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aya CHUAH POH LAY

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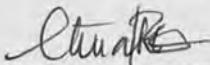
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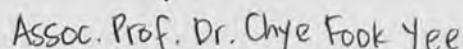
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**EFFICACY OF BANANA (*Musa paradisiaca* cv. Mysore)
INFLORESCENCE AGAINST FOODBORNE
PATHOGENS IN FOOD MODEL SYSTEM**

CHUAH POH LAY

**THESIS SUBMITTED IN PARTIAL FULLFILLMENT
FOR THE DEGREE OF BACHELOR OF FOOD
SCIENCE WITH HONOURS
(FOOD TECHNOLOGY AND BIOPROCESS)**

**SCHOOL OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2010**



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DECLARATION

I hereby declare that the materials in this thesis are my own except for quotations, excerpts, summaries and references, which have been duly acknowledged.

10 August 2010



Chuah Poh Lay
HN2006-3148



CERTIFICATION

NAME : CHUAH POH LAY
MATRIC NO. : HN2006-3148
TITLE : EFFICACY OF BANANA (*Musa paradisiaca* cv. Mysore) INFLORESCENCE AGAINST FOODBORNE PATHOGENS IN FOOD MODEL SYSTEM
DEGREE : BACHELOR OF FOOD SCIENCE WITH HONOURS (FOOD TECHNOLOGY AND BIOPROCESS)
VIVA DATE : 14 MAY 2010

DECLARED BY

1. SUPERVISOR
Assoc. Prof. Dr. Chye Fook Yee
2. EXAMINER 1
Pn. Nor Qhairul Izzreen Mohd Noor
3. EXAMINER 2
Dr. Muhammad Iqbal Hashmi
4. DEAN
Assoc. Prof. Dr. Mohd Ismail Abdullah



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Chuah Poh Lay
10 August 2010



ABSTRACT

Musa paradisiaca cv. Mysore inflorescence was investigated for its potential antimicrobial activity and the efficacy of its isolated fraction was evaluated in different models of food components and food properties *in vitro* against selected foodborne pathogens. The isolated water fraction of methanolic extract exhibited the most potent antimicrobial activity as compared to other fractions, with the inhibition zones of 16.10 ± 0.14 mm, 14.90 ± 0.14 mm, 13.00 ± 0.00 mm and 11.75 ± 0.35 mm against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Vibrio parahaemolyticus* respectively. Water fraction showed the lowest Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC : MBC) for *Bacillus cereus* (8mg/ml : 10mg/ml), *Staphylococcus aureus* (12mg/ml : 16mg/ml), *Listeria monocytogenes* (22mg/ml : 26mg/ml) and *Vibrio parahaemolyticus* (24mg/ml : 27mg/ml) respectively. The results in broth model system indicated that high concentration of proteins (>5%) and oils (>10%) significantly decreased the antimicrobial activity of water fraction by 2 times ($p<0.05$). However, the addition of starches at concentration up to 30% adds to the water fraction efficacy, whereby the percentage of reduction was significantly higher (10%) as compared to starch concentrations of 1%. Similarly, high concentration of NaCl (>8%) also aids to the further reduction of *Listeria monocytogenes* by water fraction, while *Staphylococcus aureus* is optimally inhibited at low concentration of NaCl (<0.5%). The antimicrobial activity of water fraction was found to be most efficient at pH 4.0. Incubation temperature did not reveal any significant effect on the efficacy of water fraction. Overall, this study has shown that *Musa paradisiaca* water fraction could be potential source of natural antimicrobials to be used in foods when applied to foods containing a lower levels of proteins and fats, at pH 4.0-7.0, as well as moderate levels of carbohydrates.

ABSTRAK

KEBERKESANAN JANTUNG PISANG (*Musa paradisiaca* cv. *Mysore*) TERHADAP PATOGEN BAWAAN MAKANAN DALAM SISTEM MODEL MAKANAN

Jantung pisang (*Musa paradisiaca* cv. *Mysore*) dikajikan untuk mengetahui aktiviti antimikrobialnya serta keberkesanannya dalam model berlainan yang mengandungi komponen makanan dan sifat makanan terhadap pathogen bawaan makanan juga dikenalpastikan. Pecahan air daripada ekstrak methanol menunjukkan aktiviti antimikroial yang paling kuat berbanding dengan pecahan lain, dengan mempamerkan zon penyekatan 16.10 ± 0.14 mm, 14.90 ± 0.14 mm, 13.00 ± 0.00 mm dan 11.75 ± 0.35 mm terhadap *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* dan *Vibrio parahaemolyticus* masing-masing. Pechan air menunjukkan "Minimum Inhibitory Concentration" dan "Minimum Bactericidal Concentration" (MIC : MBC) bagi *Bacillus cereus* (8mg/ml : 10mg/ml), *Staphylococcus aureus* (12mg/ml : 16mg/ml), *Listeria monocytogenes* (22mg/ml : 26mg/ml) dan *Vibrio parahaemolyticus* (24mg/ml : 27mg/ml) masing-masing. Keputusan menunjukkan bahawa tahap protein (<5%) dan minyak (<10%) yang tinggi menyebabkan aktiviti antimikroial bagi pechan air menurun dengan signifikasi sebanyak 2 kali ganda ($p < 0.05$). Akan tetapi, penambahan kanji pada kepekatan 30% menambahkan keberkesanannya pecahan air, di mana peratus pengurangan adalah lebih tinggi (10%) berbanding dengan kepekatan kanji pada 1%. Selain itu, tahap NaCl yang tinggi (>8%) juga membantu dalam pengurangan bagi *Listeria monocytogenes*, manakala *Staphylococcus aureus* disekat optimal pada tahap NaCl yang rendah (<0.5%). Aktiviti antimikroial bagi pechan air didapati paling efisien pada pH 4.0. Suhu pengeraman tidak menunjukkan sebarang pengaruh yang signifikasi terhadap keberkesanannya pecahan air. Keseluruhananya, kajian ini menunjukkan bahawa pechan air *Musa paradisiaca* merupakan sumber berpotensi sebagai antimikroial natural untuk digunakan dalam makanan apabila diaplilikasikan ke dalam model makanan yang mempunyai kandungan protein dan minyak yang rendah, pada pH dalam lingkungan 4.0-7.0, serta kandungan sederhana tinggi dalam karbohidrat.

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LIST OF SYMBOLS

mm	millimeter
μl	microliter
ml	milliliter
L	liter
μg	microgram
mg	milligram
g	gram
min	minute
hr	hour
w	weight
v	volume
CFU	colony forming unit
CFU/ml	colony forming unit/ml
$^{\circ}\text{C}$	degree Celcius
%	percentage

LIST OF ABBREVIATIONS

BC	<i>Bacillus cereus</i>
SA	<i>Staphylococcus aureus</i>
LM	<i>Listeria monytogenes</i>
VP	<i>Vibrio parahaemolyticus</i>
ATCC	American type of culture collection
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
DRT	Decimal reduction time
OD	Optical density
TSA	Tryptic soy agar
TSB	Tryptic soy broth
SPSS	Statistical Package of Science Social
ANOVA	Analysis of Variance
NCCLS	National Committee for Clinical Laboratory Standards
WHO	World Health Organization
MOH	Ministry of Health

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CHAPTER 1

INTRODUCTION

The microbiological safety of foods is a major concern to consumers, regulatory agencies and food industries throughout the world due to its wide economic and public health impact. Thus, many food preservation systems have been developed to overcome the illness caused by the consumption of contaminated foods or food products (Mead *et al.*, 1999). Apart from various foods processing such as thermal or cooling process, addition of preservatives is one of the most commonly used systems to enhance microbiological safety and stability of food products (Guillier *et al.*, 2007).

A variety of different chemical and synthetic compounds have been used as antimicrobial agents to inhibit bacteria in foods (Rahman & Kang, 2008). Synthetic antimicrobial agents such as organic acids, nitrite and sulfides were used for years to control the growth of microorganisms in foods (Kim *et al.*, 2001; Moosavy *et al.*, 2008). However, due to the identified and potential toxicity of chemical food preservatives, there has been increased demand on food preservatives from natural sources (Rahman & Kang, 2008). Therefore, a wide range of natural systems from animals, plants and microorganisms is being studied for the use in food preservation to replace the synthetic antimicrobial agents. The consumer demands for more natural antimicrobial agents have driven food scientists to investigate the effectiveness of extracts from plants (Nguefack *et al.*, 2004; Shin *et al.*, 2004).

Plants, including herbs and spices are widely used in the food industry as flavors and fragrances and some of them also exhibit useful antimicrobial properties. Many plant-derived antimicrobial compounds exhibited a wide spectrum of activity against bacteria, yeast and molds which has lead to suggestions that they could be used as natural food preservatives (Cho *et al.*, 2008). Wilkins & Board (1989) has been reported that 1340 plants are known to be potential sources of natural antimicrobial compounds. The major components with antimicrobial activity found

in plants, herbs and spices are phenolic compounds, terpenes, aliphatic alcohols, aldehydes, ketones, acids and isoflavonoids (Davidson *et al.*, 2005).

Generally, the antibacterial efficacy of antimicrobial agents may reduce when apply in multiple food system as compared to the microbiological system (Naitali *et al.*, 2009). Additionally, the antimicrobial activity of these plant extracts toward foodborne pathogens which is manifested in microbiological system is inadequate to give the justification that the similar antimicrobial activity shall be obtained when it had been incorporated into a real food system (Ferrer *et al.*, 2009). Therefore, the application of antimicrobials using food model system is still essential due to the results obtained from the *in vitro* study could be hardly incorporated to a complex food system. The presence of various food components may influence the growth and behavior of microorganisms, and consequently affect the antimicrobial efficacy of the antimicrobial agents (Devlieghere *et al.*, 2004). In previous studies, it has been found that the antimicrobial efficacy of essential oils was reduced considerably when added to food system due to the high level of fat and protein content (Aureli *et al.*, 1992; Pandit & Shelef, 1994; Tassou *et al.*, 1995; Garcia-Gonzalez *et al.*, 2009).

Banana (*Musa sp.*) is one of the most common of the edible tropical fruits from the order *Zingiberales* and family *Musaceae* (Fereidoon & Marrian, 2004). Referring to FAO statistic database (FAOSTAT, 2008), the world's annual production in the year 2008 was 90 million tonnes for bananas and 34 million tonnes for plantains. In Malaysia, the production of banana and plantains is the second highest among all fruit crops with the total production of 325,353 tonnes in 2007 (MOA, 2007). According to Julies & Robert (2008), edible banana and plantain are divided into dessert bananas, cooking bananas, and beer bananas and plantains categories. Dessert bananas are eaten raw, while the other types are processed for cooking or beer before consumption. Additionally, other parts of the banana such as banana inflorescence or banana heart which is the male buds that removed from the centre of pseudostem can also be eaten. In India and Ethiopia, people consume the heart of the stem as a food delicacy and in the Far East and Sri Lanka consumers eat the male buds as salads (Kamatou *et al.*, 2007).

The major phenolics compounds of banana pulp include catecholamines, naringin and rutin (Griffiths, 1959; Drell, 1970; Kanazawa & Sakakibara, 2000). Dopamine and norepinephrine (α -(aminomethyl)-3,4-hydroxybenzyl alcohol) are the predominant catecholamines in banana peel and pulp; phenolics accumulate mainly in the peel (Kanazawa & Sakakibara, 2000). Dopamine displays anti-inflammatory activity and protects against intestinal injury as modulator for eicosanoid synthesis (MacNaughton & Wallace, 1989; Alanko *et al.*, 1992).

Bananas and plantains are multipurpose perennial plants which offer a wide range of uses with its fruits, leaves, flowers and stalks (Fagbemi *et al.*, 2009). Various parts of banana plant have been reported posses antimicrobial activities as well as antioxidant activities. According to Gurumaa (2008), the banana fruit has been used as part of anti-ulcer diet in combination with pineapple, blueberries, cloves, ginger and cinnamon. Besides, previous study showed that extract from *Musa, AAA cv Cavendish* fruits peel posses antibacterial and antioxidant properties capable to against pathogens (Mokbel & Hashinaga, 2005). The banana *Musa Paradisiaca* juice is being verified to have anti-venom properties by Someya *et al.* (2002). The antioxidant power of banana peel and banana pulp extract was also studied by Haruhiro *et al.* (1998).

Previous studies have been done on the various parts of banana plant in which performed the inhibitory effects towards foodborne pathogens, hence banana plant should be considered to be a potential natural source of antimicrobial as well as antioxidant compounds. However, there is still lack of information and studies on the functional properties and antimicrobial effectiveness of banana and plantain inflorescences, as opposed to its fruits and peels. Therefore, the aim of this study is performed to evaluate the antibacterial activity of banana by-product, which is the banana inflorescences and to investigate the antibacterial efficacy of banana inflorescences against foodborne pathogens in broth model system.

The specific objectives of the study are:

1. To evaluate the antimicrobial activity of banana inflorescence.
2. To determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of banana inflorescence water fraction against foodborne pathogens.
3. To investigate the influence of various food components and food properties towards the antimicrobial efficacy of banana inflorescence water fraction in broth model system.



CHAPTER 2

LITERATURE REVIEW

2.1 Banana and Plantain

Banana and plantain (*Musa spp.*) are major food crops in the humid and sub-humid parts over the world and also a major source of energy for millions of people (Adenji *et al.*, 2006). Bananas are widely consumed, primarily because of their low cost and their potential as functional and nutraceutical food (Reddy *et al.*, 2003). They are considered to be one of the health promoting fruits since it consist of beneficial nutrients such as vitamin A, B, C and fibre which are essential for maintaining good health, improve digestion and prevent constipation (Ismail, 2000; Rukayah, 2000; Torre-Gutierrez *et al.*, 2008).

Bananas (*Musa spp.*) belong to the order *Zingiberales* and family *Musaceae*. Members of this *Musaceae* family are large plants in 2-9 metres tall with aerial trunk consisting compacted leaf sheaths which grow directly from the top of corm (Anon, 1992). Upon flowering, the true stem emerges from the centre of tightly rolled bunch of leaves. The pseudostem supports a canopy consisting of 6-20 or more varying sizes of leaves (Rieger, 2006). According to Somson (1992), *Musaceae* consists of two types of genera which are *Musa* and *Ensete*. The genus of *Musa* will produces suckers and having small seeds. On the other hand, the genus *Ensete* is monocarpic, non-sucker and have large size seeds.

2.1.1 Differences between Banana and Plantain Cultivars

Banana (*Musa spp.*) cultivation is exclusively tropical and suited to the warm, high-rainfall regions of the lowland tropics (Lassoudiere, 2007). The banana plants are large fruit-bearing herb with great biodiversity due to the plenty types of cultivars found worldwide. The different edible fruit cultivars are a man-made complex based on cross-breeding two wild diploid species originating from regions around South-East Asia (Aurore *et al.*, 2009). These two wild diploid species refer to *Musa acuminata* Colla (AA), which is highly polymorphous with spindly plants that grown



in clumps and *Musa balbisiana* Colla (BB), a homogeneous hardy plant with a massive pseudo-trunk. The next generations of diploid, triploid and tetraploid genome groups can be produced through such cross-breeding. The main genome groups include AA, AB, AAA, AAB and ABB as classified in Table 2.1. All varieties derived from *Musa acuminate* are more sugary and mainly eaten raw as a dessert fruit. On the other hand, the varieties of *Musa Balbisiana* have a slower process of ripening characteristics when starch is converted into sugar (Bakry *et al.*, 1997; Robinson, 1996; Stover & Simmonds, 1987).

Besides differentiating by types of genus, it can also be differentiated as banana and plantain. Plants of banana and plantain are alike physiologically. The main difference between banana and plantain is in the fruit itself. The term banana is typically used to describe sweet dessert banana cultivars (AA or AAA) that usually eaten raw when ripe while plantains (AAB, ABB or BBB) are generally the larger and more angular starchy cultivars which intended for cooking prior to consumption, but also edible raw when fully ripe (Nogrove & Hauser, 2002; Zhang *et al.*, 2005).

According to Rukayah (2000), there are about 70 banana cultivars planted in Malaysia with different nutritional values and classified into banana that being eaten raw and cooking or processed bananas which known as plantain. Banana that are being eaten as raw in Malaysia are pisang mas, pisang rastali, pisang embun, pisang berangan and pisang lemak manis. The varieties that are being subjected to cook or processing are pisang tanduk, pisang awak, pisang abu, pisang raja and pisang nangka (Zainun, 1992).

Table 2.1: The main cultivars of banana and plantain.

Group	Subgroup	Cultivar	Fruit usage	Geographic distribution
AA	Sucrier	Frayssinette Figue sucree	Sweet dessert	All continents
	Pisang Lilin		Dessert	Indonesia, Malaysia
	Pisang Berangan Lakatan		Dessert	
			Dessert	
AAA	Gros Michel Cavendish	Gros Michel Locatan, Poyo, Grand Nain, Williams	Dessert Dessert	All continents Exported countries
	Figue rose Lujugira	Figue rose Intuntu	Dessert Cooking, Beer	East Africa. highland
		Mujuba	Cooking, Beer	
AAAA	Champa Nasik		Dessert	
AAAB	Goldfinger	Goldfinger	Dessert	America, Australia
AB	Ney Poovan	Safet Velchi Sukari	Dessert acid Dessert acid	India, East Africa
AAB	Figue Pomme Pome Mysore Plantain	Maca, Silk Prata	Dessert acid Dessert acid	All continents Brazil, India India Africa, Caribbean
ABB	Bluggoe	French, Horn Corne Bluggoe	Cooking Cooking Cooking	Philippines, America
	Poteau Pisang Awak	Fougamou	Cooking Dessert	
ABBB		Klue Terapod		
BBB	Saba	Saba	Cooking	Indonesia, Malaysia

Source: Bakry *et al.*, 1997; Nakasone & Paull, 1999; Rieger, 2006; Aurore *et al.*, 2009

2.1.2 Production of Banana and Plantain

Banana and plantain are important starchy foods, reported as being the fourth most produced global food group after rice, wheat and milk in terms of gross value (FAO, 2007). They are major crops of west and central Africa and grown in 120 developing countries throughout the world (Qi *et al.*, 2000). According to the Food and Agriculture Organization of the United Nations Statistics (FAOSTAT, 2008), the annual world production of banana accounted for 90 million tonnes and estimated a total of 34 million tonnes for plantains in year 2008. As can be seen from the Table 2.2, the world banana and plantain production quantity was increased steadily between 2004-2008 periods. The main producing countries for banana were India, China, Brazil, Philippines and Ecuador which alone produced more than 60% of total world banana production. While Uganda was the top producing country for plantain accounted for more than 20% of the world production (Appendix B). The *Cavendish* variety is the most exploited, corresponding to about 1/3 worldwide production (FAOSTAT, 2006) (Appendix B).

Table 2.2: The world's annual production of bananas and plantains.

	2004	2005	2006	2007	2008
Bananas					
Production (Tonnes)	75680849	78861753	84320784	89099503	90705922
Average yield (Tonnes/Hectares)	163749	169091	171954	176942	188282
Area harvested (Hectares)	4621741	4663837	4903676	5035499	4817511
Plantains					
Production (Tonnes)	33972622	33499339	33945755	34670709	34343343
Average yield (Tonnes/Hectares)	62323	62405	63217	64114	63708
Area harvested (Hectares)	5358704	5368037	5369708	5407644	5390731

Source: Food and Agriculture Organization of the United Nations Statistics, 2008

Although countries like India and Brazil are the major producers in the world, however they are not the top exporters due to the fact that most bananas are planted for sale in local markets or self-consumption, and only a small fraction produced are traded in the global market (FAO, 2003). The four leading banana exporting countries in 2006 were Ecuador, Costa Rica, Philippines and Colombia (Appendix B). Meanwhile the main importing areas are the European Union, the United States of America and Japan,, which accounted for more than 70% of world total imports in 2006 (Appendix B).

Banana and plantains are one of the major fruits which are planted in Malaysia and there are about 170 cultivars of bananas are found all over Malaysia (Ahaidah & Samaludin, 1993). According to FAO (2007), bananas graded as 7th among top 20 important food and agricultural commodities in Malaysia in 2007 (Appendix B). According to Ministry of Agriculture Malaysia (MOA, 2007), banana is ranked as second high production among various fruits as followed by the production of durian in Malaysia. As shown in Table 2.3, banana production has found to be increased from year 2004 to year 2007, which are 317,104 tonnes and 325,353 tonnes respectively. During the year 2007, the total hectarage of banana plantation was 26,079 hectares and 21,540 hectares were producing areas. From the statistics of MOA (2003), the Johor state has shown to contribute the main production of banana and plantain in Malaysia with 124,307 tonnes, followed by Sarawak with 28,854 tonnes and Sabah with 26,364 tonnes (Table 2.4). During the year 2007, 41,852 tonnes of banana and plantain was produced in Sabah, which is 28.8% of the total fruit crops production (Department of Agriculture, Sabah, 2007) (Appendix B). When the production of banana was being reviewed according to districts in Sabah, it was found that Kota Belud, Tawau, Tuaran were the three districts providing the highest banana production sequentially throughout year 2007 (Appendix B).

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