo, Japan). The images of gold-coated samples were taken by using a Hitachi S-3000 N SEM (Hitachi High-Technologies Co., Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV.

Table 5.2 Treatment conditions for *in vitro* gastrointestinal experiments

Treatment conditions	Abbreviations
Lactobacillus casei 01 (LC)	
Free cells in distill water (control)	LC-F
Free cells in maoluang juice	LC-FM
Reconstituted probiotic-mao juice powder plus 20% maltodextrin in distill water (1:4, w/v)	LC-RM
Reconstituted probiotic-mao juice powder plus 10% maltodextrin and 10% *T. triandra* gum in distill water (1:4, w/v)	LC-RMY
Reconstituted probiotic-mao juice powder plus 10% maltodextrin and 10% inulin in distill water (1:4, w/v)	LC-RMI
Lactobacillus acidophilus LA5 (LA)	
Free cells in distill water (control)	LA-F
Free cells in maoluang juice	LA-FM
Reconstituted probiotic-mao juice powder plus 20% maltodextrin in distill water (1:4, w/v)	LA-RM
Reconstituted probiotic-mao juice powder plus 10% maltodextrin and 10% *T. triandra* gum in distill water (1:4, w/v)	LA-RMY
Reconstituted probiotic-mao juice powder plus 10% maltodextrin and 10% inulin in distill water (1:4, w/v)	LA-RMI

5.2.7 *In vitro* stomach and small intestine experiments

1) Preparation of simulated stomach and small intestinal fluids

Preparation of gastric, duodenal and bile fluids was carried out by mixing sets of inorganic, organic and accessory substances as described in Table 5.1, augmenting

them to 500 ml with distilled water and subsequently adjusting them to pH 1.4, 8.1 and 8.1, respectively with either 1 M sodium hydroxide (Merck, Darmstadt, Germany) or 37% hydrochloric acid (Merck, Darmstadt, Germany). These fluids were prepared a day prior to use, and then stored at 4°C. All these fluids were warmed to 37°C prior to being use.

2) Batch experiment in simulated stomach and small intestinal sections

The various treatment compositions are shown in Table 5.2. For free cell treatments, cell pellets of both probiotics as prepared in Section 5.2.2 were then aseptically inoculated into acidified sterile distill water (pH 1.4) or sterile maoluang juice (pH 1.4) to provide the concentration of 10^8 CFU/ml. To reconstitute probiotic-mao powders, 20 g of powder were well-mixed with 80 ml warmed distill water (pH 1.4, 37°C) for 10 min. Subsequently, 2 ml of all mixed solutions were inoculated into 15 ml of gastric fluid (pH 1.4) in sterile glass bottles sealed with a rubber cap and a metal ring. The mixtures were incubated anaerobically at 37°C with gentle shaking at 100 rpm for 1 h. After the incubation period in the simulated stomach, 10 ml of duodenal fluid and 5 ml of bile fluid (pH 8.1) were fed into the system (pH 6.5), which was then allowed to incubate for a further 4 h.

To assess the viable cells, 1 ml of incubated fluid was withdrawn and diluted with 9 ml of 0.2 M sterile phosphate buffer (Merck, Darmstadt, Germany). Several dilutions were made with 0.1% (w/v) peptone water (Hi-Media, Mumbai, India) and then plated on MRS agar. Colonies appeared after incubation under anaerobic conditions at 37°C for 48–72 h.

5.2.8 *In vitro* colon experiment

1) Preparation of carbohydrate-based medium

The carbohydrate-based medium was prepared according to Van de Wiele et al. (2004) by mixing the following substances; 4 g potato starch (O.V. Chemical, Chiang Mai, Thailand), 1 g (+)-arabinogalactan (Sigma-Aldrich, St. Louis, MO, USA), 2 g pectin (Sigma-Aldrich, St. Louis, MO, USA), 1 g xylan (Sigma-Aldrich, St. Louis, MO, USA), 0.4 g *D*-(+)-glucose (Merck, Darmstadt, Germany), 3 g yeast extract (Oxoid, Hampshire, UK), 1 g special peptone water (Oxoid, Hampshire, UK), 4 g mucin (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 g *L*-cysteine (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 1 L deionized water (RCI Labscan, Bangkok, Thailand). This medium was

then sterilized at 121 °C for 15 min and adjusted with either 0.5 M hydrochloric acid or 0.5 M sodium hydroxide to pH 5.5.

2) Preparation of fecal fluid

The fecal fluid was prepared by collecting fecal samples from six healthy adults (aged 20-32 years) who had no history of antibiotic treatment within last 6 months. All fresh fecal samples were homogeneously mixed. Consequently, 25 g of mixed fecal were diluted with 150 ml of 0.7 M phosphate buffer (pH 7; Merck, Darmstadt, Germany), containing 1.5 g of sodium thioglycolate (Sigma-Aldrich, St. Louis, MO, USA) as a reducing agent, then homogenized for 10 min using a stomacher and centrifuged at 2,000 rpm for 3 min. The supernatant was separated and used as “fecal fluid” (Chaikham et al., 2013).

3) Batch experiment in colon section

The colon fluid was prepared by mixing 50 ml fecal fluid with 50 ml carbohydrate-based medium in a sterile bottle and used as “colon fluid” for a control condition. For treatment conditions (Table 5.2), 250 ml of fecal fluid, 150 ml of carbohydrate-based medium and 100 ml of diluted cells or reconstituted samples were well-mixed in 500 ml of sterile bottle and subsequently flushed with nitrogen gas to simulate an anaerobic environment. All simulated colon bottles were then incubated at 37 °C for 24 h with gentle shaking at 100 rpm. During the incubation period, the colon fluids were collected at the initial stage, 6, 12 and 24 h for further analysis.

4) Determination of lactic acid and ammonia

Lactic acid were quantified using the UV spectrophotometric method at λ_{max} 340 nm (Perkin Elmer UV WINLAB, Perkin Elmer, Waltham, MA, USA) for the determination of *D*- and *L*-lactic acid, according to the manufacturer's instructions (R-Biopharm AG, Darmstadt, Germany).

To release ammonia gas from the colon fluids, an excess of magnesium oxide (Merck, Darmstadt, Germany) was added into 1 ml sample. The mixture was then distilled using a Kjeldahl Apparatus Vapodest 30 S (Kjeldahl, Gerhardt, Germany) to enable vaporization of ammonia which subsequently was trapped in boric acid solution (Merck, Gerhardt, Germany). The entrapped ammonia solution was then titrated with 0.02 M hydrochloric acid. The concentration of ammonia was calculated by the following equation (Chaikham et al., 2012):

$$\text{Ammonia concentration (mg/L)} = [(V_{sample} - V_{blank}) \times 0.02 \times 14 \times 1{,}000]/V_{sample}$$

where, V_{sample} and V_{blank} are volumes (ml) of titers used to titrate the sample and blank (deionized water).

5) Determination of short-chain fatty acids

SCFA were determined according to the procedure of Chaikham et al. (2012) with some modifications. Two milliliters of sample were mixed with 0.4 g sodium chloride and acidified by adding 0.5 ml sulfuric acid conc. (Ajax, Sydney, Australia). A volume of 0.1 ml 2-methylhexanoic acid (as internal standard) (2.8 ml in 1 L deionized water; Fluka, Buchs, Switzerland) and 2 ml diethylether (Merck, Darmstadt, Germany) were added, and subsequently SCFA were extracted using a shaker for 10 min and centrifuged at 1,000 rpm for 5 min. A 1-µl aliquot of the diethylether layer (top layer) was injected and measured with a Shimadzu GCMS-QP2010 gas chromatography (Shimadzu Cooperation Analytical & Measuring Instruments Division, Kyoto, Japan) equipped with a flame ionization detector. The gas chromatograph was equipped with a capillary free fatty acid-packed column (25 m × 0.53 mm, film thickness 1.2 µm: Superchrom, Milan, Italy). Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. The column temperature was 130 °C and the temperature of the injection port and detector was 195 °C.

6) Enumeration of colon microbial communities

Enumeration of colon microbial communities including lactobacilli, bifidobacteria, coliforms, clostridia and total anaerobic bacteria was determined using plate counting methods (Chaikham et al., 2012). Decimal dilutions in 0.1% (w/v) sterile peptone water of samples were placed on 5 types of selective media as follows; LAMVAB agar (Hartemink, Domenech, & Rombouts, 1997) for lactobacilli (72 h, anaerobic), RB-agar (Hartemink, Kok, Weenk, & Rombouts, 1996) for bifidobacteria (96 h, anaerobic), McConkey agar (Hi Media, Mumbai, India) for fecal coliforms (24 h, aerobic), TSC agar (Hi Media, Mumbai, India) containing *Clostridium perfringens* selective supplement (Merck, Darmstadt, Germany) for clostridia (24 h, anaerobic) and BHI agar (Oxoid, Hampshire, UK) for total anaerobic bacteria (24 h, anaerobic). All plated media were then incubated at 37 °C for colony counting.

5.2.9 *In vivo* animal model experiments

Five-weeks-old male Sprague –Dawley (SD) rats (*n* = 14) with weight around 130 to 160 grams were purchased from the National Laboratory Animal Center at Salaya Campus, Mahidol University. All rats were housed at Faculty of Tropical Medicine, Mahidol University in according with the rules and regulations of the Animal Care Ethical Committee of Laboratory Animal Science Center, Faculty of Tropical Medicine, Mahidol University (Ethic number FTM-ACUC 013/2017). After allowing to accustom in a new environment for a week, one rat wasl then housed per cage in solid bottom, open top at an automatic ambient humidity (65±10%), temperature (24±2°C) and light-dark (12:12) controlled room and continue with free access to standard chow diet and water until the end of experiment (12 days).

After 1 week of acclimation, 14 male SD rats was randomly into mao juice (M; *n* = 7) and spray-dried probiotic-mao juice (PM; *n* = 7) groups. M and PM groups were administrated the spray-dried mao juice or probiotic-mao juice powders subsequently at the dose of 1 g/kg of rat body weight. The oral gavage was performed at 10.00 am every day for 12 days. Thereafter, the feces of all tested ratds were collected every-4-day interval and preserved at -25°C for further analysis. SCFA and colon microbial communities were determined following the methods in Section 5.2.8.

5.2.10 Statistical data analysis

All data were the means of triplicate determinations with standard deviations (means ± SD). Analysis of variance (ANOVA) was carried out using SPSS version 15 (SPSS Inc., Chicago, IL, USA).Determination of significant differences among treatment means was analyzed by general linear model and Duncan's multiple range test ($P < 0.05$).

5.3 Results and discussion

5.3.1 Microstructure of spray dried probiotic-mao juice powders

According to our previous study, the outlet temperature of 80°C was an optimal temperature as it provided microparticles of probiotics with high survival cells, low moisture content and low a_w (Kingwatee et al., 2015). Thus, in this study, the spray-drying of *L. casei* 01 and *L. acidophilus* LA5 in maoluang juice was performed with the outlet temperature 80 °C using 3 different encapsulating materials including pure and mixtures of maltodextrin. Figure 5.1 shows the SEM images of the *L. casei* 01 in

maoluang juice entrapped with 20% maltodextrin and 10% maltodextrin mixed with 10% *T. triandra* gum and/or 10% inulin, respectively. Microparticles of probiotic coated with 20% maltodextrin were spherical in shape with a small size and wrinkled surface (Figure 5.1-a). Figure 5.1-b displays irregularly spherical particles with some smooth and some shrinkage and dents on its surface, as affected by 10% *T. triandra* gum addition. The mixture of 10% maltodextrin and 10% inulin not only increased the average size of the probiotic loaded beads but it also smoothened the surface of microparticles (Figure 5.1-c). In this case, the morphology of microparticles depends on the types of carrier materials (Solanki et al., 2013). For examples, slowly-formed gel of products containing milk provided an irregular shape in the granules (Reid et al., 2005). The microcapsules with smooth surface devoid the porosity resulted in a better protective property of coated microcapsules (Zanjani et al., 2014). Size and smoothness of the microparticles might affect its properties (e.g. solubility, water activity) and influencing the number of survival cells (Fritzen-Freire et al., 2012).

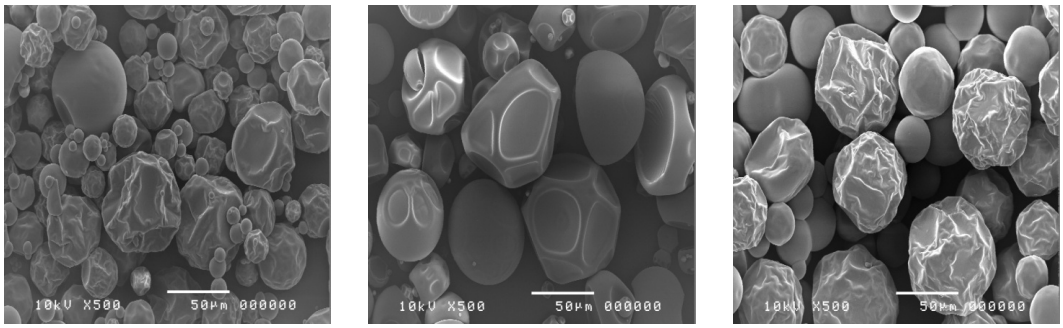

Figure 5.1 SEM images of mao juice plus *L. casei* 01 powders spray dried at 80±2ºC outlet temperature using different carriers; (a) 20% maltodextrin, (b) 10% maltodextrin plus 10% *T. triandra* gum and (c) 10% maltodextrin plus 10% inulin.

5.3.2 Survival of probiotics after spray drying

Survival of probiotics is a critical concern when spray drying is employed. Therefore, after the drying, the counts of *L. casei* 01 (LC) and *L. acidophilus* LA5 (LA) were determined and reported as shown in Table 5.3. For both lactobacilli, the survival cells were higher than 8 log CFU/g of dry powder, irrespective of the encapsulating materials. The treatments with maltodextrin alone showed significantly lower cell viability ($P < 0{\cdot}05$) compared to the mixtures of maltodextrin with *T. triandr*a gum and/or with inulin (Table 5.3). However, no significant difference in viable counts was obtained between encapsulating matrices added with *T. triandra* gum or inulin for both lactobacilli

species. To achieve highest recovery, these data suggested that either *T. triandra* gum or inulin could be used as a protective carrier for LC and LA immobilization. Several findings previously reported that encapsulating materials containing different prebiotics *viz*. inulin, polydextrose and fructooligosaccharide could improve the viability of probiotics, such as *L. rhamnosus*, *L. casei*, *L. plantarum* and *B. lactis*, during dehydration via spray drying technique (Corcoran et al., 2004; Kingwatee et al., 2015; Rajam and Anandharamakrishnan, 2015). Moreover, Lian et al. (2002) reported that gelatin, gum arabic and soluble starch also increased the survival of bifidobacteria after spray drying.

Besides the encapsulation and survival of probiotics after the spray drying, the *in vitro* gastrointestinal studies were performed in which reconstituted probiotic-mao powder and free cells of LC and LA were exposed to simulated gastric, small intestinal and colon fluids. The free cells (fresh cells) and reconstituted cells were prepared into 10 different treatment conditions as shown in Table 5.2.

Table 5.3 Viable of probiotics after spray drying with different carrier materials at 80°C outlet temperature

Treatment conditions	Viable cells (CFU/g)
Lactobacillus casei 01 (LC)	
20% maltodextrin	$4.43\pm1.04^{b}\times10^{8}$
10% maltodextrin plus 10% *T. triandra* gum	$5.01\pm1.87^{ab}\times10^{8}$
10% maltodextrin plus 10% inulin	$5.45\pm0.92^{ab}\times10^{8}$
Lactobacillus acidophilus LA5 (LA)	
20% maltodextrin	$5.83\pm1.27^{ab}\times10^{8}$
10% maltodextrin plus 10% *T. triandra* gum	$6.38\pm0.81^{a}\times10^{8}$
10% maltodextrin plus 10% inulin	$6.59\pm1.18^{a}\times10^{8}$

Means in the same column with the same small letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

5.3.3 Antagonistic activity

In this study, we found that the reactivated probiotics (spry dried-probiotic mao juice powder) have been shown to be insignificantly different in antagonistic activities compared to pure cultures (data not shown). In addition, *L. casei* 01 produced

antibacterial substances against all five selected pathogenic bacteria, whereas the antibacterial substances produced by *L. acidophilus* LA5 could inhibit only *E. coli* TISTR780, *B. cereus* TISTR687 and *M. luteus* TISTR884, suggesting that *L. casei* 01 had greater antibacterial activity than *L. acidophilus* LA5.

5.3.4 Viable probiotics during passage through *in vitro* stomach and small intestinal models

Survival cells of LC and LA in stimulated stomach and small intestinal environments are illustrated in Table 5.4. The initial amounts of LC and LA were 4.53-5.40×10^7 CFU/ml and 7.09-7.62×10^7CFU/ml, respectively. The numbers of viable cells in all treatments was gradually reduced over 3 log CFUs within 1 h (P < 0·05). Comparing between free cells and reconstituted probiotic cells under simulated stomach environment, the reconstituted cells of LC tended to have apparent higher viability, especially, when entrapped with maltodextrin added with *T. triandra* gum or inulin (P < 0·05). The benefits of immobilization are highlighted when the cells were successfully passed though small intestine fluid. Similarly, Hernandez-Hernandez et al. (2012) found that lactulose (prebiotic) could enhance the survivability of *L. bulgaricus* ATCC7517 and *L. plantarum* WCFS1 embedded in simulated stomach and small intestine fluids, as compared to those without prebiotic addition. Kingwatee et al. (2015) also exhibited that spray dried *L. casei* 01 with lychee juice and inulin showed better impact on the survival rate of probiotic in gastric and bile juices. Moreover, Pankasemsuk et al. (2016) reported that microencapsulated *L. casei* 01 with 2% alginate and 1% hi-maize starch enabled optimal survival in both gastric and bile fluids. In contrast, there was insignificant difference (P > 0.05) in the number of survival cells of LA for all treatments under the stimulated stomach condition. Unfortunately, when LA *was* subsequently transferred to simulated intestinal environment, both free cells in distill water (F) and free cells in maoluang juice (FM) showed the least number of viable cells; whereas the survival of reconstituted probiotic cells was significantly higher in simulated gastric and intestinal fluids. In overall, free and encapsulate cells of LA showed significantly (P < 0.05) higher survivability than all forms of LC after incubation at 37°C for 5 h. This finding was agreed with the reports of Krasaekoopt et al. (2004) and Chaikham et al. (2013).

Table 5.4 Survival of free cells and reconstitute probiotic-mao juice powders in simulated gastric fluid (1 h) followed by small intestinal fluid (4 h) during incubation under anaerobic environment at 37°C for 5 h

Treatment conditions	Survival cells (CFU/ml) during incubation at 37°C						
	Simulated stomach environment					Simulated small intestinal environment	
	Initial stage	0.25 h	0.5 h	0.75 h	1 h	3 h	5 h
Lactobacillus casei 01 (LC)							
LC-F	$4.98\pm0.67^{bA}\times10^{7}$	$5.04\pm0.63^{cB}\times10^{6}$	$6.19\pm0.89^{bC}\times10^{5}$	$8.36\pm2.02^{aD}\times10^{4}$	$5.93\pm0.29^{cdE}\times10^{3}$	$5.08\pm1.28^{cdE}\times10^{3}$	$9.08\pm0.63^{dF}\times10^{2}$
LC-FM	$5.04\pm0.52^{bA}\times10^{7}$	$1.38\pm0.48^{dB}\times10^{6}$	$8.27\pm1.15^{dC}\times10^{4}$	$2.09\pm0.77^{cD}\times10^{4}$	$4.12\pm1.31^{dE}\times10^{3}$	$3.24\pm0.46^{eE}\times10^{3}$	$8.94\pm1.72^{dF}\times10^{2}$
LC-RM	$4.53\pm0.80^{bA}\times10^{7}$	$4.02\pm0.95^{cB}\times10^{6}$	$2.08\pm1.42^{cC}\times10^{5}$	$4.04\pm1.05^{bD}\times10^{4}$	$6.01\pm0.65^{cE}\times10^{3}$	$3.60\pm1.20^{deF}\times10^{3}$	$9.60\pm1.18^{dG}\times10^{2}$
LC-RMY	$5.12\pm0.75^{bA}\times10^{7}$	$4.18\pm1.01^{cB}\times10^{6}$	$2.53\pm0.56^{cC}\times10^{5}$	$5.13\pm0.63^{bD}\times10^{4}$	$1.92\pm1.06^{bE}\times10^{4}$	$7.85\pm0.79^{bF}\times10^{3}$	$2.65\pm1.28^{bcG}\times10^{3}$
LC-RMI	$5.40\pm0.34^{bA}\times10^{7}$	$4.20\pm0.41^{cB}\times10^{6}$	$1.92\pm0.94^{cC}\times10^{5}$	$5.65\pm1.52^{bD}\times10^{4}$	$1.08\pm0.77^{bE}\times10^{4}$	$8.14\pm0.91^{abF}\times10^{3}$	$3.43\pm0.64^{bG}\times10^{3}$
Lactobacillus acidophilus LA5 (LA)							
LA-F	$7.32\pm0.78^{aA}\times10^{7}$	$3.85\pm0.41^{aB}\times10^{7}$	$7.02\pm1.17^{aC}\times10^{6}$	$1.85\pm1.04^{aD}\times10^{5}$	$4.12\pm0.86^{aE}\times10^{4}$	$6.80\pm1.73^{bcF}\times10^{3}$	$1.14\pm1.40^{cdG}\times10^{3}$
LA-FM	$7.45\pm1.06^{aA}\times10^{7}$	$2.23\pm0.50^{bB}\times10^{7}$	$7.48\pm0.73^{aC}\times10^{6}$	$9.15\pm0.81^{aD}\times10^{4}$	$4.04\pm1.68^{aE}\times10^{4}$	$5.99\pm1.05^{cF}\times10^{3}$	$1.65\pm1.61^{cG}\times10^{3}$
LA-RM	$7.09\pm0.63^{aA}\times10^{7}$	$3.05\pm0.44^{abB}\times10^{7}$	$6.11\pm1.02^{aC}\times10^{6}$	$9.02\pm0.43^{aD}\times10^{4}$	$3.11\pm0.40^{aE}\times10^{4}$	$6.02\pm0.28^{cF}\times10^{3}$	$2.64\pm0.75^{bcG}\times10^{3}$
LA-RMY	$7.40\pm0.19^{aA}\times10^{7}$	$3.62\pm0.67^{aB}\times10^{7}$	$6.50\pm0.60^{aC}\times10^{6}$	$8.24\pm0.35^{aD}\times10^{4}$	$3.52\pm0.27^{aE}\times10^{4}$	$9.47\pm0.55^{aF}\times10^{3}$	$5.05\pm0.31^{aG}\times10^{3}$
LA-RMI	$7.62\pm0.81^{aA}\times10^{7}$	$3.45\pm1.05^{aB}\times10^{7}$	$6.83\pm2.16^{aC}\times10^{6}$	$9.08\pm1.75^{aD}\times10^{4}$	$4.01\pm0.74^{aE}\times10^{4}$	$8.38\pm1.30^{abF}\times10^{3}$	$4.89\pm1.80^{abG}\times10^{3}$

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

Table 5.5 Viable cells of colon lactobacilli in simulated colon environment during fermentation with various treatment conditions at 37°C for 24 h

Treatment conditions	Numbers of colon lactobacilli (CFU/ml) during fermentation at 37°C				
	Initial stage	6 h	12 h	18 h	24 h
Colon fluid	$8.85\pm1.83^{cC}\times10^{4}$	$9.15\pm0.64^{cC}\times10^{4}$	$1.48\pm0.40^{cB}\times10^{5}$	$2.32\pm1.07^{gAB}\times10^{5}$	$2.69\pm0.70^{fA}\times10^{5}$
Lactobacillus casei 01 (LC)					
LC-F	$6.13\pm0.63^{bD}\times10^{7}$	$8.59\pm1.50^{aC}\times10^{7}$	$1.90\pm1.18^{abB}\times10^{8}$	$9.68\pm0.84^{aA}\times10^{8}$	$2.53\pm1.15^{bB}\times10^{8}$
LC-FM	$6.84\pm1.08^{abD}\times10^{7}$	$7.03\pm0.49^{bCD}\times10^{7}$	$8.05\pm0.92^{bC}\times10^{7}$	$3.17\pm0.22^{dA}\times10^{8}$	$1.09\pm0.96^{cB}\times10^{8}$
LC-RM	$6.25\pm0.70^{bD}\times10^{7}$	$7.44\pm0.81^{abD}\times10^{7}$	$9.65\pm0.55^{bC}\times10^{7}$	$6.83\pm1.02^{bA}\times10^{8}$	$2.57\pm1.01^{bB}\times10^{8}$
LC-RMY	$7.02\pm1.82^{abD}\times10^{7}$	$7.32\pm0.53^{bD}\times10^{7}$	$1.10\pm0.42^{abC}\times10^{8}$	$9.61\pm0.88^{aA}\times10^{8}$	$5.22\pm0.35^{aB}\times10^{8}$
LC-RMI	$6.75\pm0.94^{abD}\times10^{7}$	$7.60\pm0.69^{abD}\times10^{7}$	$1.83\pm0.75^{aC}\times10^{8}$	$9.86\pm2.15^{aA}\times10^{8}$	$5.14\pm1.38^{aB}\times10^{8}$
Lactobacillus acidophilus LA5 (LA)					
LA-F	$7.35\pm1.61^{abC}\times10^{7}$	$8.69\pm0.61^{aC}\times10^{7}$	$9.93\pm1.09^{bBC}\times10^{7}$	$5.43\pm1.84^{bcA}\times10^{8}$	$1.60\pm0.40^{bcB}\times10^{8}$
LA-FM	$7.21\pm0.85^{aC}\times10^{7}$	$8.01\pm0.25^{aBC}\times10^{7}$	$8.60\pm0.61^{bAB}\times10^{7}$	$9.14\pm0.40^{fA}\times10^{7}$	$8.05\pm1.05^{eAB}\times10^{7}$
LA-RM	$7.09\pm0.44^{abD}\times10^{7}$	$7.92\pm0.43^{abCD}\times10^{7}$	$8.77\pm0.29^{bB}\times10^{7}$	$1.13\pm0.18^{eA}\times10^{8}$	$8.38\pm0.24^{dBC}\times10^{7}$
LA-RMY	$7.60\pm1.09^{aC}\times10^{7}$	$8.16\pm1.09^{aC}\times10^{7}$	$1.53\pm0.80^{abB}\times10^{8}$	$4.00\pm1.27^{cA}\times10^{8}$	$2.53\pm0.47^{bB}\times10^{8}$
LA-RMI	$7.92\pm0.58^{aB}\times10^{7}$	$8.24\pm0.56^{aB}\times10^{7}$	$2.03\pm1.48^{aA}\times10^{8}$	$4.10\pm0.91^{cA}\times10^{8}$	$2.82\pm1.09^{bA}\times10^{8}$

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

5.3.5 Viable cells of colon lactobacilli under *in vitro* colon section

The simulated colon fluid initially prepared from fecal fluid with carbohydrate-based medium contained 8.58×10^{4} CFU/ml lactobacilli. In the case of negative control, the colon fluid was incubated at 37°C. The number of lactobacilli gradually increased to 2.69×10^{5} CFU/ml in 24 h ($P < 0{\cdot}05$). For the studied traits, the initial inoculum concentrations for LC and LM were $6.13\text{-}7.02\times10^{7}$ and $7.09\text{-}7.92\times10^{7}$ CFU/ml, respectively. The number of viable cells remained stable at the initial concentration after 6 h. Then the viable counts gradually increased ($P < 0{\cdot}05$) over 8 log CFUs within 24 h with only two exceptions (LA-FM and LA-RM). Interestingly, the transition from encapsulated lactobacilli to reconstituted cells showed a high recovery level in which they have a similar activity as their fresh cells. This might imply that the drying condition as well as the encapsulating carrier compounds used induced minimal cellular injury. In general, coating materials including maltodextrin, *T. triandra* gum, inulin and other oligosaccharides mainly provided a carbon source to assist the growth of microbes in the colon compartment. Our results were in agreement with several reports previously. Pankasemsuk et al. (2016) reported that entrapped *L. casei* 01 with hi-maize starch had great influence on the augmentation of indigenous lactobacilli during 24 h fermentation in an *in vitro* colon model. Gmeiner et al. (2000) fed milk-based product supplemented with *L. acidophilus* 74-2 plus fructooligosaccharide into a Simulator of the Human Intestinal Microbial Ecosystem (SHIME reactor). They found that the amounts of beneficial bacteria including lactobacilli and bifidobacteria increased in all colon compartments (i.e. ascending, transverse and descending colons), while the numbers of *E. coli* and enterobacteria were noticeably diminished. Van de Wiele et al. (2007) also reported that inulin could enhance the viable cells of colon lactobacilli after fermentation with colon bacteria in the SHIME system.

Table 5.6 Formation of lactic acid and ammonia in simulated colon environment after fermentation with various treatment conditions at 37°C for 24 h

Treatment conditions	Lactic acid and ammonia concentrations (mg/L) after fermentation at 37°C							
	L-lactic acid		*D*-lactic acid		Total lactic acid		Ammonia	
	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h
Colon fluid	148.59±2.29^{dF}	183±10.25^{gE}	208.91±8.15^{bD}	310.01±8.55^{eC}	357.49±10.30^{cB}	493.93±9.48^{fA}	221.14±10.22^{aB}	280.15±3.53^{aA}
Lactobacillus casei 01 (LC)								
LC-F	168.03±5.29^{aF}	287.15±7.10^{dD}	247.16±13.21^{aE}	653.15±7.57^{aB}	415.19±3.92^{aC}	940.30±30.41^{bcA}	222.76±9.73^{aB}	253.29±4.10^{deA}
LC-FM	165.66±4.63^{aF}	288.52±8.89^{dD}	248.50±10.26^{aE}	612.17±9.44^{cB}	414.50±13.84^{abC}	900.70±12.10^{cA}	223.48±5.94^{aB}	255.89±2.04^{deA}
LC-RM	156.43±1.12^{bF}	291.84±6.78^{cdD}	244.75±11.62^{aE}	615.77±9.34^{bcB}	401.19±6.51^{bC}	907.61±17.13^{cA}	220.81±6.82^{aB}	250.63±5.49^{eA}
LC-RMY	161.43±6.68^{abF}	318.27±14.63^{bD}	243.26±5.88^{aE}	647.40±8.45^{aB}	404.68±11.46^{bC}	965.67±20.88^{bA}	217.73±2.85^{aB}	235.13±2.12^{gA}
LC-RMI	158.21±5.76^{abF}	348.57±12.57^{aD}	245.73±4.58^{aE}	649.00±9.35^{aB}	403.94±10.34^{bC}	997.57±11.47^{aA}	220.13±4.01^{aB}	241.05±0.78^{fA}
Lactobacillus acidophilus LA5 (LA)								
LA-F	155.25±1.53^{bF}	255.39±6.42^{fD}	239.16±3.50^{aE}	585.55±11.00^{dB}	391.41±5.03^{bC}	840.95±17.40^{eA}	225.64±7.72^{aB}	268.72±5.13^{bA}
LA-FM	156.11±1.78^{bF}	263.82±4.70^{efD}	243.78±4.08^{aE}	586.81±5.77^{dB}	398.89±5.50^{bC}	850.62±9.66^{eA}	221.05±6.30^{aB}	260.18±0.71^{cA}
LA-RM	153.11±1.67^{cF}	272.31±10.41^{deD}	243.83±3.15^{aE}	592.16±13.55^{cdB}	396.95±1.84^{bC}	864.47±1.66^{dA}	218.80±6.11^{aB}	257.16±2.01^{dA}
LA-RMY	153.03±0.73^{cF}	290.78±9.70^{cdD}	245.07±2.77^{aE}	621.31±7.56^{bB}	398.10±13.00^{bC}	912.09±17.10^{cA}	220.01±5.25^{aB}	250.17±1.14^{eA}
LA-RMI	152.89±4.91^{cdF}	298.50±2.63^{cD}	250.91±9.20^{aE}	617.98±13.58^{bcB}	403.50±6.21^{bC}	916.48±10.97^{cA}	220.11±3.62^{aB}	251.82±0.92^{eA}

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

5.3.6 Formation of lactic acid and ammonia on *in vitro* colon experiments

Lactobacilli are able to ferment sugars into two forms of lactic acid namely *L*-lactic acid and *D*-lactic acid. In fact, lactic acid promotes human health by lowering the gut pH against the growth of pathogenic bacteria. In our study, lactic acid, including *L*-form and *D*-form, were monitored under simulated colon environment as shown in Table 6. For the negative control, the level of lactic acid was lesser, but ammonia was higher as compared to those of the colon fluid treated with LA and LC ($P < 0{\cdot}05$). In this study, significant higher levels of lactic acid produced by LC was observed as compared with LA ($P < 0{\cdot}05$), irrespective of cell states, free and reconstituted cells, or the encapsulating materials used. This was in agreement with our previous study (Chaikham et al., 2012). In addition, these two lactobacilli stimulated the production of *D*-form than the *L*-form, especially LC. Specifically, LC increased *D*-lactic acid and *L*-lactic acid by an average of 389.62 mg/L and 144.92 mg/L, respectively. Regarding LC, the greater lactic acid reflects the higher number of survival counts. For example, as the count of LC-RMY and LC-RMI were double than that of LC-F, the formation of lactic acid by LC-RMY and LC-RMI were almost two-fold higher than that of LC-F. However, the residue survival cells of LA did not showed the clear correlation between functionality of lactic acid and ammonia production.

In our study, the decrease of the ammonia production was observed when the colon fluid was treated with both lactobacilli (Table 5.6). For the control, ammonia sharply increased from 221.14 to 280.15 mg/L within 24 h ($P < 0{\cdot}05$), while ammonia increased from 217.73-223.48 and 218.80-225.64 mg/L to 235.13-255.89 and 250.17-268.75 mg/L in treatments with LC and LA, respectively. Interestingly, comparing to free cells, the encapsulated cells of both LC and LA were highly capable of inhibiting ammonia production. Especially, lactobacilli encapsulated with *T. triandra* gum and inulin showed the most effective activities on reducing ammonia formation. The beneficial effects of probiotics especially decrease of ammonia production have been reported in many previous studies (Chaikham et al., 2012; Fooladi et al., 2013). The decrease of ammonia production is associated with the reduction of urease activity of bacterial microflora and ammonia adsorption (Fooladi et al., 2013; Kanauchi et al., 1999). As ammonia is considered as a potential tumor promoters in the colon (Gråsten et al., 2000). Therefore, the low production of ammonia in the colon could be able to promote a beneficial health effect.

Table 5.7 Formation of short-chain fatty acids (SCFA) in simulated colon environment after fermentation with various treatment conditions at 37°C for 24 h

Treatment conditions	SCFA concentration (mg/L) after fermentation at 37°C							
	Acetate		Propionate		Butyrate		Total SCFA	
	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h
Colon fluid	848.15±22.75^{aD}	1,056.43±53.12^{iC}	315.98±11.43^{aF}	419.18±19.49^{hE}	178.64±15.91^{aH}	231.50±22.47^{gG}	1,580.64±71.50^{aB}	2,100.51±42.19^{gA}
Lactobacillus casei 01 (LC)								
LC-F	864.64±40.13^{aD}	1,444.80±50.01^{fC}	320.04±24.04^{aG}	605.32±10.44^{eE}	185.03±10.09^{aH}	393.61±10.60^{eF}	1,600.03±59.32^{aB}	2,909.63±60.61^{dA}
LC-FM	870.00±63.45^{aC}	1,600.61±84.22^{dB}	321.82±15.60^{aF}	687.44±12.90^{dD}	180.67±11.32^{aG}	432.10±14.78^{dE}	1,612.55±50.18^{aB}	3,141.10±45.12^{cA}
LC-RM	860.10±21.41^{aD}	1,990.30±58.10^{bB}	319.67±19.01^{aG}	735.50±25.99^{cE}	183.82±17.02^{aH}	503.22±23.41^{cF}	1,599.46±32.05^{aC}	3,913.44±31.25^{bA}
LC-RMY	875.23±25.22^{aD}	2,245.77±30.73^{aB}	328.69±20.03^{aF}	915.05±50.38^{aD}	190.04±15.68^{aG}	601.84±19.80^{aE}	1,636.83±42.63^{aC}	4,402.35±50.15^{aA}
LC-RMI	883.14±61.59^{aE}	2,390.55±90.46^{aB}	327.30±11.75^{aG}	967.68±19.72^{aD}	185.50±20.09^{aH}	625.39±19.11^{aF}	1,604.73±29.17^{aC}	4,415.62±61.53^{aA}
Lactobacillus acidophilus LA5 (LA)								
LA-F	861.04±30.07^{aD}	1,178.46±25.60^{hC}	314.66±30.62^{aF}	481.91±30.05^{gE}	179.36±10.22^{aG}	317.25±42.70^{fF}	1,593.67±26.00^{aB}	2,225.62±30.00^{fA}
LA-FM	865.92±22.38^{aD}	1,362.73±33.32^{gC}	340.60±29.11^{aF}	568.53±22.51^{fE}	178.92±23.41^{aG}	390.12±26.99^{eF}	1,605.44±30.12^{aB}	2,560.38±28.18^{eA}
LA-RM	859.05±44.53^{aD}	1,555.33±10.02^{eC}	327.83±20.58^{aG}	618.40±13.88^{eE}	179.00±17.71^{aH}	452.02±18.01^{dF}	1,595.04±28.41^{aB}	3,145.75±53.91^{cA}
LA-RMY	867.78±38.90^{aD}	1,700.10±59.49^{cdB}	320.06±18.59^{aG}	793.60±26.10^{bE}	182.64±20.14^{aH}	550.31±10.01^{bF}	1,623.17±40.53^{aC}	3,900.01±32.65^{bA}
LA-RMI	860.11±19.24^{aD}	1,748.69±51.05^{cB}	328.06±29.43^{aG}	769.50±19.11^{bE}	180.55±16.73^{aH}	568.90±21.28^{bF}	1,620.85±25.42^{aC}	3,895.09±44.27^{bA}

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

In this context, our result suggested the selection of encapsulating material provided positive health benefits for human by stimulating the survival of probiotics and their activities. The maltodextrin mixed with *T. triandra* gum and/or inulin showed positive influence on either supporting cell survival or changing the levels of lactic acid and ammonia, as fermentation end products.

5.3.7 Formation of SCFA on *in vitro* colon experiments

The benefit of SCFA by produced by probiotic bacteria have been reported in a countless number of studies, for examples, reducing potentially pathogenic clostridia by lowering pH and improving functions associated with ion transport (Topping, 1996; Wong et al., 2006). As shown in Table 5.7, the fermentation of lactobacilli in simulated colon environment produced significant increase in SCFA including acetate, propionate and butyrate ($P < 0{\cdot}05$). Irrespective of tested conditions, the SCFA profile sharply increased during probiotics fermentation ($P < 0{\cdot}05$), especially acetate. The encapsulated lactobacilli increased SCFA by more than two-fold as compared with SCFA yielded in the negative control (only colon fluid). The effect of the treatment conditions on the SCFA profiles exhibited a similar pattern of production, yielding the highest acetate, propionate and butyrate in RMI treatment followed by RMY > RM > FM > F treatments, respectively. The only exception was LA-RMY which produced higher propionate and butyrate than LA-RMI.

Moreover, the significantly higher SCFA were observed in the reconstituted probiotic-mao juice powders encapsulated with maltodextrin plus *T. triandra* gum or maltodextrin plus inulin. This might suggest that dietary fibers *viz. T. triandra* gum and inulin could have beneficial effects beyond SCFA formation. Many previous studies have reported that dietary fiber fractions, for example, inulin, oligofructose, lactulose and galactooligosaccharides, efficiently promote the growth of probiotics and they also stimulate the accumulation of SCFA (Nomoto, 2005). SCFA formation may be possibly explained by bifidobacterial biomass (Table 5.8), creating by the bifidogenic effect from treatment addition. Hosseini et al. (2011) revealed that propionate was believed to lower lipogenesis, serum cholesterol levels, and carcinogenesis in other tissues, while butyrate could protect colon from inflammatory bowel disease (Van Immerseel et al., 2010). Furthermore, increasing the SCFA synthesis could create more acidic environment to the colon which was important in terms of colonizing resistance against pathogens (Van de Wiele et al., 2004).

Table 5.8 Enumeration of other colon bacteria in simulated colon environment after fermentation with various treatment conditions at 37°C for 24 h

Treatment conditions	Numbers of other colon bacteria (CFU/ml) after fermentation at 37°C							
	Bifidobacteria		Fecal coliforms		Clostridia		Total anaerobic bacteria	
	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h
Colon fluid	$6.42\pm0.81^{aA}\times10^{6}$	$7.04\pm1.03^{bcA}\times10^{6}$	$1.65\pm0.48^{aC}\times10^{5}$	$1.51\pm0.60^{aC}\times10^{5}$	$7.83\pm1.22^{aB}\times10^{5}$	$7.94\pm0.85^{aB}\times10^{5}$	$6.42\pm1.53^{aA}\times10^{6}$	$6.30\pm2.08^{aA}\times10^{6}$
Lactobacillus casei 01 (LC)								
LC-F	$6.53\pm0.64^{aB}\times10^{6}$	$1.22\pm0.41^{aA}\times10^{7}$	$1.82\pm0.36^{aE}\times10^{5}$	$7.30\pm0.42^{cF}\times10^{4}$	$8.12\pm0.74^{aC}\times10^{5}$	$6.42\pm0.73^{cdF}\times10^{4}$	$6.83\pm0.41^{aB}\times10^{6}$	$5.68\pm0.54^{cD}\times10^{5}$
LC-FM	$6.80\pm0.45^{aA}\times10^{6}$	$6.85\pm1.30^{cA}\times10^{6}$	$1.70\pm0.25^{aD}\times10^{5}$	$4.12\pm1.20^{deE}\times10^{4}$	$7.80\pm0.56^{aB}\times10^{5}$	$5.10\pm0.11^{eE}\times10^{4}$	$7.02\pm1.17^{aA}\times10^{6}$	$4.03\pm0.29^{dC}\times10^{5}$
LC-RM	$6.43\pm0.50^{aB}\times10^{6}$	$8.34\pm0.66^{bA}\times10^{6}$	$1.63\pm0.71^{aE}\times10^{5}$	$3.36\pm0.53^{eG}\times10^{4}$	$7.94\pm0.63^{aC}\times10^{5}$	$6.32\pm0.35^{dF}\times10^{4}$	$6.75\pm0.97^{aB}\times10^{6}$	$4.31\pm0.46^{dD}\times10^{5}$
LC-RMY	$6.40\pm1.02^{aB}\times10^{6}$	$1.05\pm0.47^{aA}\times10^{7}$	$1.65\pm0.60^{aD}\times10^{5}$	$8.90\pm0.15^{hF}\times10^{3}$	$8.04\pm1.15^{aC}\times10^{5}$	$4.02\pm0.27^{fE}\times10^{4}$	$6.39\pm1.05^{aB}\times10^{6}$	$2.18\pm1.22^{eD}\times10^{5}$
LC-RMI	$6.42\pm0.55^{aB}\times10^{6}$	$9.82\pm0.73^{aA}\times10^{6}$	$1.62\pm0.42^{aD}\times10^{5}$	$9.72\pm0.29^{gF}\times10^{3}$	$7.85\pm1.80^{aC}\times10^{5}$	$3.87\pm0.90^{fE}\times10^{4}$	$6.45\pm0.60^{aB}\times10^{6}$	$2.36\pm1.12^{eD}\times10^{5}$
Lactobacillus acidophilus LA5 (LA)								
LA-F	$6.69\pm0.87^{aB}\times10^{6}$	$9.53\pm0.60^{aA}\times10^{6}$	$1.65\pm0.37^{aD}\times10^{5}$	$8.01\pm0.20^{bE}\times10^{4}$	$7.60\pm1.48^{aC}\times10^{5}$	$8.14\pm0.45^{aE}\times10^{4}$	$6.50\pm0.92^{aB}\times10^{6}$	$8.14\pm1.63^{bC}\times10^{5}$
LA-FM	$6.42\pm1.14^{aA}\times10^{6}$	$6.80\pm1.03^{cA}\times10^{6}$	$1.61\pm0.89^{aD}\times10^{5}$	$5.79\pm0.88^{dF}\times10^{4}$	$7.62\pm0.44^{aB}\times10^{5}$	$7.02\pm0.28^{cE}\times10^{4}$	$6.52\pm0.45^{aA}\times10^{6}$	$6.58\pm0.45^{cC}\times10^{5}$
LA-RM	$6.56\pm0.39^{aB}\times10^{6}$	$8.28\pm0.52^{bA}\times10^{6}$	$1.74\pm0.55^{aD}\times10^{5}$	$4.68\pm1.03^{deF}\times10^{4}$	$8.01\pm2.43^{aC}\times10^{5}$	$7.14\pm0.50^{cE}\times10^{4}$	$6.83\pm1.65^{aB}\times10^{6}$	$6.07\pm1.06^{cC}\times10^{5}$
LA-RMY	$6.50\pm0.61^{aB}\times10^{6}$	$9.72\pm0.30^{aA}\times10^{6}$	$1.81\pm0.71^{aE}\times10^{5}$	$1.18\pm0.24^{fgG}\times10^{4}$	$7.90\pm1.13^{aC}\times10^{5}$	$5.38\pm0.64^{eF}\times10^{4}$	$6.42\pm0.58^{aB}\times10^{6}$	$4.02\pm0.71^{deD}\times10^{5}$
LA-RMI	$6.50\pm1.60^{aB}\times10^{6}$	$9.93\pm1.06^{aA}\times10^{6}$	$1.57\pm0.68^{aE}\times10^{5}$	$1.92\pm0.73^{fG}\times10^{4}$	$7.74\pm0.86^{aC}\times10^{5}$	$5.60\pm0.31^{eF}\times10^{4}$	$6.45\pm0.70^{aB}\times10^{6}$	$3.86\pm0.98^{deD}\times10^{5}$

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

5.3.8 Changes of other colon bacteria on *in vitro* colon experiments

In this study, the results in Table 5.8 showed that the modulation of gut microbiome was associated with either probiotics or treatment conditions (i.e. free cells/encapsulated cells and encapsulating materials). The free cells in water (LC-F and LA-F) and the encapsulated lactobacilli in RMY and RMI markedly increased the bifidobacteria proliferation ($P < 0{\cdot}05$); in contrast, no significant difference between two lactobacilli either on FM or RM and negative control ($P > 0{\cdot}05$). Irrespective of the treatments and lactobacilli species, fecal coliforms, clostridia and total anaerobes were efficiently inhibited ($P < 0{\cdot}05$) by encapsulated probiotic cells more than uncoated cells, especially treatments RMY and RMI. The percentages of inhibition as affected by most treatments LC and LA for fecal coliforms, clostridia and total anaerobes were ranged around 51.45-94.61%, 89.29-95.07% and 87.48-96.59%, respectively. In this case, it was worth to mention that LC seemed to have the stronger activity on enhancing the bifidobateria growth and limiting number of the fecal coliforms ($P < 0{\cdot}05$).

Some studies have been reported the beneficial of probiotics and prebiotics, either alone or together, on gut microbioflora modulation. Hsieh et al. (2013) and Zhang et al. (2014) reported that lactobacilli increased numbers of healthy bacteria (genera *Lactobacillus* and *Bifidobacterium*), but decreased number of pathogenic bacteria (*E. coli* and *Clostridium* species). Furthermore, prebiotics, mainly carbohydrate, especially polysaccharides or oligosaccharides, served as the selective nutrients for healthy bacteria (Nagpal et al., 2012). Besides, they also blocked the adhesion of pathogenic bacteria to intestinal epithelial cells, serving as antimicrobial compounds (Gibson et al., 2005; Shoaf et al., 2006).

To confirm the oral administrative effects of probiotic-mao juice powders and pur mao juice on colon microbiota, *in vivo* animal models were done to show that the microbiome in rat feces showed in different SCFA and colon community patterns after feeding with probiotic-mao juice powder and pur mao juice (data not shown). The SCFA concentrations *viz.* acetate, propionate and butyrate sharply increased ($P < 0{\cdot}05$) after admistration probiotic-mao juice powders compare with pur mao juice. The effects of probiotic-mao juice powders could directly modulate the colon bacterial communities. probiotic-mao juice powders modulated the microbiome in the rat feces, by increasing SCFA, lactobacilli and bifidobaccteria, while the populations of clostridia, fecal coliforms and total anaerobes decreased noticetably (data not shown).

5.4 Conclusion

In this study, the probiotic lactobacilli in maoluang juice powder encapsulated with maltodextrin plus *T. triandra* gum and maltodextrin plus inulin similarly showed protective ability on probiotic bacteria against adverse condition of simulated stomach and small intestinal fluids. Spray dried probiotic along with maoluang juice containing *T. triandra* gum also modulated the microbiome in the colon model, by increasing lactic acid, SCFA, lactobacilli and bifidobaccteria. Significant decreases of toxic ammonia, clostridia, fecal coliforms and total anaerobes were also observed. Therefore, this concluded that *T. triandra* gum can be used as an effective co-encapsulating material for spray drying probiotic. The benefit impacts of this probiotic product for improving of health status were confirmed by the *in vivo* models.

CHAPTER 6

CONCLUSIONS

In this research, the overall conclusions could be presented as follow:

1) The results from this experiment revealed that no significant changes in total soluble solids, pH, and viscosity of thermally and ultra-sonic treated mao juices could be observed. Ultra-sonication had a noticeable effect on color parameters, but sensorial characteristics of treated juice were no different from fresh juice. However, variations in the ripeness of mao used in this study could be a limitation on the parameters investigated in this study. Although pasteurization is normally applied to extend shelf-life of fruit juices, this method damages the desired characteristics and antioxidant constituents of fruit juice products. Ultra-sonication could be an alternative mao processing method to obtain a juice with high retention of bioactive compounds and antioxidant capacities, and low residual PPO and POD activities as well as microbial counts.

2) The probiotic-supplemented Mao juice powder in vacuum-sealed package stored at 4ºC could maintain high levels of *L. casei* viability and bioactive compounds than those in the other packaging conditions throughout the storage period. Some properties of the powders *viz*. moisture content, a_w, bulk density and solubility were found to change significantly. Refrigerated storage allowed the powder, especially that in the vacuum sealing package, to have the optimal parameters. Thus, this indicates that stability of probiotic, powder properties and bioactive compounds in spray dried probiotic-supplemented Mao juice could be preserved for a long time when using the appropriate packages and storage conditions.

3) The probiotic lactobacilli in maoluang juice powder encapsulated with maltodextrin plus *T. triandra* gum and maltodextrin plus inulin similarly showed protective ability on probiotic bacteria against adverse condition of simulated stomach and small intestinal fluids. Spray dried probiotic along with maoluang juice containing *T. triandra* gum also modulated the microbiome in the colon model, by increasing lactic acid, SCFA, lactobacilli and bifidobaccteria. Significant decreases of toxic ammonia, clostridia, fecal coliforms and total anaerobes were also observed. Therefore, this concluded that *T. triandra* gum can be used as an effective co-encapsulating material

for spray drying probiotic. The benefit impacts of this probiotic product for improving of health status were confirmed by the *in vivo* models.

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APPENDIX

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Original paper

Ultra-Sonication Effects on Quality Attributes of Maoberry (*Antidesma bunius* L.) Juice

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Maoberry (*Antidesma bunius* L.) juice is popularly consumed as a pasteurized juice because of its high levels of antioxidants. Although pasteurization is normally applied to extend the shelf life of fruit juices, this method damages the desired characteristics and antioxidant constituents of fruit juice products. Ultra-sonication is an alternative process to treat fruit juices without impairing their health benefits and consumer acceptance. This study is the first report on ultra-sonication effects on the physicochemical properties, bioactive components, antioxidant activities, and sensorial characteristics of maoberry juice. After ultra-sonication processing at a frequency of 20 kHz and amplitude levels between 20% and 80% for 30 min and when compared with fresh and heated (75℃, 30 min) juices, we found that the physicochemical properties viz. total soluble solids, pH, and viscosity of processed juices did not change. However, a noticeable effect on the color parameters of ultra-sonicated juices shown by decreased lightness and increased redness values was found. The highest chroma value (*C) and total different colors (Δ*E*) were observed in the heated one. Microbial count levels, polyphenol oxidase and peroxidase activities were low in all treatments, in particular thermally and 80% amplitude treated batches. Total anthocyanins and phenolic compounds increased in the ultra-sonicated maoberry juices treated at 60% and 80% amplitudes. The contents of ascorbic acid in maoberry juice decreased significantly at 80% amplitude and at 75℃. The antioxidant activities (DPPH and FRAP assays) of ultra-sonicated products still retained high levels with no significant difference from the fresh sample. The sensorial attributes of ultra-sonicated juice showed no difference from the control (fresh juice), but higher than the heated sample. In general, ultra-sonication technology could be an appropriate processing technique to maintain the desired quality attribute characteristics of maoberry juice.**

Keywords: maoberry juice, *Antidesma bunius* L., ultra-sonication, bioactive compounds, antioxidative property

Introduction

In Thailand, maoberry or maoluang (*Antidesma bunius* L.) products are popularly consumed, particularly as pasteurized juice. Maoberry juice is commercially recommended as a functional beverage because of its high levels of antioxidants, particularly anthocyanins, phenolic acids, flavonoids, and ascorbic acid (Butkhup and Samappito 2008). These compounds are well known to help prevent cancer, diabetes, and cardiovascular and inflammatory diseases (Jorjong *et al.* 2015). The beneficial effects of maoberry by-products have also been reported in several studies.

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Puangpronpitag *et al.* (2011) found that the extracts of maoberry seed and skin-pulp residue showed anti-apoptotic and anti-inflammatory effects in human breast epithelial cells, and also displayed inhibitory effects against some pathogenic and spoilage bacteria (Butkhup and Samappito 2011). Kukongviriyapan *et al.* (2013) illustrated that maoberry pomace supplementation reduced blood pressure and improved the hemodynamic status of induced hypertensive rats.

Consumers today favor natural fresh foods with high nutritional values. Although pasteurization is one of the most popular methods used to successfully extend the shelf life of numerous fruit juices, this method generally damages the original qualities (i.e. color, flavor, taste etc.) and also causes losses in the antioxidant constituents of the products (Aadil *et al.* 2013). Therefore, a non-thermal technique such as ultra-sonication is an alternative to pasteurize processed fruit juices, without impairing their health benefits and consumer acceptance (Zafra-Rojas *et al.* 2013).

The effects of ultra-sonication on the characteristics of various berry juices such as blackberry, strawberry, and mulberry have been investigated by Engmann *et al.* (2015), Tiwari *et al.* (2009a) and Wong *et al.* (2010). The frequency of ultra-sonication is applied at 20 kHz to maintain the fruit's quality and reduce the incidence of decay and infection of microorganisms. However, no studies have investigated the effect of this novel processing technique on the qualities of maoberry juice. Hence, this study aimed to determine the alteration of physicochemical, antioxidative and microbiological properties, as well as sensory attributes of maoberry juice after ultra-sonic treatments at a frequency of 20 kHz and different amplitude levels of 20 − 80% for 30 min.

Materials and Methods

Maoberry juice preparation Maoberry fruits were harvested from an orchard in Sakon Nakhon province, Northeastern Thailand, from August to September of 2014. The fruits were washed and preserved at −20℃ until further experiments. Juice was extracted using a fruit juice extractor. The extract was mixed with distilled water at a ratio of 1:1 (w/w) before adjusting to the total soluble solids of 12°Brix with fructose sugar. A 100-mL of maoberry juice was treated in a 150-mL glass bottle using a high intensity ultra-sonic processor (VCX 130 PB 130 W, Sonics & Materials Inc., Newtown, CT). The ultra-sonic probe was immersed into the juice to half the depth of the sample which produced a 20 kHz wave frequency. The maoberry juice was exposed to different amplitude levels of 20 − 80% for 30 min (Santhirasegaram *et al.* 2013). At the final stage of processing, the temperatures of the samples increased from the baseline of 10.79 ± 0.53℃ to 41.63 ± 2.36, 51.71 ± 1.50, 58.70 ± 1.23, and 75.46 ± 1.63℃ for ultra-sonication with wave amplitudes of 20%, 40%, 60%, and 80%, respectively. For thermal treatment, a 100-mL of fresh juice was filled in a 150-mL glass bottle and heated at 75.34 ± 2.48℃ for 30 min. Afterward, all the processed juices were cooled down to 10.27 ± 1.40℃ using an ice-water bath prior to analyzing.

Color measurement Color parameters of fresh, ultra-sonic and thermally treated juices were measured using a colorimeter (Minolta Chroma Meter CR-300, Kyoto, Japan). Analytical data were expressed as L (lightness), a^* (redness) and b^* (yellowness) parameters. In addition, chroma value (C^*) and total different colors (ΔE) were calculated using equations (1) and (2).

$$\text{Chroma value } (C^*) = [(a^*)^2 + (b^*)^2]^{1/2} \quad \cdots\cdots \text{Eq. 1}$$

$$\text{Total different colors } (\Delta E) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \cdots\cdots \text{Eq. 2}$$

Measurements of total soluble solids, pH, and viscosity Total soluble solids and pH values of all the samples were determined using a refractometer (N-10E, Atago, Japan) and a Sartorius PB-20 pH meter (Sartorius, Gottingen, Germany). Dynamic viscosity of fresh and treated samples was measured using a control stress AR 2000 rheometer (TA Instruments, Inc., New Castle, DE) combined with commercial computer software (Rheology Advantage Analysis software Version 4.1). A concentric cylinder geometry (stator inner radius 15 mm, rotor outer radius 14 mm, cylinder immersed height 42 mm, gap 5,920 μm) was used. Juice (19.6 mL) was poured into the stationary cup and allowed to equilibrate to 25 ± 2℃, which was controlled by a circulating water system. Viscosity was calculated from the average of five points of the flow curves obtained in the shear rate range between 1 and 10 s^{-1}.

Crude enzyme extraction To extract the crude enzymes, 10 mL of samples were stirred with a mixture of 40 mL of 50 mM potassium phosphate, 1 M potassium chloride and 2% polyvinylpolypyrrolidone at 150 rpm for 20 min. The mixed solution was centrifuged at 4,200 rpm for 20 min before filtering through Whatman paper No. 1 (Apichartsrangkoon *et al.* 2013).

Polyphenol oxidase (PPO) activity PPO activity was determined according to the procedure described by Apichartsrangkoon *et al.* (2013). Briefly, 0.05 mL crude enzyme extract was poured into a mixture of 2.2 mL of 0.1 M potassium phosphate buffer (pH 6.5) and 0.25 mL of 0.2 M pyrocatechol. The absorbance of the mixed solution was recorded every 1 min for 5 min using a UV-Vis spectrophotometer (Perkin Elmer series Lambda 35, USA). One unit of enzymatic activity was defined as an increase of 0.1 unit of absorbance per min at 420 nm.

Peroxidase (POD) activity POD activity was measured following a modified method of Apichartsrangkoon *et al.* (2013) using a spectrophotometer at 470 nm. A 0.1-mL supernatant of crude enzyme extract was added into a mixture of 2.15 mL of 0.01 M sodium acetate buffer (pH 6), 0.25 mL of 0.1% hydrogen peroxide and 0.5% guaiacol. Subsequently, the increase in absorbance was recorded at every 1 min for 5 min. One unit of POD activity was defined as an increase of 0.1 unit of absorbance per min.

Determination of total anthocyanins Total anthocyanins were analyzed according to the pH differential method (Lee *et al.* 2005). First, 0.2-mL of the juice was added into 1.8 mL of 0.03 M potassium

chloride buffer (pH 1.0) or into 1.8 mL sodium acetate buffer (pH 4.5). Consequently, the absorbance of the well-mixed solution was measured at 520 and 700 nm using a spectrophotometer. Total anthocyanins were expressed as cyanidin 3-glucoside equivalent (mg CE/100 mL).

Determination of total phenolic compounds Total phenolic compounds were determined following the modified method of Chaikham and Apichartsrangkoon (2012). Accordingly, 2 mL of the juice were mixed with 8 mL of 100% cooled ethanol for 20 min before centrifuging at 4,500 rpm for 10 min. After that, 0.5 mL of supernatant was poured into 2.5 mL of 10% Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) and allowed to react for 5 min. A 2-mL of saturated sodium carbonate solution was then added to the mixture and held for 2 h at room temperature. The apparent blue complex solution was measured at 765 nm using a spectrophotometer. Total phenolic contents were expressed as mg gallic acid equivalent per 100 mL sample (mg GAE/100 mL).

Determination of ascorbic acid The concentrations of ascorbic acid in fresh and ultra-sonic treated maoberry juices were determined using a HPLC system (Chaikham and Apicartsrangkoon 2012). Before injection, 2 mL of the juice were mixed with 18 mL of diluted sulphuric acid (pH 2.2; Merck, Munich, Germany) by stirring at 150 rpm for 15 min, and centrifuged at 4,500 rpm at 4℃ for 10 min. Next, the supernatant was filtered through a 0.20-μm nylon membrane (Vertical, Bangkok, Thailand). The HPLC system (Shimadzu LC-10AD; Shimadzu, Kyoto, Japan) consisted of a low-pressure pump and a photodiode array detector (SPD-M20A; Shimadzu) adjusted to a λ_{max} 250 nm. Chromatographic separation was performed with a C18 column (YMC-Pack ODS-AM, 5 μm, 4.6 mm ID × 250 mm; YMC, Kyoto Japan). The isocratic system used 0.1 M acetic acid (Merck) in deionized water (RCI Lab-Scan, Bangkok, Thailand) as a mobile phase with a flow rate of 1.5 mL/min at 30℃. A 20-μL filtrate was injected into the column. The peak area of each component was determined and converted to concentration.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity DPPH radical scavenging activity was determined according to the procedure of Chaikham and Apicartsrangkoon (2012). In brief, 2 mL of maoberry juice were poured into 8 mL of 100% methanol for 10 min before mixing for 10 min, and then was centrifuged at 4,500 rpm for 10 min. Afterward, 1.6 mL supernatant or methanol (control) was well-mixed with 0.4 mL of 1.5 μM DPPH radical in methanol, and allowed to stand for 30 min at room temperature before measuring the absorbance at 517 nm. DPPH radical scavenging activity (% inhibition) was calculated using equation (3), where A_0 = absorbance of the control and A_1 = absorbance of the sample.

$$\text{DPPH radical scavenging activity} = [1 - (A_1/A_0)] \times 100 \quad \cdots\cdots \text{Eq. 3}$$

Ferric-reducing antioxidant power (FRAP) assay The ability to reduce ferric ions was analyzed using the method of Benzie and Strain (1996). Accordingly, 1 mL of the sample was mixed with 9 mL deionized water and filtered through a Whatman paper No. 1. After that, 3 mL of FRAP reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-triazine solution and 20 mM $FeCl_3.6H_2O$ solution) was added into the filtrate before incubating at 37℃ for 30 min. The absorbance of the mixture was measured at 593 nm. The antioxidant capacity based on the ability to reduce ferric ions was expressed as mM $FeSO_4$ per 100 mL sample (mM $FeSO_4$/100 mL).

Microbiological assessments Total plate counts, yeasts and molds, and fecal coliforms in fresh and processed maoberry juices were determined following the Bacteriological Analytical Manual (US Food and Drug Administration, 2001).

Sensory evaluation Sensory evaluation was carried out by consumers recruited within a 5-km radius of the research center. Thirty trained volunteers were enrolled and asked to rate the degree of preference between the samples. A 9-point hedonic scale test; 9 = like extremely much, 5 = neither like nor dislike and 1 = dislike extremely much was applied. Triplicate sets of 20 mL of fresh, heated and ultra-sonicated maoberry juices were served at 4℃ during the evaluation. Before starting the evaluation, participants were instructed to rinse their mouths with water after tasting the sample, as this could influence the result.

Statistical analysis Data consist of the means of six replications with standard deviations. Analysis of variance (ANOVA) was carried out using a SPSS Version 11.5. Differences among treatment means were compared by Duncan's multiple range tests with a level of significance of $P < 0.05$.

Results and Discussion

Physicochemical qualities Color is one of the most important visual criteria to which consumers refer with regards to the overall fruit juice quality. Table 1 depicts the effects of ultra-sonication and heating on color parameters in maoberry juice. For ultra-sonicated samples, it was found that the lightness (*L* parameter) in the juice tended to decrease with the rising wave amplitudes, while an increase in other parameters were observed. In overall, maoberry juices treated at 80% amplitude and at 75℃ were significantly lower in *L* value than the others, but showed the highest values of *a** (reddish), *b** (yellowish), *C** (chroma) and Δ*E* (total different colors) parameters. As indicated by the reduction of the lightness parameter, the increasing darkness and yellowish color in the juices could be caused by enzymatic browning involving PPO and POD, because the residual activities of both enzymes still remained in the products (Table 2). In addition, the decreased lightness and increased redness values in the ultra-sound treated juices might be because of the better extraction of anthocyanin pigments. Anthocyanins are the predominant polyphenolic compounds present in maoberry fruits and are responsible for the redness and blueness of this fruit (Butkhup and Samappito 2008). These results were confirmed by the levels of total anthocyanins in the samples

Table 1. Color parameters of fresh, heated and ultra-sonicated maoberry juices

Samples	Color parameters				
	L	*a**	*b**	*C**	Δ*E*
Fresh juice	16.59 ± 0.30^{a}	2.53 ± 0.06^{c}	2.31 ± 0.10^{c}	3.43 ± 0.05^{cd}	-
HT 75℃	13.92 ± 0.27^{d}	2.77 ± 0.06^{b}	2.50 ± 0.03^{b}	3.73 ± 0.03^{b}	2.83 ± 0.26^{a}
US 20%	16.55 ± 0.06^{a}	2.49 ± 0.04^{c}	2.27 ± 0.04^{cd}	3.37 ± 0.05^{d}	0.24 ± 0.05^{d}
US 40%	16.03 ± 0.11^{b}	2.79 ± 0.07^{b}	2.19 ± 0.04^{d}	3.54 ± 0.08^{c}	0.80 ± 0.11^{c}
US 60%	15.90 ± 0.07^{b}	2.63 ± 0.09^{bc}	2.25 ± 0.08^{cd}	3.47 ± 0.10^{cd}	0.87 ± 0.07^{c}
US 80%	14.48 ± 0.15^{c}	3.11 ± 0.10^{a}	2.68 ± 0.03^{a}	4.11 ± 0.05^{a}	2.35 ± 0.15^{b}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). HT is heat treatment and US is ultra-sonication.

Table 2. Physicochemical properties and enzymatic browning activities of fresh, heated and ultra-sonicated maoberry juices

Samples	Physicochemical properties			Enzymatic browning activity			
	TSS (°Brix)	pH	Viscosity (mPa.s)	PPO activity (Unit/m/mL)	Relative activity(%)	POD activity (Unit/m/mL)	Relative activity(%)
Fresh juice	12.77 ± 0.21^{a}	3.46 ± 0.03^{a}	5.18 ± 0.42^{a}	89.23 ± 3.71^{a}	100.00 ± 0.00^{a}	125.41 ± 6.32^{a}	100.00 ± 0.00^{a}
HT 75℃	12.79 ± 0.05^{a}	3.46 ± 0.02^{a}	5.25 ± 0.51^{a}	50.99 ± 2.79^{c}	57.30 ± 5.59^{e}	34.27 ± 2.82^{d}	27.35 ± 0.73^{e}
US 20%	12.73 ± 0.12^{a}	3.44 ± 0.02^{a}	5.55 ± 0.34^{a}	88.16 ± 1.95^{a}	98.86 ± 2.08^{b}	111.72 ± 3.89^{b}	89.17 ± 3.65^{b}
US 40%	12.80 ± 0.08^{a}	3.45 ± 0.02^{a}	5.29 ± 0.64^{a}	84.17 ± 3.66^{a}	94.34 ± 2.45^{c}	106.06 ± 4.40^{b}	84.59 ± 0.91^{c}
US 60%	12.84 ± 0.10^{a}	3.45 ± 0.01^{a}	5.43 ± 0.22^{a}	72.48 ± 2.59^{b}	81.38 ± 6.01^{d}	82.29 ± 2.84^{c}	65.80 ± 5.49^{d}
US 80%	12.81 ± 0.05^{a}	3.47 ± 0.04^{a}	5.61 ± 0.56^{a}	46.49 ± 1.62^{d}	52.18 ± 3.17^{f}	33.78 ± 4.07^{d}	26.98 ± 3.44^{e}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). TSS is total soluble solids, HT is heat treatment and US is ultra-sonication.

Table 3. Bioactive components and antioxidant capacities of fresh, heated and ultra-sonicated maoberry juices

Samples	Total anthocyanins (mg CE/100 mL)	Total phenolics (mg GAE/100 mL)	Ascorbic acid (mg/100 mL)	DPPH inhibition (%)	FRAP value (mM $FeSO_4$/100 mL)
Fresh juice	44.32 ± 5.02^{a}	274.65 ± 9.16^{b}	26.14 ± 2.12^{a}	56.52 ± 5.45^{a}	24.18 ± 1.27^{a}
HT 75℃	44.65 ± 5.68^{a}	280.04 ± 6.37^{b}	16.09 ± 1.38^{c}	50.47 ± 6.02^{b}	18.68 ± 2.42^{c}
US 20 %	45.80 ± 3.61^{a}	290.16 ± 5.62^{ab}	25.35 ± 1.05^{a}	58.91 ± 2.11^{a}	25.60 ± 2.00^{a}
US 40 %	48.12 ± 4.30^{a}	288.59 ± 8.12^{ab}	24.05 ± 3.16^{a}	60.43 ± 4.07^{a}	26.08 ± 3.49^{a}
US 60 %	50.63 ± 3.18^{a}	300.12 ± 6.89^{a}	25.07 ± 2.08^{a}	62.14 ± 1.92^{a}	23.95 ± 2.68^{ab}
US 80 %	49.01 ± 6.75^{a}	292.88 ± 5.21^{a}	18.19 ± 2.33^{b}	59.12 ± 2.85^{a}	22.32 ± 0.86^{b}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). HT is heat treatment and US is ultra-sonication.

which were enhanced by ultra-sonic conditions (Table 3). Therefore, anthocyanins may also protect the darkness effects from PPO. The ultra-sonication effects on color parameters of other fruit juices were previously reported by several researchers. For instance, Abid *et al.* (2013) and Bhat *et al.* (2011) respectively illustrated that the *L* and *a** parameters of ultra-sonicated apple and kasturi lime juices were lower than the fresh juices, whereas the *b** parameters increased noticeably. The significant decreases of those parameters in both grapefruit and Chokanan mango juices (Aadil *et al*. 2013; Santhirasegaram *et al*. 2013) were also observed. Moreover, Engmann *et al.* (2015) discovered that the *C** and Δ*E* values for most of the ultra-sonic treated mulberry juices were significantly higher than the control.

The influences of heat and ultra-sonic treatments on total soluble solids, pH and viscosity of maoberry juice are displayed in Table 2. The results indicated that thermal and ultra-sound processes did not induce any changes in the total soluble solids, pH and viscosity of the samples ($P > 0.05$). These values remained stable when the wave amplitudes increased. Our findings are supported by several other studies. For instance, Zafra-Rojas *et al.* (2013) reported that ultra-sonication treatments had no effect on the pH of purple cactus juice. In addition, Tiwari *et al.* (2008a) and Bhat *et al.* (2011) respectively found that orange and kasturi lime juices showed no significant changes in total soluble solids and pH after ultra-sonic processing. Cruz-Cansino *et al.* (2013) illustrated that the ultra-sonic process induced slight changes in green cactus pear juice pH and total soluble solids. In this study, the dynamic viscosity of processed maoberry juices did not change when

compared to the control. This outcome was different to a report of Santhirasegaram *et al.* (2013), which revealed that the application of ultra-sonic processing decreased the viscosity of Chokanan mango juice. They suggested that ultra-sonic cavitation caused the breakdown of large macromolecules and particles such as pectin in the juice, and decreased the viscosity of the juice. Ultra-sonication for 30 min reported to improve the quality of juice compared to control by inactivating spoilage microorganisms and increasing in extractability of bioactive compounds. Thus, 30 min treatment was also chosen in our study.

PPO and POD activities PPO and POD are the principal enzymes involved in the browning reactions of non-thermally processed fruit juices. Browning is an important aspect that influences consumer acceptance. The residual PPO and POD activities in fresh, thermally and ultra-sonic treated maoberry juices are described in Table 2. It was found that increasing the wave amplitudes reduced the activities of both enzymes, especially the enzymatic inactivation of POD. Inactivation levels of PPO and POD by thermal and ultra-sonic processing at 80% amplitude were markedly higher than those of the other juices ($P < 0.05$). The application of ultra-sonication with different amplitudes of 20%, 40%, 60% and 80% inhibited PPO by 1.14%, 5.66%, 18.62% and 47.82%, and POD by 10.83%, 15.41%, 34.20% and 73.02%, respectively. In this case, we noted that wave amplitudes had inhibitory effects on both of PPO and POD, but these were more pronounced for POD activity. Likewise, Rithmanee and Intipunya (2012) found that ultrasonic treatment at 100% amplitude and a frequency of 20 kHz for 30 min could inhibit PPO and POD activities in longan pulp by 70.68% and 94.06%, respectively, compared to an untreated sample. Costa *et al.* (2013) treated pineapple juice at 75% amplitude for 10 min, and they discovered that the PPO activity diminished by 20%. Moreover, Ercan and Soysal (2011) reported that POD activity in tomato extracts noticeably declined with increased ultrasonic amplitude power, and the reduction of POD activity was achieved at 50% and 75% amplitudes for 2.5 and 1.5 min, respectively. They revealed that physical stress because of bubble collapse can contribute toward enzymatic inactivation. Enzymatic inhibition can be affected by high shear generated by the interaction of cavitating bubbles with the acoustic field. da Rocha Cordeiro Dias *et al.* (2015) also explained that the extreme agitation created by microstreaming can disrupt Van der Waals interactions and hydrogen bonds in the polypeptide, resulting in protein denaturation. Temperatures above 60℃ can also denature the protein structures of the enzymes, but may negatively affect consumer acceptance and nutritional values of the products.

Bioactive components and antioxidant activity We investigated the effect of heat and ultra-sonic treatments on the concentrations of some phytochemicals viz. total anthocyanins, total phenolic compounds and ascorbic acid in maoberry juice. The results in Table 3 depicted that the processing conditions had no significant influence on the total anthocyanins in maoberry juice ($P > 0.05$). Alighourchi *et al.* (2013) found that the contents of anthocyanins in ultra-sonicated pomegranate juices did not decrease substantially; in fact they increased slightly at some amplitude levels and times. However, Engmann *et al.* (2015) and Tiwari *et al.* (2008b) respectively reported the reduction of anthocyanins after ultra-sonic treatments in mulberry and strawberry juices. They explained that the degradation of these compounds might be related to oxidation reactions promoted by the interaction of free radicals formed during ultra-sonication.

Moreover, our results also demonstrated that an increase ($P < 0.05$) of total phenolic compounds occurred in the juices treated at 60% and 80% amplitudes compared with the other samples (Table 3). These components were also found to increase significantly in kasturi lime juice (Bhat *et al.* 2011), grapefruit juice (Aadil *et al.* 2013) and purple cactus pear juice (Zafra-Rojas *et al.* 2013) after ultra-sonic processing, compared with the fresh juices. The increased phenolic compounds in the products might be a result of the higher intensities of wave amplitudes which could enhance the interruption of plant-cell walls to facilitate the release of their contents (Chaikham and Prangthip, 2015).

Besides anthocyanins and phenolics, the results in Table 3 illustrated that the contents of ascorbic acid in maoberry juices treated at 20 − 60% amplitudes did not show any significant change ($P > 0.05$). At a higher amplitude however, it decreased significantly ($P < 0.05$). In this case, after processing at 80% amplitude for 30 min, the temperature of the sample markedly increased to roughly 75.46℃. The declining level of this component was also observed in the heated juice. Therefore, the reduction of ascorbic acid could be primarily because of degradation from the heat. Previously, Abid *et al.* (2013) found no significant change in ascorbic acid concentration in apple juice after ultra-sonication at a 70% amplitude, frequency of 25 kHz and a controlled temperature of 20℃ for 30 min. This finding was similar to a report of Bhat *et al.* (2011) with kasturi lime juice. Under the same conditions, Aadil *et al.* (2013) revealed that sonication significantly improved ascorbic acid levels in grape juice by ~ 14.30% compared with the control. In contrast, Adekunte *et al.* (2010) found a significant decrease of this component in tomato juice. Similarly, Lee and Feng (2011) depicted that ascorbic acid in orange juice was also reduced by processing, possibly because of the generation of free radicals. This suggests that heat and oxidation reactions are mainly responsible for ascorbic acid degradation during ultra-sonication treatments (de São José *et al.* 2014). Furthermore, several researchers compared the effects of ultra-sonic and thermal processing on the retention of ascorbic acid in fruit juices, such as tomato juice (Ercan and Soysal, 2011) and orange juice (Tiwari *et al.* 2009b). They concluded that thermal treatments on juices resulted in a significantly lower level of ascorbic acid than ultra-sonication treatment.

Table 4. Microbiological qualities of fresh, heated and ultra-sonicated maoberry juices

Samples	Total plate counts (CFU/mL)	Yeasts & molds (CFU/mL)	Fecal coliforms (CFU/mL)
Fresh juice	$5.15 \pm 1.23 \times 10^{6a}$	$6.42 \pm 1.40 \times 10^{4a}$	$3.50 \pm 0.80 \times 10^{2a}$
HT 75℃	nd^{c}	nd^{e}	nd^{c}
US 20%	$3.83 \pm 2.01 \times 10^{4a}$	$2.18 \pm 0.57 \times 10^{2b}$	$0.80 \pm 0.50 \times 10^{b}$
US 40%	$8.24 \pm 1.52 \times 10^{2b}$	$0.98 \pm 0.15 \times 10^{2c}$	nd^{c}
US 60%	$2.49 \pm 0.74 \times 10^{2d}$	$0.52 \pm 0.23 \times 10^{2d}$	nd^{c}
US 80%	nd^{c}	nd^{e}	nd^{c}

Means in the same column followed by the same letters are not significantly different ($P > 0.05$). HT is heat treatment, US is ultra-sonication and nd is not detected.

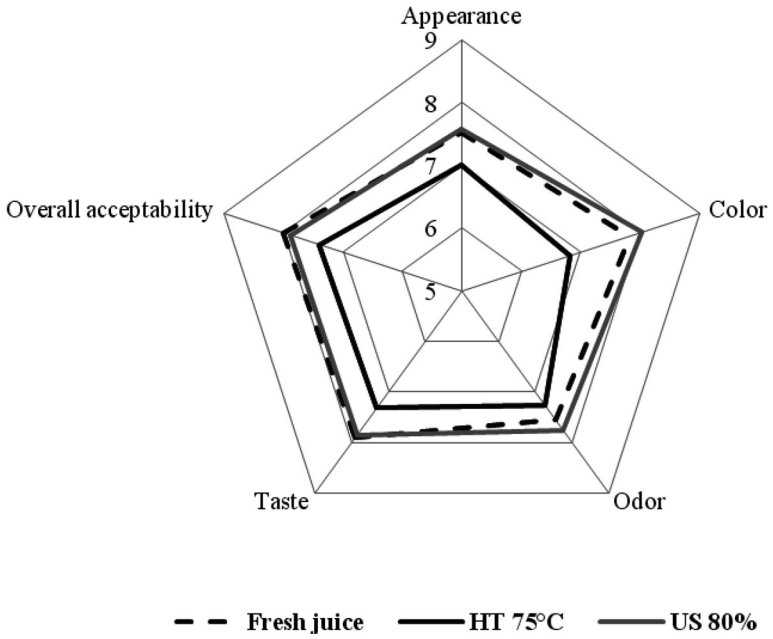

Fig. 1. Sensory evaluation of fresh, heated and ultra-sonicated maoberry juices. HT is heat treatment and US is ultra-sonication.

Antioxidants are reducing agents which possess the ability to protect humans and organisms from cell damage and homeostatic disruption caused by free radical-induced oxidative stress. The reducing properties of plant phytochemicals are related to the presence of phenolic constituents and some vitamins which exert their action by breaking the free-radical chain by donating a hydrogen atom (Jorjong *et al.* 2015). In this study, the antioxidant capacities including DPPH radical inhibition and FRAP values of fresh, thermally and ultra-sonic treated maoberry juices were investigated. Table 3 shows that the DPPH radical scavenging activities of ultra-sonicated maoberry juices were not significantly different ($P > 0.05$) from the fresh sample, and the percentages of radical inhibition ranged from $59.12 \pm 2.85\%$ to $62.14 \pm 1.92\%$; whereas this value was apparently lower in heated sample ($P < 0.05$). In this case, the lowest FRAP value was found in the juices treated at 80% amplitude and at 75℃ respectively, which had significantly lower reducing power than the other batches ($P < 0.05$). This phenomena was interpreted by Namiesnik *et al.* (2013), who revealed that the DPPH method is generally employed with aqueous-organic extracts containing hydrophilic and lipophilic compounds, while FRAP assay is appropriate only for hydrophilic compounds. However, the processed juice still retained a high level of antioxidant activity. Identical results were found with ultra-sonicated kasturi lime juice (Bhat *et al.* 2011) and purple cactus pear juices (Zafra-Rojas *et al.* 2013).

Microbiological assessments The microbiological count results are exhibited in Table 4, where the initial amounts of microorganisms present in fresh maoberry juice were 6.71, 4.81, and 2.54 log CFU/mL for total plate counts, yeasts and molds, and fecal coliforms, respectively. After ultra-sonication at 80% amplitude and heating at 75℃, all the indicator microbes in the treated juices were acceptably eliminated by the processing and complied with the limits of the Thai Community Product Standard (TCPS No. 486/2004) for ready-to-drink maoberry juice (Thai Industrial Standard Institute, 2004). Our results were similar to that observed by Abid *et al.* (2013) with apple juice. The microbial reduction was because of the enhancement of biocides by cavitation. The formation of free radicals and hydrogen peroxide

during ultra-sonication treatment can lead to microbial elimination. In addition, cavitation also creates shock waves that ultimately cause damage to the living microbes, in particular vegetative cells (Abid *et al.* 2013; Bhat *et al.* 2011).

Sensory evaluation With regards to the microbiological assessments, it was found that ultra-sonication at 80% amplitude and heating completely eliminated the general microorganisms in maoberry juice (Table 4). Therefore, these samples were selected for sensory evaluation compared to control (fresh juice). Figure 1 elucidates the sensorial attributes of fresh and processed juices which were evaluated by 30 trained panelists. The data showed that the liking scores of appearance, color, odor, taste, and overall acceptability of ultra-sonic treated juice were not significantly different ($P > 0.05$) compared to the fresh sample (appearance = 7.53 − 7.58, color = 7.82 − 8.03, odor = 7.54 − 7.76, taste = 7.85 − 7.90, and overall acceptability = 7.88 − 8.01), and were significantly higher ($P < 0.05$) than those from the heated product (appearance = 7.02, color = 6.83, odor = 7.25, taste = 7.32, and overall acceptability = 7.39). Although an alteration of color parameters was observed after ultra-sonication (Table 1), there were no major changes in the appearance of the juice, as indicated by the sensorial scores. Similar outcomes were observed between fresh, pasteurized and ultra-sonicated apple juice, where ultra-sonic treated juice was also more accepted than thermally treated juice (Ertugay and Başlar 2014).

Conclusion

The results from this experiment revealed that no significant changes in total soluble solids, pH, and viscosity of thermally and ultra-sonic treated maoberry juices could be observed. Ultra-sonication had a noticeable effect on color parameters, but sensorial characteristics of treated juice were no different from fresh juice. However, variations in the ripeness of maoberrie used in this study could be a limitation on the parameters investigated in this study. Although pasteurization is normally applied to extend shelf-life of fruit juices, this method damages the desired characteristics and antioxidant constituents of fruit juice products. Ultra-sonication could be an alternative maoberry processing method to obtain a juice with high retention of bioactive compounds and antioxidant capacities, and low residual PPO and POD activities as well as microbial counts.

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Spray drying probiotics along with maoluang juice plus *Tiliacora triandra* gum for exposure to the *in vitro* gastrointestinal environments

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ABSTRACT

Application of potential probiotic bacteria in dehydrated fruit juices could be an optional diversity for the consumers who are aware of functional beverages. This study purposed to investigate the impact of *Tiliacora triandra* gum on the survival of *Lactobacillus casei* 01 and *Lactobacillus acidophilus* LA5 along with maoluang juice after spray drying and their activities under simulated gut models, as compared to inulin addition. The results showed that probiotic-maoluang juice powders as immobilized by *Tiliacora triandra* gum and inulin similarly showed protective ability on probiotic bacteria against adverse conditions of simulated gastrointestinal (SGI) model. After 6 h incubation in SGI, the survival rates of spray dried *L. casei* 01 and *L. acidophilus* LA5 with maoluang juice and *Tiliacora triandra* gum or inulin were higher than the free cells, by increasing around 15–20% and 20–21%, respectively. According to the *in vitro* colon experiments, *Tiliacora triandra* gum and inulin apparently enhanced the accumulation of lactic acid, short-chain fatty acids (SCFA) and beneficial colon bacteria (i.e. lactobacilli and bifidobacteria), whilst the levels of toxic ammonia and the populations of harmful bacteria (i.e. clostridia and fecal coliforms) were diminished. Therefore, this indicates that *Tiliacora triandra* gum displayed prebiotic impacts on the human colon microbiome.

1. Introduction

Probiotic foods and beverages are dietary supplements containing potentially beneficial bacteria or yeasts. According to the definition of FAO/WHO (2001), probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*". Probiotics consider as "protective" intestinal bacteria which show several health benefits, viz. decreasing the lactose intolerance symptoms, reducing plasmatic cholesterol levels, controlling intestinal infections, stimulation of the immune system and anti-carcinogenic activity (Sanders et al., 2013). Furthermore, these microbes could also enhance the formation of essential mucosal nutrients including short-chain fatty acids (SCFA) and amino acids (i.e. arginine, cysteine and glutamine) (Bengmark, 1996). However, to provide health and clinical benefits, probiotic bacteria must possess a number of properties ($>10^6$–10^7 CFU/g or CFU/ml) when implanted in the human digestive tract (Chaikham, 2015a). There are various techniques to preserve these microbes such as immobilization via spray drying (Kingwatee et al., 2015; Riveros, Ferrer, & Bórquez, 2009).

Spray drying is one of the effective processes to entrap probiotic cells because of it is relatively inexpensive. In drying process, probiotic cell loses viability due to physical injury to microencapsule and heat generation. However, the loss of probiotic cells can be reduced by using the proper protectants such as prebiotics (i.e. inulin, xylose, fructo-oligosacccharides, etc.) and hydrocolloids (i.e. xanthan gum, guar gum, locust bean gum, etc.) for spray drying (Avila-Reyes, Garcia-Suarez, Jiménez, Martín-Gonzalez, & Bello-Perez, 2014; Solanki et al., 2013). Several findings exhibited that spray dried probiotics with optimal forms can protect the living cells from the adverse conditions of storage and digestion (Corcoran, Ross, Fitzgerald, & Stanton, 2004; Lian, Hsiao, & Chou, 2002). The findings of Kingwatee et al. (2015) illustrated that maltodextrin and inulin have potentially increased the survival rate

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of *Lactobacillus casei* 01 during spray drying, storage and under *in vitro* gastrointestinal environments. They also suggested that these materials could reduce the porous matrix of the microcapsule and improved the stability of immobilized probiotics.

Tiliacora triandra, namely "*Yanang*", is an indigenous vegetable used in many Thai cuisines. It leaves contain high levels of minerals (i.e. calcium and iron), beta-carotene, vitamin E and phenolic compounds (Chaveerach et al., 2016; Singthong, Oonsivilai, Oonmetta-aree, & Ningsanond, 2014). Hydrocolloid/gum in *Tiliacora triandra* leaves could be another source of natural polysaccharides and are interesting additives for food industry. The chemical structure of viscous component in *Tiliacora triandra* gum was similar to xylan. The predominant monosaccharide of *Tiliacora triandra* gum was xylose, together with substantial amounts of other neutral sugars (Singthong, Ningsanond, & Cui, 2009). Thus, the addition of *Tiliacora triandra* gum to maltodextrin may expect to provide an excellent material for protection the probiotics from heat and others adverse conditions.

Functional synbiotic soft-drinks or dehydrated fruit juices including maoluang (*Antidesma bunius* Linn.) juice containing probiotics and prebiotics are the challenging implementation for this research area. Maoluang juice has become more popular consumption in Thailand because it contains high amounts of bioactive constituents including ascorbic acid and phenols (i.e. anthocyanins and flavonoids), with antioxidant properties (Chaikham & Baipong, 2016; Jorjong, Butkhup, & Samappito, 2015). Puangpronpitag et al. (2011) and Butkhup and Samappito (2011) found that maoluang seed and skin-pulp extracts exhibited anti-apoptotic and anti-inflammatory effects in human breast epithelial cells as well as inhibitory effects against several pathogenic microorganisms. In addition, induced hypertensive rats fed with maolaung pomace found to be have lowering blood pressure and improving hemodynamic status (Kukongviriyapan et al., 2013).

Up to date, there is no information on the impact of *Tiliacora triandra* gum as carrier material for enhancing the survival of probiotics inmaolung juice after spray drying and with the *in vitro* gut models. Therefore, this research aimed to monitor the survivability of *Lactobacillus casei* 01 and *Lactobacillus acidophilus* LA5 after spray drying with maoluang juice plus maltodextrin, *Tiliacora triandra* gum and/or inulin, and under simulated gastrointestinal fluids. Changes of colon microbiome due to probiotic-maoluang juice powders in an *in vitro* colon model were also studied.

2. Materials and methods

2.1. Preparation of Tiliacora triandra gum

Tiliacora triandra leaves were freshly harvested from an orchard in Phranakhon Si Ayutthaya province, Thailand. Upon arrival at the laboratory, the leaves were washed with running tap water before dehydration at 65 °C for 4 h using a convective hot-air oven (FED series, Binder, Hauppauge, NY, USA). The dehydrated leaves were blended using a blender (MX-20G 250 W, National, Bangkok, Thailand) at high speed for 5 min and then extracted with distill water (a ratio of 1:6.6, w/v) at 85 °C for 100 min. After that, the extract was filtered through a vacuum filter and concentrated to half of its volume using a rotary vacuum evaporator (AV 10, IKA® Works, Inc. Wilmington, NC, USA) before being precipitated with three volumes of absolute ethanol (Merck, Darmstadt, Germany). Later, the precipitate was dried using a vacuum oven (XF050, France Etuves, Chelles, France) and powdered using a National blender (Singthong et al., 2009).

2.2. Activation of probiotic cultures

Freeze dried probiotics including *L. casei* 01 and *L. acidophilus* LA5 were purchased from Chr. Hansen (Hørsholm, Denmark). The cell pellets of both probiotics were prepared following the procedures of Chaikham, Apichartsrangkoon, Jirarattanarangsri, and Van de Wiele (2012). In brief, freeze dried cultures were rehydrated, activated and anaerobically incubated in de Man Rogosa and Sharp (MRS) broth (Hi-media, Mumbai, India) at 37 °C for 16 and 18 h respectively to reach their stationary phases. Both activated cultures were harvested by centrifugation at 4500 rpm and 4 °C for 20 min (Rotina 46 R, Hettich, Tuttlingen, Germany) and then washed twice with 0.85% (w/v) sterile saline water (Merck, Darmstadt, Germany). The cell pellets were then diluted to provide the concentration of 10^{12} CFU/ml using sterile saline water before use.

2.3. Spray drying procedure

Maoluang fruits (*Antidesma bunius* Linn.) from an orchard in Sakon Nakhon province, Thailand were cleaned and extracted using a juice extractor (HR 1871/10 Collection Juicer, Philips, Singapore). The extracted juice was separately blended with either 20% (w/v) maltodextrin (10.5 dextrose equivalent, DE: Maltrin®, Grain Processing Corporation, Muscatine, IA, USA) or mixture of 10% (w/w) maltodextrin containing 10% (w/w) *Tiliacora triandra* gum and/or 10% (w/w) inulin (Sigma-Aldrich, St. Louis, MO, USA) before pasteurization at 90 °C for 1 min (Chaikham, 2015b). After cooling down to 25 °C, the probiotic cultures were individually inoculated into the pasteurized juices to obtain a bacterial concentration of roughly 10^{10} CFU/ml before spray drying. A spray dryer (JCM Engineering concept, Bangkok, Thailand) equipped with a fluid atomizer (inside diameter of 5 mm) was operated in a co-current manner. Drying condition for the entire experiment was adjusted as follows: 25 °C feeding temperature, 0.6–1 L/h feeding rate, 15 psi atomizing pressure and 160 °C hot-air-inlet temperature to generate 80 °C outlet temperature (Kingwatee et al., 2015). Later, the collected powders were vacuum sealed in laminated bags (polyethylene tetraphthalate/polypropylene/aluminum: Siam Pack, Chiang Mai, Thailand) and kept in a refrigerator for further analysis.

2.4. Enumeration of viable probiotics after spray drying

To release the entrapped cells, 1 g powder was mixed with 99 ml sterile saline water using a stomacher (IUL Instruments, Barcelona, Spain) for 10 min at room temperature. After that, serial dilutions were made with sterile saline water and they were subsequently plated on MRS agar and anaerobically incubated at 37 °C for 48 and 72 h prior to determination the survivors (CFU/g) of *L. casei* 01 and *L. acidophilus* LA5, respectively.

2.5. Microstructure analysis of spry dried probiotic-maoluang juice powders

A scanning electron microscope (SEM) was used to examine external microstructure of spry dried probiotic-maoluang juice powders. The powders were coated with 10 nm of gold using a Hitachi E1010 ion sputter (Hitachi Science Systems Co. Ltd, Tokyo, Japan). The images of gold-coated samples were taken by using a Hitachi S-3000 N SEM (Hitachi High-Technologies Co. Ltd. Tokyo, Japan) at an accelerating voltage of 15 kV.

2.6. In vitro stomach and small intestine experiments

2.6.1. Preparation of simulated stomach and small intestinal fluids

Preparation of gastric, duodenal and bile fluids was carried out by mixing sets of inorganic, organic and accessory substances as described in Table 1, augmenting them to 500 ml with distilled water and subsequently adjusting them to pH 1.4, 8.1 and 8.1, respectively with either 1 M sodium hydroxide (Merck, Darmstadt, Germany) or 37% hydrochloric acid (Merck, Darmstadt, Germany). These fluids were prepared a day prior to use, and then stored at 4 °C. All these fluids were warmed to 37 °C prior to being use.

2.6.2. Batch experiment in simulated stomach and small intestinal sections

The various treatment compositions are shown in Table 2. For free cell treatments, cell pellets of both probiotics as prepared in Section 2.2 were then aseptically inoculated into acidified sterile distill water (pH 1.4) or sterile maoluang juice (pH 1.4) to provide the concentration of 10^8 CFU/ml. To reconstitute probiotic-maoluang powders, 20 g of powder were well-mixed with 80 ml warmed distill water (pH 1.4, 37 °C) for 10 min. Subsequently, 2 ml of all mixed solutions were inoculated into 15 ml of gastric fluid (pH 1.4) in sterile glass bottles sealed with a rubber cap and a metal ring. The mixtures were incubated anaerobically at 37 °C with gentle shaking at 100 rpm for 1 h. After the incubation period in the simulated stomach, 10 ml of duodenal fluid and 5 ml of bile fluid (pH 8.1) were fed into the system (pH 6.5), which was then allowed to incubate for a further 4 h.

To assess the viable cells, 1 ml of incubated fluid was withdrawn and diluted with 9 ml of 0.2 M sterile phosphate buffer (Merck, Darmstadt, Germany). Several dilutions were made with 0.1% (w/v) peptone water (Hi-Media, Mumbai, India) and then plated on MRS agar. Colonies appeared after incubation under anaerobic conditions at 37 °C for 48–72 h.

2.7. In vitro colon experiment

2.7.1. Preparation of carbohydrate-based medium

The carbohydrate-based medium was prepared according to Van de Wiele, Boon, Possemiers, Jacobs, and Verstraete (2004) by mixing the following substances; 4 g potato starch (O.V. Chemical, Chiang Mai, Thailand), 1 g (+)-arabinogalactan (Sigma-Aldrich, St. Louis, MO, USA), 2 g pectin (Sigma-Aldrich, St. Louis, MO, USA), 1 g xylan (Sigma-Aldrich, St. Louis, MO, USA), 0.4 g *D*-(+)-glucose (Merck, Darmstadt, Germany), 3 g yeast extract (Oxoid, Hampshire, UK), 1 g special peptone water (Oxoid, Hampshire, UK), 4 g mucin (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 g L-cysteine (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 1 L deionized water (RCI Labscan, Bangkok, Thailand). This medium was then sterilized at 121 °C for 15 min and adjusted with either 0.5 M hydrochloric acid or 0.5 M sodium hydroxide to pH 5.5.

2.7.2. Preparation of fecal fluid

The fecal fluid was prepared by collecting fecal samples from six healthy adults (aged 20–32 years) who had no history of antibiotic treatment within last 6 months. All fresh fecal samples were homogeneously mixed. Consequently, 25 g of mixed fecal were diluted with 150 ml of 0.7 M phosphate buffer (pH 7; Merck, Darmstadt, Germany), containing 1.5 g of sodium thioglycolate (Sigma-Aldrich, St. Louis, MO, USA) as a reducing agent, then homogenized for 10 min using a stomacher and centrifuged at 2000 rpm for 3 min. The supernatant was separated and used as "fecal fluid" (Chaikham et al., 2013).

2.7.3. Batch experiment in colon section

The colon fluid was prepared by mixing 50 ml fecal fluid with 50 ml carbohydrate-based medium in a sterile bottle and used as "colon fluid" for a control condition. For treatment conditions (Table 2), 250 ml of fecal fluid, 150 ml of carbohydrate-based medium and 100 ml of diluted cells or reconstituted samples were well-mixed in 500 ml of sterile bottle and subsequently flushed with nitrogen gas to simulate an anaerobic environment. All simulated colon bottles were then incubated at 37 °C for 24 h with gentle shaking at 100 rpm. During the incubation period, the colon fluids were collected at the initial stage, 6, 12 and 24 h for further analysis.

2.7.4. Determination of lactic acid and ammonia

Lactic acid were quantified using the UV spectrophotometric method at λ_{max} 340 nm (Perkin Elmer UV WINLAB, Perkin Elmer, Waltham, MA, USA) for the determination of *D*- and L-lactic acid, according to the manufacturer's instructions (R-Biopharm AG, Darmstadt, Germany).

To release ammonia gas from the colon fluids, an excess of magnesium oxide (Merck, Darmstadt, Germany) was added into 1 ml sample. The mixture was then distilled using a Kjeldahl Apparatus Vapodest 30 S (Kjeldahl, Gerhardt, Germany) to enable vaporization of ammonia which subsequently was trapped in boric acid solution (Merck, Gerhardt, Germany). The entrapped ammonia solution was then titrated with 0.02 M hydrochloric acid. The concentration of ammonia was calculated by the following equation (Chaikham et al., 2012):

Table 1
Compositions of synthetic gastric and small intestinal fluids of the *in vitro* digestive experiment (Oomen et al., 2003).

	Gastric fluid (pH 1.4)		Duodenal fluid (pH 8.1)		Bile fluid (pH 8.1)	
Inorganic substances	NaCl	2752 mg	NaCl	7012 mg	NaCl	5259 mg
	NaH_2PO_4	266 mg	$NaHCO_3$	5607 mg	$NaHCO_3$	5785 mg
	KCl	824 mg	NaH_2PO_4	80 mg	KCl	376 mg
	$CaCl_2$	400 mg	KCl	564 mg	HCl (conc.)	180 μl
	NH_4Cl	306 mg	$MgCl_2$	50 mg		
	HCl (conc.)	6.5 ml	HCl (conc.)	180 μl		
Organic substances	Glucose	650 mg	Urea	100 mg	Urea	250 mg
	Glucuronic acid	20 mg				
	Urea	85 mg				
	Glucosamine hydrochloride	330 mg				
Other accessories	Bovine serum albumin	1000 mg	$CaCl_2$	200 mg	$CaCl_2$	222 mg
	Mucin	3000 mg	Bovine serum albumin	1000 mg	Bovine serum albumin	1800 mg
	Pepsin	2500 mg	Pancreatin	9000 mg	Bile	30,000 mg
			Lipase	1500 mg		

Table 2
Treatment conditions for *in vitro* gastrointestinal experiments.

Treatment conditions	Abbreviations
Lactobacillus casei 01 (LC)	
Free cells in distill water (control)	LC-F
Free cells in maoluang juice	LC-FM
Reconstituted probiotic-maoluang juice powder plus 20% maltodextrin in distill water (1:4, w/v)	LC-RM
Reconstituted probiotic-maoluang juice powder plus 10% maltodextrin and 10% *Tiliacora triandra* gum in distill water (1:4, w/v)	LC-RMY
Reconstituted probiotic-maoluang juice powder plus 10% maltodextrin and 10% inulin in distill water (1:4, w/v)	LC-RMI
Lactobacillus acidophilus LA5 (LA)	
Free cells in distill water (control)	LA-F
Free cells in maoluang juice	LA-FM
Reconstituted probiotic-maoluang juice powder plus 20% maltodextrin in distill water (1:4, w/v)	LA-RM
Reconstituted probiotic-maoluang juice powder plus 10% maltodextrin and 10% *Tiliacora triandra* gum in distill water (1:4, w/v)	LA-RMY
Reconstituted probiotic-maoluang juice powder plus 10% maltodextrin and 10% inulin in distill water (1:4, w/v)	LA-RMI

$$\text{Ammonia concentration}(\text{mg/L}) = \left[\left(V_{\text{sample}} - V_{\text{blank}}\right) \times 0.02 \times 14 \times 1000\right] \times \Big/ V_{\text{sample}}$$

where, V_{sample} and V_{blank} are volumes (ml) of titers used to titrate the sample and blank (deionized water).

2.7.5. Determination of short-chain fatty acids

SCFA were determined according to the procedure of Chaikham et al. (2012) with some modifications. Two milliliters of sample were mixed with 0.4 g sodium chloride and acidified by adding 0.5 ml sulfuric acid conc. (Ajax, Sydney, Australia). A volume of 0.1 ml 2-methylhexanoic acid (as internal standard) (2.8 ml in 1 L deionized water; Fluka, Buchs, Switzerland) and 2 ml diethylether (Merck, Darmstadt, Germany) were added, and subsequently SCFA were extracted using a shaker for 10 min and centrifuged at 1000 rpm for 5 min. A 1-μl aliquot of the diethylether layer (top layer) was injected and measured with a Shimadzu GCMS-QP2010 gas chromatography (Shimadzu Cooperation Analytical & Measuring Instruments Division, Kyoto, Japan) equipped with a flame ionization detector. The gas chromatograph was equipped with a capillary free fatty acid-packed column (25 m × 0.53 mm, film thickness 1.2 μm: Superchrom, Milan, Italy). Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. The column temperature was 130 °C and the temperature of the injection port and detector was 195 °C.

2.7.6. Enumeration of colon microbial communities

Enumeration of colon microbial communities including lactobacilli, bifidobacteria, coliforms, clostridia and total anaerobic bacteria was determined using plate counting methods (Chaikham et al., 2012). Decimal dilutions in 0.1% (w/v) sterile peptone water of samples were placed on 5 types of selective media as follows; LAMVAB agar (Hartemink, Domenech, & Rombouts, 1997) for lactobacilli (72 h, anaerobic), RB-agar (Hartemink, Kok, Weenk, & Rombouts, 1996) for bifidobacteria (96 h, anaerobic), McConkey agar (Hi Media, Mumbai, India) for fecal coliforms (24 h, aerobic), TSC agar (Hi Media, Mumbai, India) containing *Clostridium perfringens* selective supplement (Merck, Darmstadt, Germany) for clostridia (24 h, anaerobic) and BHI agar (Oxoid, Hampshire, UK) for total anaerobic bacteria (24 h, anaerobic). All plated media were then incubated at 37 °C for colony counting.

2.8. Statistical data analysis

All data were the means of triplicate determinations with standard deviations (means ± SD). Analysis of variance (ANOVA) was carried out using SPSS version 15 (SPSS Inc. Chicago, IL, USA). Determination of significant differences among treatment means was analyzed by general linear model and Duncan's multiple range test ($P \leq 0.05$).

3. Results and discussion

3.1. Microstructure of spray dried probiotic-maoluang juice powders

According to our previous study, the outlet temperature of 80 °C was an optimal temperature as it provided microparticles of probiotics with high survival cells, low moisture content and low a_w (Kingwatee et al., 2015). Thus, in this study, the spray-drying of *L. casei* 01 and *L. acidophilus* LA5 in maoluang juice was performed with the outlet temperature 80 °C using 3 different encapsulating materials including pure and mixtures of maltodextrin. Fig. 1 shows the SEM images of the *L. casei* 01 in maoluang juice entrapped with 20% maltodextrin and 10% maltodextrin mixed with 10% *Tiliacora triandra* gum and/or 10% inulin, respectively. Microparticles of probiotic coated with 20% maltodextrin were spherical in shape with a small size and wrinkled surface (Fig. 1-a). Fig. 1-b displays irregularly spherical particles with some smooth and some shrinkage and dents on its surface, as affected by 10% *Tiliacora triandra* gum addition. The mixture of 10% maltodextrin and 10% inulin not only increased the average size of the probiotic loaded beads but it also smoothened the surface of microparticles (Fig. 1-c). In this case, the morphology of microparticles depends on the types of carrier materials (Solanki et al., 2013). For examples, slowly-formed gel of products containing milk provided an irregular shape in the granules (Reid et al., 2005). The microcapsules with smooth surface devoid the porosity resulted in a better protective property of coated microcapsules (Zanjani, Tarzi, Sharifan, & Mohammadi, 2014). Size and smoothness of the microparticles might affect its properties (e.g. solubility, water activity) and influencing the number of survival cells (Fritzen-Freire et al., 2012).

3.2. Survival of probiotics after spray drying

Survival of probiotics is a critical concern when spray drying is employed. Therefore, after the drying, the counts of *L. casei* 01 (LC) and *L. acidophilus* LA5 (LA) were determined and reported as shown in Table 3. For both lactobacilli, the survival cells were higher than 8 log CFU/g of dry powder, irrespective of the encapsulating materials. The treatments with maltodextrin alone showed significantly lower cell viability ($P \leq 0{\cdot}05$) compared to the mixtures of maltodextrin with *Tiliacora triandra* gum and/or with inulin (Table 3). However, no significant difference in viable counts was

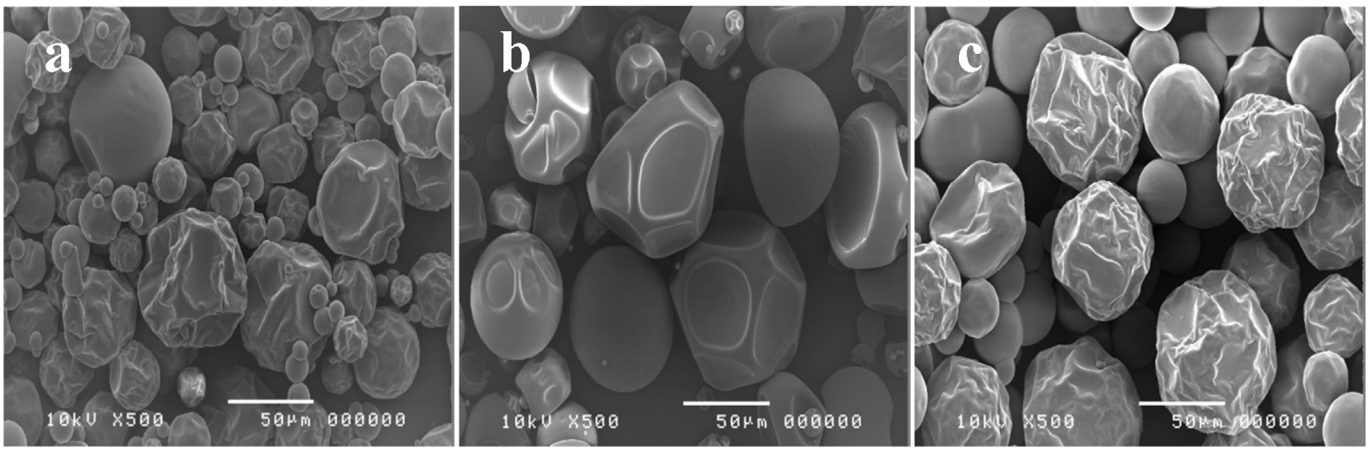

Figure 1. SEM images of maolung juice plus *L. casei* 01 powders spray dried at 80±2 °C outlet temperature using different carriers; (a) 20% maltodextrin, (b) 10% maltodextrin plus 10% *Tiliacora triandra* gum and (c) 10% maltodextrin plus 10% inulin.

Table 3
Viable of probiotics after spray drying with different carrier materials at 80°C outlet temperature.

Treatment conditions	Viable cells (CFU/g)
Lactobacillus casei 01 (LC)	
20% maltodextrin	$4.43 \pm 1.04^{b} \times 10^{8}$
10% maltodextrin plus 10% *Tiliacora triandra* gum	$5.01 \pm 1.87^{ab} \times 10^{8}$
10% maltodextrin plus 10% inulin	$5.45 \pm 0.92^{ab} \times 10^{8}$
Lactobacillus acidophilus LA5 (LA)	
20% maltodextrin	$5.83 \pm 1.27^{ab} \times 10^{8}$
10% maltodextrin plus 10% *Tiliacora triandra* gum	$6.38 \pm 0.81^{a} \times 10^{8}$
10% maltodextrin plus 10% inulin	$6.59 \pm 1.18^{a} \times 10^{8}$

Means in the same column with the same small letter indicate no significant difference (P > 0.05). Each data point is the average of three replications.

obtained between encapsulating matrices added with *Tiliacora triandra* gum or inulin for both lactobacilli species. To achieve highest recovery, these data suggested that either *Tiliacora triandra* gum or inulin could be used as a protective carrier for LC and LA immobilization. Several findings previously reported that encapsulating materials containing different prebiotics viz. inulin, polydextrose and fructooligosaccharide could improve the viability of probiotics, such as *Lactobacillus rhamnosus*, *L. casei*, *Lactobacillus plantarum* and *Bifodobacterium lactis*, during dehydration via spray drying technique (Corcoran et al., 2004; Kingwatee et al., 2015; Rajam & Anandharamakrishnan, 2015). Moreover, Lian et al. (2002) reported that gelatin, gum arabic and soluble starch also increased the survival of bifidobacteria after spray drying.

Besides the encapsulation and survival of probiotics after the spray drying, the *in vitro* gastrointestinal studies were performed in which reconstituted probiotic-maoluang powder and free cells of LC and LA were exposed to simulated gastric, small intestinal and colon fluids. The free cells (fresh cells) and reconstituted cells were prepared into 10 different treatment conditions as shown in Table 2.

3.3. Viable probiotics during passage through in vitro stomach and small intestinal models

Survival cells of LC and LA in stimulated stomach and small intestinal environments are illustrated in Table 4. The initial amounts of LC and LA were 4.53–5.40 $\times$ 10^{7} CFU/ml and 7.09–7.62 $\times$ 10^{7} CFU/ml, respectively. The numbers of viable cells in all treatments was gradually reduced over 3 log CFUs within 1 h ($P \leq 0{\cdot}05$). Comparing between free cells and reconstituted probiotic cells under simulated stomach environment, the reconstituted cells of LC tended to have apparent higher viability, especially, when entrapped with maltodextrin added with *Tiliacora triandra* gum or inulin ($P \leq 0{\cdot}05$). The benefits of immobilization are highlighted when the cells were successfully passed though small intestine fluid. Similarly, Hernandez-Hernandez et al. (2012) found that lactulose (prebiotic) could enhance the survivability of *Lactobacillus bulgaricus* ATCC7517 and *L. plantarum* WCFS1 embedded in simulated stomach and small intestine fluids, as compared to those without prebiotic addition. Kingwatee et al. (2015) also exhibited that spray dried *L. casei* 01 with lychee juice and inulin showed better impact on the survival rate of probiotic in gastric and bile juices. Moreover, Pankasemsuk, Apichartsrangkoon, Worametrachanon, and Techarang (2016) reported that microencapsulated *L. casei* 01 with 2% alginate and 1% hi-maize starch enabled optimal survival in both gastric and bile fluids. In contrast, there was insignificant difference ($P > 0.05$) in the number of survival cells of LA for all treatments under the stimulated stomach condition. Unfortunately, when LA was subsequently transferred to simulated intestinal environment, both free cells in distill water (F) and free cells in maoluang juice (FM) showed the least number of viable cells; whereas the survival of reconstituted probiotic cells was significantly higher in simulated gastric and intestinal fluids. In overall, free and encapsulate cells of LA showed significantly ($P \leq 0.05$) higher survivability than all forms of LC after incubation at 37 °C for 5 h. This finding was agreed with the reports of Krasaekoopt, Bhandari, and Deeth (2004) and Chaikham et al. (2013).

3.4. Viable cells of colon lactobacilli under in vitro colon section

The simulated colon fluid initially prepared from fecal fluid with carbohydrate-based medium contained 8.58 $\times$ 10^{4} CFU/ml lactobacilli. In the case of negative control, the colon fluid was incubated at 37 °C. The number of lactobacilli gradually increased to 2.69 $\times$ 10^{5} CFU/ml in 24 h ($P \leq 0{\cdot}05$). For the studied traits, the initial inoculum concentrations for LC and LM were 6.13–7.02 $\times$ 10^{7} and 7.09–7.92 $\times$ 10^{7} CFU/ml, respectively. The number of viable cells remained stable at the initial concentration after 6 h. Then the viable counts gradually increased ($P \leq 0{\cdot}05$) over 8 log CFUs within 24 h with only two exceptions (LA-FM and LA-RM) (Table 5). Interestingly, the transition from encapsulated lactobacilli to reconstituted cells showed a high recovery level in which they have

Table 4
Survival of free cells and reconstitute probiotic-maoluang juice powders in simulated gastric fluid (1 h) followed by small intestinal fluid (4 h) during incubation under anaerobic environment at 37 °C for 5 h.

Treatment conditions	Survival cells (CFU/ml) during incubation at 37 °C						
	Simulated stomach environment					Simulated small intestinal environment	
	Initial stage	0.25 h	0.5 h	0.75 h	1 h	3 h	5 h
Lactobacillus casei 01 (LC)							
LC-F	$4.98 \pm 0.67^{bA} \times 10^7$	$5.04 \pm 0.63^{cB} \times 10^6$	$6.19 \pm 0.89^{bC} \times 10^5$	$8.36 \pm 2.02^{aD} \times 10^4$	$5.93 \pm 0.29^{cdE} \times 10^3$	$5.08 \pm 1.28^{cdE} \times 10^3$	$9.08 \pm 0.63^{dF} \times 10^2$
LC-FM	$5.04 \pm 0.52^{bA} \times 10^7$	$1.38 \pm 0.48^{dB} \times 10^6$	$8.27 \pm 1.15^{dC} \times 10^4$	$2.09 \pm 0.77^{cD} \times 10^4$	$4.12 \pm 1.31^{dE} \times 10^3$	$3.24 \pm 0.46^{eE} \times 10^3$	$8.94 \pm 1.72^{dF} \times 10^2$
LC-RM	$4.53 \pm 0.80^{bA} \times 10^7$	$4.02 \pm 0.95^{cB} \times 10^6$	$2.08 \pm 1.42^{cC} \times 10^5$	$4.04 \pm 1.05^{bD} \times 10^4$	$6.01 \pm 0.65^{cE} \times 10^3$	$3.60 \pm 1.20^{deF} \times 10^3$	$9.60 \pm 1.18^{dG} \times 10^2$
LC-RMY	$5.12 \pm 0.75^{bA} \times 10^7$	$4.18 \pm 1.01^{cB} \times 10^6$	$2.53 \pm 0.56^{cC} \times 10^5$	$5.13 \pm 0.63^{bD} \times 10^4$	$1.92 \pm 1.06^{bE} \times 10^4$	$7.85 \pm 0.79^{bF} \times 10^3$	$2.65 \pm 1.28^{bcG} \times 10^3$
LC-RMI	$5.40 \pm 0.34^{bA} \times 10^7$	$4.20 \pm 0.41^{cB} \times 10^6$	$1.92 \pm 0.94^{cC} \times 10^5$	$5.65 \pm 1.52^{bD} \times 10^4$	$1.08 \pm 0.77^{bE} \times 10^4$	$8.14 \pm 0.91^{abF} \times 10^3$	$3.43 \pm 0.64^{bG} \times 10^3$
Lactobacillus acidophilus LA5 (LA)							
LA-F	$7.32 \pm 0.78^{aA} \times 10^7$	$3.85 \pm 0.41^{aB} \times 10^7$	$7.02 \pm 1.17^{aC} \times 10^6$	$1.85 \pm 1.04^{aD} \times 10^5$	$4.12 \pm 0.86^{aE} \times 10^4$	$6.80 \pm 1.73^{bcF} \times 10^3$	$1.14 \pm 1.40^{cdG} \times 10^3$
LA-FM	$7.45 \pm 1.06^{aA} \times 10^7$	$2.23 \pm 0.50^{bB} \times 10^7$	$7.48 \pm 0.73^{aC} \times 10^6$	$9.15 \pm 0.81^{aD} \times 10^4$	$4.04 \pm 1.68^{aE} \times 10^4$	$5.99 \pm 1.05^{cF} \times 10^3$	$1.65 \pm 1.61^{cG} \times 10^3$
LA-RM	$7.09 \pm 0.63^{aA} \times 10^7$	$3.05 \pm 0.44^{abB} \times 10^7$	$6.11 \pm 1.02^{aC} \times 10^6$	$9.02 \pm 0.43^{aD} \times 10^4$	$3.11 \pm 0.40^{aE} \times 10^4$	$6.02 \pm 0.28^{cF} \times 10^3$	$2.64 \pm 0.75^{bcG} \times 10^3$
LA-RMY	$7.40 \pm 0.19^{aA} \times 10^7$	$3.62 \pm 0.67^{aB} \times 10^7$	$6.50 \pm 0.60^{aC} \times 10^6$	$8.24 \pm 0.35^{aD} \times 10^4$	$3.52 \pm 0.27^{aE} \times 10^4$	$9.47 \pm 0.55^{aF} \times 10^3$	$5.05 \pm 0.31^{aG} \times 10^3$
LA-RMI	$7.62 \pm 0.81^{aA} \times 10^7$	$3.45 \pm 1.05^{aB} \times 10^7$	$6.83 \pm 2.16^{aC} \times 10^6$	$9.08 \pm 1.75^{aD} \times 10^4$	$4.01 \pm 0.74^{aE} \times 10^4$	$8.38 \pm 1.30^{abF} \times 10^3$	$4.89 \pm 1.80^{abG} \times 10^3$

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

Table 5
Viable cells of colon lactobacilli in simulated colon environment during fermentation with various treatment conditions at 37 °C for 24 h.

Treatment conditions	Numbers of colon lactobacilli (CFU/ml) during fermentation at 37 °C				
	Initial stage	6 h	12 h	18 h	24 h
Colon fluid	$8.85 \pm 1.83^{cC} \times 10^4$	$9.15 \pm 0.64^{cC} \times 10^4$	$1.48 \pm 0.40^{cB} \times 10^5$	$2.32 \pm 1.07^{gAB} \times 10^5$	$2.69 \pm 0.70^{fA} \times 10^5$
Lactobacillus casei 01 (LC)					
LC-F	$6.13 \pm 0.63^{bD} \times 10^7$	$8.59 \pm 1.50^{aC} \times 10^7$	$1.90 \pm 1.18^{abB} \times 10^8$	$9.68 \pm 0.84^{aA} \times 10^8$	$2.53 \pm 1.15^{bB} \times 10^8$
LC-FM	$6.84 \pm 1.08^{abD} \times 10^7$	$7.03 \pm 0.49^{bCD} \times 10^7$	$8.05 \pm 0.92^{bC} \times 10^7$	$3.17 \pm 0.22^{dA} \times 10^8$	$1.09 \pm 0.96^{cB} \times 10^8$
LC-RM	$6.25 \pm 0.70^{bD} \times 10^7$	$7.44 \pm 0.81^{abD} \times 10^7$	$9.65 \pm 0.55^{bC} \times 10^7$	$6.83 \pm 1.02^{bA} \times 10^8$	$2.57 \pm 1.01^{bB} \times 10^8$
LC-RMY	$7.02 \pm 1.82^{abD} \times 10^7$	$7.32 \pm 0.53^{bD} \times 10^7$	$1.10 \pm 0.42^{abC} \times 10^8$	$9.61 \pm 0.88^{aA} \times 10^8$	$5.22 \pm 0.35^{aB} \times 10^8$
LC-RMI	$6.75 \pm 0.94^{abD} \times 10^7$	$7.60 \pm 0.69^{abD} \times 10^7$	$1.83 \pm 0.75^{aC} \times 10^8$	$9.86 \pm 2.15^{aA} \times 10^8$	$5.14 \pm 1.38^{aB} \times 10^8$
Lactobacillus acidophilus LA5 (LA)					
LA-F	$7.35 \pm 1.61^{abC} \times 10^7$	$8.69 \pm 0.61^{aC} \times 10^7$	$9.93 \pm 1.09^{bBC} \times 10^7$	$5.43 \pm 1.84^{bcA} \times 10^8$	$1.60 \pm 0.40^{bcB} \times 10^8$
LA-FM	$7.21 \pm 0.85^{aC} \times 10^7$	$8.01 \pm 0.25^{aBC} \times 10^7$	$8.60 \pm 0.61^{bAB} \times 10^7$	$9.14 \pm 0.40^{fA} \times 10^7$	$8.05 \pm 1.05^{eAB} \times 10^7$
LA-RM	$7.09 \pm 0.44^{abD} \times 10^7$	$7.92 \pm 0.43^{abCD} \times 10^7$	$8.77 \pm 0.29^{bB} \times 10^7$	$1.13 \pm 0.18^{eA} \times 10^8$	$8.38 \pm 0.24^{dBC} \times 10^7$
LA-RMY	$7.60 \pm 1.09^{aC} \times 10^7$	$8.16 \pm 1.09^{aC} \times 10^7$	$1.53 \pm 0.80^{abB} \times 10^8$	$4.00 \pm 1.27^{cA} \times 10^8$	$2.53 \pm 0.47^{bB} \times 10^8$
LA-RMI	$7.92 \pm 0.58^{aB} \times 10^7$	$8.24 \pm 0.56^{aB} \times 10^7$	$2.03 \pm 1.48^{aA} \times 10^8$	$4.10 \pm 0.91^{cA} \times 10^8$	$2.82 \pm 1.09^{bA} \times 10^8$

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

a similar activity as their fresh cells. This might imply that the drying condition as well as the encapsulating carrier compounds used induced minimal cellular injury. In general, coating materials including maltodextrin, *Tiliacora triandra* gum, inulin and other oligosaccharides mainly provided a carbon source to assist the growth of microbes in the colon compartment. Our results were in agreement with several reports previously. Pankasemsuk et al. (2016) reported that entrapped *L. casei* 01 with hi-maize starch had great influence on the augmentation of indigenous lactobacilli during 24 h fermentation in an *in vitro* colon model. Gmeiner et al. (2000) fed milk-based product supplemented with *L. acidophilus* 74-2 plus fructooligosaccharide into a Simulator of the Human Intestinal Microbial Ecosystem (SHIME reactor). They found that the amounts of beneficial bacteria including lactobacilli and bifidobacteria increased in all colon compartments (i.e. ascending, transverse and descending colons), while the numbers of *Escherichia coli* and enterobacteria were noticeably diminished. Van de Wiele, Boon, Possemiers, Jacobs, and Verstraete (2007) also reported that inulin could enhance the viable cells of colon lactobacilli after fermentation with colon bacteria in the SHIME system.

3.5. Formation of lactic acid and ammonia on in vitro colon experiments

Lactobacilli are able to ferment sugars into two forms of lactic acid namely L-lactic acid and D-lactic acid. In fact, lactic acid promotes human health by lowering the gut pH against the growth of pathogenic bacteria. In our study, lactic acid, including *L*-form and *D*-form, were monitored under simulated colon environment as shown in Table 6. For the negative control, the level of lactic acid was lesser, but ammonia was higher as compared to those of the colon fluid treated with LA and LC ($P \leq 0{\cdot}05$). In this study, significant higher levels of lactic acid produced by LC was observed as compared with LA ($P \leq 0{\cdot}05$), irrespective of cell states, free and reconstituted cells, or the encapsulating materials used. This was in agreement with our previous study (Chaikham et al., 2012). In addition, these two lactobacilli stimulated the production of *D*-form than the *L*-form, especially LC. Specifically, LC increased D-lactic acid and L-lactic acid by an average of 389.62 mg/L and 144.92 mg/L, respectively. Regarding LC, the greater lactic acid reflects the higher number of survival counts. For example, as the count of LC-RMY and LC-RMI were double than that of LC-F, the formation of lactic acid by LC-RMY and LC-RMI were almost two-fold higher than that of LC-F. However, the residue survival cells

Table 6
Formation of lactic acid and ammonia in simulated colon environment after fermentation with various treatment conditions at 37 °C for 24 h.

Treatment conditions	Lactic acid and ammonia concentrations (mg/L) after fermentation at 37 °C							
	L-lactic acid		D-lactic acid		Total lactic acid		Ammonia	
	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h
Colon fluid	148.59 ± 2.29^{dF}	183 ± 10.25^{gE}	208.91 ± 8.15^{bD}	310.01 ± 8.55^{eC}	357.49 ± 10.30^{cB}	493.93 ± 9.48^{fA}	221.14 ± 10.22^{aB}	280.15 ± 3.53^{aA}
Lactobacillus casei 01 (LC)								
LC-F	168.03 ± 5.29^{aF}	287.15 ± 7.10^{dD}	247.16 ± 13.21^{aE}	653.15 ± 7.57^{aB}	415.19 ± 3.92^{aC}	940.30 ± 30.41^{bcA}	222.76 ± 9.73^{aB}	253.29 ± 4.10^{deA}
LC-FM	165.66 ± 4.63^{aF}	288.52 ± 8.89^{dD}	248.50 ± 10.26^{aE}	612.17 ± 9.44^{cB}	414.50 ± 13.84^{abC}	900.70 ± 12.10^{cA}	223.48 ± 5.94^{aB}	255.89 ± 2.04^{deA}
LC-RM	156.43 ± 1.12^{bF}	291.84 ± 6.78^{cdD}	244.75 ± 11.62^{aE}	615.77 ± 9.34^{bcB}	401.19 ± 6.51^{bC}	907.61 ± 17.13^{cA}	220.81 ± 6.82^{aB}	250.63 ± 5.49^{eA}
LC-RMY	161.43 ± 6.68^{abF}	318.27 ± 14.63^{bD}	243.26 ± 5.88^{aE}	647.40 ± 8.45^{aB}	404.68 ± 11.46^{bC}	965.67 ± 20.88^{bA}	217.73 ± 2.85^{aB}	235.13 ± 2.12^{gA}
LC-RMI	158.21 ± 5.76^{abF}	348.57 ± 12.57^{aD}	245.73 ± 4.58^{aE}	649.00 ± 9.35^{aB}	403.94 ± 10.34^{bC}	997.57 ± 11.47^{aA}	220.13 ± 4.01^{aB}	241.05 ± 0.78^{fA}
Lactobacillus acidophilus LA5 (LA)								
LA-F	155.25 ± 1.53^{bF}	255.39 ± 6.42^{fD}	239.16 ± 3.50^{aE}	585.55 ± 11.00^{dB}	391.41 ± 5.03^{bC}	840.95 ± 17.40^{eA}	225.64 ± 7.72^{aB}	268.72 ± 5.13^{bA}
LA-FM	156.11 ± 1.78^{bF}	263.82 ± 4.70^{efD}	243.78 ± 4.08^{aE}	586.81 ± 5.77^{dB}	398.89 ± 5.50^{bC}	850.62 ± 9.66^{eA}	221.05 ± 6.30^{aB}	260.18 ± 0.71^{cA}
LA-RM	153.11 ± 1.67^{cF}	272.31 ± 10.41^{deD}	243.83 ± 3.15^{aE}	592.16 ± 13.55^{cdB}	396.95 ± 1.84^{bC}	864.47 ± 1.66^{dA}	218.80 ± 6.11^{aB}	257.16 ± 2.01^{dA}
LA-RMY	153.03 ± 0.73^{cF}	290.78 ± 9.70^{cdD}	245.07 ± 2.77^{aE}	621.31 ± 7.56^{bB}	398.10 ± 13.00^{bC}	912.09 ± 17.10^{cA}	220.01 ± 5.25^{aB}	250.17 ± 1.14^{eA}
LA-RMI	152.89 ± 4.91^{cdF}	298.50 ± 2.63^{cD}	250.91 ± 9.20^{aE}	617.98 ± 13.58^{bcB}	403.50 ± 6.21^{bC}	916.48 ± 10.97^{cA}	220.11 ± 3.62^{aB}	251.82 ± 0.92^{eA}

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

of LA did not showed the clear correlation between functionality of lactic acid and ammonia production.

In our study, the decrease of the ammonia production was observed when the colon fluid was treated with both lactobacilli (Table 6). For the control, ammonia sharply increased from 221.14 to 280.15 mg/L within 24 h ($P \leq 0{\cdot}05$), while ammonia increased from 217.73 to 223.48 and 218.80–225.64 mg/L to 235.13–255.89 and 250.17–268.75 mg/L in treatments with LC and LA, respectively. Interestingly, comparing to free cells, the encapsulated cells of both LC and LA were highly capable of inhibiting ammonia production. Especially, lactobacilli encapsulated with *Tiliacora triandra* gum and inulin showed the most effective activities on reducing ammonia formation. The beneficial effects of probiotics especially decrease of ammonia production have been reported in many previous studies (Chaikham et al., 2012; Fooladi, Hosseini, Nourani, Khani, & Alavian, 2013). The decrease of ammonia production is associated with the reduction of urease activity of bacterial microflora and ammonia adsorption (Fooladi et al., 2013; Kanauchi et al., 1999). As ammonia is considered as a potential tumor promoters in the colon (Gråsten et al., 2000). Therefore, the low production of ammonia in the colon could be able to promote a beneficial health effect.

In this context, our result suggested the selection of encapsulating material provided positive health benefits for human by stimulating the survival of probiotics and their activities. The maltodextrin mixed with *Tiliacora triandra* gum and/or inulin showed positive influence on either supporting cell survival or changing the levels of lactic acid and ammonia, as fermentation end products.

3.6. Formation of SCFA on in vitro colon experiments

The benefit of SCFA by produced by probiotic bacteria have been reported in a countless number of studies, for examples, reducing potentially pathogenic clostridia by lowering pH and improving functions associated with ion transport (Topping, 1996; Wong, de Souza, Kendall, Emam, & Jenkins, 2006). As shown in Table 7, the fermentation of lactobacilli in simulated colon environment produced significant increase in SCFA including acetate, propionate and butyrate ($P \leq 0{\cdot}05$). Irrespective of tested conditions, the SCFA profile sharply increased during probiotics fermentation ($P \leq 0{\cdot}05$), especially acetate. The encapsulated lactobacilli increased SCFA by more than two-fold as compared with SCFA yielded in the negative control (only colon fluid). The effect of the

Table 7
Formation of short-chain fatty acids (SCFA) in simulated colon environment after fermentation with various treatment conditions at 37 °C for 24 h.

Treatment conditions	SCFA concentration (mg/L) after fermentation at 37 °C							
	Acetate		Propionate		Butyrate		Total SCFA	
	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h
Colon fluid	848.15 ± 22.75^{aD}	1056.43 ± 53.12^{iC}	315.98 ± 11.43^{aF}	419.18 ± 19.49^{hE}	178.64 ± 15.91^{aH}	231.50 ± 22.47^{gG}	1580.64 ± 71.50^{aB}	2100.51 ± 42.19^{gA}
Lactobacillus casei 01 (LC)								
LC-F	864.64 ± 40.13^{aD}	1444.80 ± 50.01^{fC}	320.04 ± 24.04^{aG}	605.32 ± 10.44^{eE}	185.03 ± 10.09^{aH}	393.61 ± 10.60^{eF}	1600.03 ± 59.32^{aB}	2909.63 ± 60.61^{dA}
LC-FM	870.00 ± 63.45^{aC}	1600.61 ± 84.22^{dB}	321.82 ± 15.60^{aF}	687.44 ± 12.90^{dD}	180.67 ± 11.32^{aG}	432.10 ± 14.78^{dE}	1612.55 ± 50.18^{aB}	3141.10 ± 45.12^{cA}
LC-RM	860.10 ± 21.41^{aD}	1990.30 ± 58.10^{bB}	319.67 ± 19.01^{aG}	735.50 ± 25.99^{cE}	183.82 ± 17.02^{aH}	503.22 ± 23.41^{cF}	1599.46 ± 32.05^{aC}	3913.44 ± 31.25^{bA}
LC-RMY	875.23 ± 25.22^{aD}	2245.77 ± 30.73^{aB}	328.69 ± 20.03^{aF}	915.05 ± 50.38^{aD}	190.04 ± 15.68^{aG}	601.84 ± 19.80^{aE}	1636.83 ± 42.63^{aC}	4402.35 ± 50.15^{aA}
LC-RMI	883.14 ± 61.59^{aE}	2390.55 ± 90.46^{aB}	327.30 ± 11.75^{aG}	967.68 ± 19.72^{aD}	185.50 ± 20.09^{aH}	625.39 ± 19.11^{aF}	1604.73 ± 29.17^{aC}	4415.62 ± 61.53^{aA}
Lactobacillus acidophilus LA5 (LA)								
LA-F	861.04 ± 30.07^{aD}	1178.46 ± 25.60^{bC}	314.66 ± 30.62^{aF}	481.91 ± 30.05^{gE}	179.36 ± 10.22^{aG}	317.25 ± 42.70^{fF}	1593.67 ± 26.00^{aB}	2225.62 ± 30.00^{fA}
LA-FM	865.92 ± 22.38^{aD}	1362.73 ± 33.32^{gC}	340.60 ± 29.11^{aF}	568.53 ± 22.51^{fE}	178.92 ± 23.41^{aG}	390.12 ± 26.99^{eF}	1605.44 ± 30.12^{aB}	2560.38 ± 28.18^{eA}
LA-RM	859.05 ± 44.53^{aD}	1555.33 ± 10.02^{eC}	327.83 ± 20.58^{aG}	618.40 ± 13.88^{eE}	179.00 ± 17.71^{aH}	452.02 ± 18.01^{dF}	1595.04 ± 28.41^{aB}	3145.75 ± 53.91^{cA}
LA-RMY	867.78 ± 38.90^{aD}	1700.10 ± 59.49^{cdB}	320.06 ± 18.59^{aG}	793.60 ± 26.10^{bE}	182.64 ± 20.14^{aH}	550.31 ± 10.01^{bF}	1623.17 ± 40.53^{aC}	3900.01 ± 32.65^{bA}
LA-RMI	860.11 ± 19.24^{aD}	1748.69 ± 51.05^{cB}	328.06 ± 29.43^{aG}	769.50 ± 19.11^{bE}	180.55 ± 16.73^{aH}	568.90 ± 21.28^{bF}	1620.85 ± 25.42^{aC}	3895.09 ± 44.27^{bA}

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

Table 8
Enumeration of other colon bacteria in simulated colon environment after fermentation with various treatment conditions at 37 °C for 24 h.

Treatment conditions	Numbers of other colon bacteria (CFU/ml) after fermentation at 37 °C							
	Bifidobacteria		Fecal coliforms		Clostridia		Total anaerobic bacteria	
	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h	Initial stage	24 h
Colon fluid	$6.42 \pm 0.81^{aA} \times 10^6$	$7.04 \pm 1.03^{bcA} \times 10^6$	$1.65 \pm 0.48^{aC} \times 10^5$	$1.51 \pm 0.60^{aC} \times 10^5$	$7.83 \pm 1.22^{aB} \times 10^5$	$7.94 \pm 0.85^{aB} \times 10^5$	$6.42 \pm 1.53^{aA} \times 10^6$	$6.30 \pm 2.08^{aA} \times 10^6$
Lactobacillus casei 01 (LC)								
LC-F	$6.53 \pm 0.64^{aB} \times 10^6$	$1.22 \pm 0.41^{aA} \times 10^7$	$1.82 \pm 0.36^{aE} \times 10^5$	$7.30 \pm 0.42^{cF} \times 10^4$	$8.12 \pm 0.74^{aC} \times 10^5$	$6.42 \pm 0.73^{cdF} \times 10^4$	$6.83 \pm 0.41^{aB} \times 10^6$	$5.68 \pm 0.54^{cD} \times 10^5$
LC-FM	$6.80 \pm 0.45^{aA} \times 10^6$	$6.85 \pm 1.30^{cA} \times 10^6$	$1.70 \pm 0.25^{aD} \times 10^5$	$4.12 \pm 1.20^{deE} \times 10^4$	$7.80 \pm 0.56^{aB} \times 10^5$	$5.10 \pm 0.11^{eE} \times 10^4$	$7.02 \pm 1.17^{aA} \times 10^6$	$4.03 \pm 0.29^{dC} \times 10^5$
LC-RM	$6.43 \pm 0.50^{aB} \times 10^6$	$8.34 \pm 0.66^{bA} \times 10^6$	$1.63 \pm 0.71^{aE} \times 10^5$	$3.36 \pm 0.53^{eG} \times 10^4$	$7.94 \pm 0.63^{aC} \times 10^5$	$6.32 \pm 0.35^{dF} \times 10^4$	$6.75 \pm 0.97^{aB} \times 10^6$	$4.31 \pm 0.46^{dD} \times 10^5$
LC-RMY	$6.40 \pm 1.02^{aB} \times 10^6$	$1.05 \pm 0.47^{aA} \times 10^7$	$1.65 \pm 0.60^{aD} \times 10^5$	$8.90 \pm 0.15^{bF} \times 10^3$	$8.04 \pm 1.15^{aC} \times 10^5$	$4.02 \pm 0.27^{fE} \times 10^4$	$6.39 \pm 1.05^{aB} \times 10^6$	$2.18 \pm 1.22^{eD} \times 10^5$
LC-RMI	$6.42 \pm 0.55^{aB} \times 10^6$	$9.82 \pm 0.73^{aA} \times 10^6$	$1.62 \pm 0.42^{aD} \times 10^5$	$9.72 \pm 0.29^{gF} \times 10^3$	$7.85 \pm 1.80^{aC} \times 10^5$	$3.87 \pm 0.90^{fE} \times 10^4$	$6.45 \pm 0.60^{aB} \times 10^6$	$2.36 \pm 1.12^{eD} \times 10^5$
Lactobacillus acidophilus LA5 (LA)								
LA-F	$6.69 \pm 0.87^{aB} \times 10^6$	$9.53 \pm 0.60^{aA} \times 10^6$	$1.65 \pm 0.37^{aD} \times 10^5$	$8.01 \pm 0.20^{bE} \times 10^4$	$7.60 \pm 1.48^{aC} \times 10^5$	$8.14 \pm 0.45^{aE} \times 10^4$	$6.50 \pm 0.92^{aB} \times 10^6$	$8.14 \pm 1.63^{bC} \times 10^5$
LA-FM	$6.42 \pm 1.14^{aA} \times 10^6$	$6.80 \pm 1.03^{cA} \times 10^6$	$1.61 \pm 0.89^{aD} \times 10^5$	$5.79 \pm 0.88^{dF} \times 10^4$	$7.62 \pm 0.44^{aB} \times 10^5$	$7.02 \pm 0.28^{cE} \times 10^4$	$6.52 \pm 0.45^{aA} \times 10^6$	$6.58 \pm 0.45^{cC} \times 10^5$
LA-RM	$6.56 \pm 0.39^{aB} \times 10^6$	$8.28 \pm 0.52^{bA} \times 10^6$	$1.74 \pm 0.55^{aD} \times 10^5$	$4.68 \pm 1.03^{deF} \times 10^4$	$8.01 \pm 2.43^{aC} \times 10^5$	$7.14 \pm 0.50^{cE} \times 10^4$	$6.83 \pm 1.65^{aB} \times 10^6$	$6.07 \pm 1.06^{cC} \times 10^5$
LA-RMY	$6.50 \pm 0.61^{aB} \times 10^6$	$9.72 \pm 0.30^{aA} \times 10^6$	$1.81 \pm 0.71^{aE} \times 10^5$	$1.18 \pm 0.24^{fgG} \times 10^4$	$7.90 \pm 1.13^{aC} \times 10^5$	$5.38 \pm 0.64^{eF} \times 10^4$	$6.42 \pm 0.58^{aB} \times 10^6$	$4.02 \pm 0.71^{deD} \times 10^5$
LA-RMI	$6.50 \pm 1.60^{aB} \times 10^6$	$9.93 \pm 1.06^{aA} \times 10^6$	$1.57 \pm 0.68^{aE} \times 10^5$	$1.92 \pm 0.73^{fG} \times 10^4$	$7.74 \pm 0.86^{aC} \times 10^5$	$5.60 \pm 0.31^{eF} \times 10^4$	$6.45 \pm 0.70^{aB} \times 10^6$	$3.86 \pm 0.98^{deD} \times 10^5$

Means in the same column with the same small letter, whereas means in the same row with the same capital letter indicate no significant difference ($P > 0.05$). Each data point is the average of three replications.

treatment conditions on the SCFA profiles exhibited a similar pattern of production, yielding the highest acetate, propionate and butyrate in RMI treatment followed by RMY > RM > FM > F treatments, respectively. The only exception was LA-RMY which produced higher propionate and butyrate than LA-RMI.

Moreover, the significantly higher SCFA were observed in the reconstituted probiotic-maoluang juice powders encapsulated with maltodextrin plus *Tiliacora triandra* gum or maltodextrin plus inulin. This might suggest that dietary fibers viz. *Tiliacora triandra* gum and inulin could have beneficial effects beyond SCFA formation. Many previous studies have reported that dietary fiber fractions, for example, inulin, oligofructose, lactulose and galactooligosaccharides, efficiently promote the growth of probiotics and they also stimulate the accumulation of SCFA (Nomoto, 2005). SCFA formation may be possibly explained by bifidobacterial biomass (Table 8), creating by the bifidogenic effect from treatment addition. Hosseini, Grootaert, Verstraete, and Van de Wiele (2011) revealed that propionate was believed to lower lipogenesis, serum cholesterol levels, and carcinogenesis in other tissues, while butyrate could protect colon from inflammatory bowel disease (Van Immerseel et al., 2010). Furthermore, increasing the SCFA synthesis could create more acidic environment to the colon which was important in terms of colonizing resistance against pathogens (Van de Wiele et al., 2004). These fatty acids had also shown an anti-inflammatory effect (Tedelind, Westberg, Kjerrulf, & Vidal, 2007) and immune-modulating agents in human colon cancer cell lines (Fu, Shi, & Mo, 2004).

3.7. Changes of other colon bacteria on in vitro colon experiments

In this study, the results in Table 8 showed that the modulation of gut microbiome was associated with either probiotics or treatment conditions (i.e. free cells/encapsulated cells and encapsulating materials). The free cells in water (LC-F and LA-F) and the encapsulated lactobacilli in RMY and RMI markedly increased the bifidobacteria proliferation ($P \leq 0{\cdot}05$); in contrast, no significant difference between two lactobacilli either on FM or RM and negative control ($P > 0{\cdot}05$). Irrespective of the treatments and lactobacilli species, fecal coliforms, clostridia and total anaerobes were efficiently inhibited ($P \leq 0{\cdot}05$) by encapsulated probiotic cells more than uncoated cells, especially treatments RMY and RMI. The percentages of inhibition as affected by most treatments LC and LA for fecal coliforms, clostridia and total anaerobes were ranged around 51.45–94.61%, 89.29–95.07% and 87.48–96.59%, respectively. In this case, it was worth to mention that LC seemed to have the stronger activity on enhancing the bifidobateria growth and limiting number of the fecal coliforms ($P \leq 0{\cdot}05$).

Some studies have been reported the beneficial of probiotics and prebiotics, either alone or together, on gut microbioflora modulation. Hsieh et al. (2013) and Zhang et al. (2014) reported that lactobacilli increased numbers of healthy bacteria (genera *Lactobacillus* and *Bifidobacterium*), but decreased number of pathogenic bacteria (*Escherichia coli* and *Clostridium* species). Furthermore, prebiotics, mainly carbohydrate, especially polysaccharides or oligosaccharides, served as the selective nutrients for healthy bacteria (Nagpal et al., 2012). Besides, they also blocked the adhesion of pathogenic bacteria to intestinal epithelial cells, serving as antimicrobial compounds (Gibson, McCartney, & Rastall, 2005; Shoaf, Mulvey, Armstrong, & Hutkins, 2006).

4. Conclusions

In this study, the probiotic lactobacilli in maoluang juice powder encapsulated with maltodextrin plus *Tiliacora triandra* gum and maltodextrin plus inulin similarly showed protective ability on

probiotic bacteria against adverse condition of simulated stomach and small intestinal fluids. Spray dried probiotic along with maoluang juice containing *Tiliacora triandra* gum also modulated the microbiome in the colon model, by increasing lactic acid, SCFA, lactobacilli and bifidobaccteria. Significant decreases of toxic ammonia, clostridia, fecal coliforms and total anaerobes were also observed. Therefore, this concluded that *Tiliacora triandra* gum can be used as an effective co-encapsulating material for spray drying probiotic. The benefit impacts of this probiotic product for improving of human health status should be further study.

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