MICROBIAL DECONTAMINATION OF MEAT WITH THAI HERB EXTRACTS, BACTERIOCINS AND HIGH HYDROSTATIC PRESSURE

Thananun Yuwang

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Technology

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การลดการปนเปื้อนจุลินทรีย์ในเนื้อด้วยสารสกัดสมุนไพรไทย แบคเทอริโอซิน และความดันไฮโดรสแตติก

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2556

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

_____
(Assoc. Prof. Dr. Jirawat Yongsawatdigul)
Chairperson

_____
(Assoc. Prof. Dr. Kanok-Orn Intarapichet)
Member (Thesis Advisor)

_____
(Assoc. Prof. Dr. Jutarat Sethakul)
Member

_____
(Asst. Prof. Dr. Anchalee Sirichote)
Member

_____
(Asst. Prof. Dr. Kaemwich Jantama)
Member

_____
(Asst. Prof. Dr. Patcharin Pakdeechanuan)
Member

_____
(Prof. Dr. Sukit Limpijumnong)
Vice Rector for Academic Affairs and Innovation

_____
(Asst. Prof. Dr. Suwayd Ningsanond)
Dean of Institute of Agricultural Technology

ธนนันต์ อยู่หว่าง : การลดการปนเปื้อนจุลินทรีย์ในเนื้อด้วยสารสกัดสมุนไพรไทย แบคเทอริโอซิน และความดันไฮโดรสแตติก (MICROBIAL DECONTAMINATION OF MEAT WITH THAI HERB EXTRACTS, BACTERIOCINS AND HIGH HYDROSTATIC PRESSURE) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. กนกอร อินทราพิเชฐ, 135 หน้า.

การศึกษาการลดการปนเปื้อนของจุลินทรีย์ในตัวอย่างเนื้อสดด้วยสมุนไพรไทย โดยใช้สารสกัดสมุนไพรไทยพื้นบ้านจำนวน 15 ชนิด ทดสอบฤทธิ์ยับยั้งการเจริญเติบโตของแบคทีเรียบ่งชี้ (indicator bacteria) 8 ชนิด และแบคทีเรียโดดเด่นจำนวน 4 ชนิด ผลการทดลองพบว่า *Spondias pinnata* (L.f.) Kurz (E1), *Garcinia mangostana* Linn. (E2) และ *Schinus terebinthifolius* (E3) มีฤทธิ์การยับยั้งแบคทีเรียตัวบ่งชี้ (indicator bacteria) สูง ใช้สารสกัดสมุนไพรทั้งสามชนิดเข้มข้นร้อยละ 10 เพื่อลดการปนเปื้อนบนผิวหน้าโดยการจุ่ม/เคลือบชิ้นตัวอย่างหมู เปรียบเทียบกับแบคเทอรีโอซินไม่บริสุทธ์ (B) ร้อยละ0.2 ที่ผลิตจาก *Lactococcus lactis* TISTR 1401 และสารทางการค้าไนซิน (nisin) ร้อยละ 0.2 บรรจุตัวอย่างเนื้อหมูในบรรจุภัณฑ์ 2 สภาวะ คือแบบมีอากาศและแบบสุญญากาศ เก็บตัวอย่างไว้ที่อุณหภูมิ 4 องศาเซลเซียส นาน 8 วัน สุ่มตัวอย่างทุกๆ 2 วัน ทำการตรวจจุลินทรีย์และคุณสมบัติเคมีกายภาพของเนื้อตัวอย่าง จำนวนจุลินทรีย์ทั้งหมดที่นับได้ (TPC) ในทุกตัวอย่างที่บรรจุแบบมีอากาศไม่มีความแตกต่างกันอย่างไรก็ตามตัวอย่างที่มีการใช้สารสกัดจากเปลือกมังคุด (E2) แสดงผลการลดจำนวนจุลินทรีย์ได้ดีกว่าสารสกัดอื่นๆในขณะที่ตัวอย่างที่มีการใช้ไนซินมีการยับยั้งจุลินทรีย์ที่ต่ำ จำนวนโคลิฟอรม์ (TCCs) ทั้งหมดของตัวอย่างที่บรรจุแบบมีอากาศ มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติเฉพาะในการเก็บสองวันแรกโดยตัวอย่างที่มีการใช้สารสกัดจากเปลือกมังคุด (E2) มีจำนวนโคลิฟอรม์น้อยกว่าตัวอย่างอื่นๆอย่างมีนัยสำคัญ ($p<0.05$) จำนวนจุลินทรีย์ทั้งหมดในตัวอย่างที่บรรจุแบบสูญญากาศตั้งแต่วันที่สองถึงวันสุดท้ายของการเก็บของตัวอย่างทั้งหมดไม่มีความแตกต่างกัน และไม่พบความแตกต่างของจำนวนโคลิ-ฟอรม์ทั้งหมดของทุกตัวอย่างที่บรรจุแบบสูญญากาศ พบว่าการสูญเสียการซึมออกของของเหลว (purge loss) การสูญเสียจากการปรุงสุก (cooking loss) ความแข็ง สี เมทไมโอโกลบิน ปริมาณกรดทั้งหมด ความชื้น และความชอบโดยรวม มีความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$) ยกเว้นค่าความเป็นกรด-ด่าง

การทดลองความดันแบบ high hydrostatic pressure (HHP) ที่ 200 และ 300 เมกกะปาสคาล ร่วมกับสารสกัดสมุนไพร E1 (1% w/v) และ E3 (1% w/v) และแบคเทอริโอซิน (0.2% w/v) กับชิ้นเนื้อหมูสดบรรจุแบบมีอากาศและแบบสูญญากาศเก็บที่อุณหภูมิ 4 องศาเซลเซียส นาน 9 วัน ในการเก็บแบบมีอากาศ การใช้ความดันที่ 200 เมกกะปาสคาล พบว่าจำนวนจุลินทรีย์ทั้งหมด (TPCs) ไม่มี

ความแตกต่างอย่างมีนัยสำคัญ ($p>0.05$) ยกเว้นการเก็บในวันที่หก ตัวอย่างที่ใช้ความดันร่วมกับสารสกัดสมุนไพร E1 มีจำนวนจุลินทรีย์ทั้งหมดมากกว่าอย่างมีนัยสำคัญ ($p<0.05$) จากทุกตัวอย่าง การใช้ความดันที่ 300 เมกกะปาสคาลเมื่อเปรียบเทียบระหว่างตัวอย่างพบว่ามีความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$) ของทุกตัวอย่าง ในขณะที่การเก็บในวันสุดท้ายตัวอย่างที่ใช้ความดันร่วมกับสารสกัดสมุนไพร E3 มีจำนวนจุลินทรีย์น้อยกว่าอย่างมีนัยสำคัญ การใช้ความดันร่วมที่ 200 เมกกะปาสคาล ในการบรรจุแบบสูญญากาศ จำนวนจุลินทรีย์ทั้งหมดไม่มีความแตกต่างกันอย่างมีนัยสำคัญอย่างไรก็ตามการเก็บในวันที่สามตัวอย่างที่ใช้ความดันร่วมกับแบคเทอรีโอซิน มีจำนวนจุลินทรีย์น้อยกว่าอย่างมีนัยสำคัญ($p<0.05$) การใช้ความดันที่ 300 เมกกะปาสคาล จำนวนจุลินทรีย์ทั้งหมดของทุกตัวอย่างมีจำนวนน้อยกว่า 10 โคโลนีต่อหนึ่งตารางเซนติเมตร คุณภาพทางกายภาพคือสีและความแข็งของตัวอย่างเนื้อหมูที่ถูกทรีตเปรียบเทียบกับตัวอย่างควบคุมมีความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$)

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2556 ลายมือชื่ออาจารย์ที่ปรึกษา _____

THANANUN YUWANG : MICROBIAL DECONTAMINATION OF MEAT WITH THAI HERB EXTRACTS, BACTERIOCINS AND HIGH HYDROSTATIC PRESSURE. THESIS ADVISOR : ASSOC. PROF. KANOK-ORN INTARAPICHET, Ph.D., 135 PP.

INDIGIENOUS THAI HERB/CRUDE BACTERIOCINS/HIGH HYDROSTATIC PRESSURE/TREATMENT/MEAT DECONTAMINATION

The purpose of this study was to investigate the potential use of indigenous Thai herbs as natural antimicrobial preservatives for fresh meat. Fifteen indigenous Thai herb extracts were examined for their antimicrobial activity against eight indicator bacteria and four dominant bacterial floras. The highly active antibacterial herb extracts were found for *Spondias pinnata* (L.f.) Kurz (E1), *Garcinia mangostana* Linn. (E2) and *Schinus terebinthifolius* (E3). The 10% (w/v) solution of each herb extract was used to decontaminate pork chop surface by dipping or coating, compared with 0.2% crude bacteriocins (B) obtained from *Lactococcus lactis* TISTR 1401 and 0.2% nisin (N), under aerobically and vacuum packed storage at 4ºC for 8 days with randomly sampling for microbial and physicochemical quality analyses every 2 days. The total plate counts (TPC) of all extracts treated and aerobically packed samples were not significantly difference ($p>0.05$). However, E2 treatment exhibited more intense antimicrobial activity than other extract treatments while nisin provided lower inhibition activity ($p<0.05$). The total coliform counts (TCC) of all pork samples were significantly difference ($p<0.05$) in Day 0 and Day 2, of which sample treated with E2 showed lower counts than other samples. The TPCs of all vacuum packed samples

were not significantly different ($p>0.05$) from Day 2 to the end of storage time and no significant differences ($p>0.05$) of TCCs were observed throughout the storage time. Significant differences were found in purge loss, cooking yield, hardness, L*, a*, b* color, metmyoglobin, total acidity, moisture and overall acceptance except pH values.

Treatments with a combination of high hydrostatic pressure (HHP) at 200 and 300 MPa and 1% of E1 and E3 and 0.2% crude bacteriocins were performed on pork chop samples, aerobically and vacuum packaged and stored at 4°C for 9 days. At 200 MPa pressure, no significant differences ($p>0.05$) of TPCs were found in the aerobically packed samples except that the HHP+E1 treated ones contained higher counts ($p<0.05$) than other treatments on Day 6. At 300 Mpa pressure, significant differences ($p<0.05$) of TPCs of all treated samples were observed throughout storage time while the HHP+E3 treated one showed lower counts ($p<0.05$) on Day 9. In vacuum packed condition, at 200 MPa, TPCs of both herb extracts combined treatments were not significantly different ($p>0.05$). However, it was found that the HHP+B showed lower TPC counts ($p<0.05$) than the other treatments on Day 3.At 300 MPa pressure, all treatments gave TPCs below 10 cfu/ cm^2. Significant differences ($p<0.05$) of color and hardness of treated pork samples compared with the control were observed due to the effect of HHP.

School of Food Technology Student's Signature _____

Academic Year 2013 Advisor's Signature _____

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CHAPTER I

INTRODUCTION

1.1 Introduction

Meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage microorganisms and common food-borne pathogens. Therefore, adequate preservation technologies must be applied in order to preserve its safety and quality. Food safety is the top priority for authorities and consumers worldwide. Consumer demands high quality, convenient, innovative, regular and safe meat products with natural flavor and taste and an extended shelf-life. Moreover, less salty, less acidified and less chemically preserved products are required. To match all these demands without compromising safety, the production and manufacture of meat products is at stage of innovative dynamics, stimulating a major research issue to develop and implement alternative technologies such as the so called non-thermal technologies or alternative, quicker, sensory-milder thermal technologies. Some promising non-thermal and thermal technologies are being considered at industrial level for decontamination of meat products, such as gamma, electron and X-ray irradiation, high pressure processing (HPP), natural antimicrobials, active packaging, ohmic heating, microwave and radiofrequency and steam among others. All these alternative technologies try to be mild, guarantee natural appearance, energy saving and environmental friendly while knocking the pathogens and spoilage microorganisms (Aymerich, Picouet and Monfort, 2008). A number of nontraditional

preservation techniques are being developed to satisfy consumer demand with regard to nutritional and sensory aspects of foods. Ensuring food safety and at the same time meeting such demands for retention of nutrition and quality attributes has resulted in increasing interest in alternative preservation techniques for inactivating microorganisms and enzymes in foods. This increasing demand has opened new dimensions for the use of natural preservatives derived from plants, animals, or microflora. A decontamination treatment of choice must have no adverse effect on product appearance or on any other sensory aspect, must pose no unacceptable risks to humans, the product and environment, and must be a low-cost application (Bolder, 1997). On these bases, the need for solutions concerning the hygienic quality of food is stated. Consumers are increasingly demanding food that is free from pathogens, with minimal processing and fewer preservatives and additives but with an unimpaired sensorial quality. As a response to these conflicting demands, current trends in the food industry include the investigation of alternative inhibitors to ensure food safety (Castellano, Belfiore, Fadd and Vignolo, 2008). Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Oussalah, Caillet, Saucier and Lacroix, 2006). Herbs and spices are known for their antimicrobial and antioxidative properties. Due to an increasing demand for natural food additives, herbs and spices have emerged as popular ingredients and have a tendency of replacing synthetic antimicrobial and antioxidant agents. Generally, essential oils of spices possess strong antibacterial properties against foodborne pathogens and contain high concentrations of phenolic compounds. These compounds exhibit a wide range

of biological effects, including antioxidant properties. Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation (Mayachiew and Devahastiny, 2008). More particularly, essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative bacteria. Essential oils have long served as flavoring agents in food and beverages, and due to their versatile content of antimicrobial compounds, they possess potential as natural agents for food preservation. The antibacterial properties of these compounds are in part associated with their lipophilic character, leading to accumulation in membranes and to subsequent membrane-associated events such as energy depletion (Oussalah, Caillet, Saucier and Lacroix, 2006). Nychas (1995) reported that phenols affect bacteria by weakening or destroying the permeability barrier of the cell membrane, resulting in the release of cellular constituents, or by altering the physiological status of the cell by changing fatty acid composition and phospholipids content of bacteria, interfering with energy metabolism, disrupting electron transport or nutrient uptake, and affecting nucleic acid synthesis. Recently, there has been a considerable interest in extracts and essential oils from common culinary herbs, spices and aromatic plants characterized by a notable antimicrobial activity. Such substances can be used to delay or inhibit the growth of pathogenic and/or toxin producing microorganisms in foods. Much attention lately has been focused on extracts from herbs and spices which have been used traditionally to improve the sensory characteristics and extend the shelf-life of foods. Essential oils in herb and spice extracts are regarded as ‘‘natural’’ alternatives to chemical preservatives and their use in foods meets the demand of consumers for minimally processed products (Chouliara, Karatapanis, Savvaidis and Kantominas,

2007). In Thailand, there are many herbs and spices that exhibit antimicrobial activity and may be used for food preservation. Few studies focused on the potential of herbs and spices as sources of antimicrobial compounds for use in meat and meat products.

High-pressure processing (HPP) is an attractive preservation technology that is mild for food but eliminates pathogenic and spoilage microorganisms; it has a good potential for the meat industry in particular. HPP may be combined with other preservation methods to increase its efficacy and commercial feasibility. Currently used and potential food additives, such as bacteriocins, potassium sorbate and carvacrol have been tested in combination with HPP. Combining phenolic compounds, which are fairly hydrophobic, with high pressure may be useful in controlling pathogens, particularly in fat-rich food such as salad to dressings and sausage (Vurma et al., 2006).

Investigation of the use of some indigenous and native Thai herb and spice extracts to encounter spoilage of fresh meats during aging and storage will be very much interesting. In addition, the use of high pressure technique in combination with the use of herb and spice extract will be expected to give better results due to synergistic effect of antimicrobial compounds from herbs and spices and high pressure treatment. The successful findings of effective herbs and spices will expectedly be great benefit to fresh meat industries in Thailand.

1.2 Research objectives

1) To screen some of Thai herbs and spices for their antimicrobial activities and for the use to decontaminate of fresh meat products.

2) To investigate the uses of selected Thai herbs and spices in comparison and combination with the in-house produced crude bacteriocins for decontamination of fresh meat products and their effects on physicochemical properties of treated fresh meat products.

3) To investigate the use of selected Thai herbs and spices in comparison and combination with high hydrostatic pressure for decontamination fresh meat products and their effects on physicochemical properties of treated fresh meat products.

1.3 Research hypothesis

The ethanolic extracts from Thai herbs and spices had an effectiveness of natural antimicrobial as inhibitor of spoilage microorganism isolated and identified from fresh meat products, i.e., pork cuts. Herb and spice extracts had ability to extend storage life of fresh meat cuts when use alone and/or in combination of crude bacteriocins and high hydrostatic pressure treatment. In addition, the use of herb and spice extracts, crude bacteriocins and high hydrostatic pressure treatments could provide the treated fresh meats with equivalent physicochemical characteristics to the regular meat cuts.

1.4 Expected results

1) The antimicrobial efficacy of some indigenous and local Thai herbs and spices on the use of decontamination of meat cuts could be obtained and be able to further upscale to be used in small-scale fresh meat industry.

2) The efficacy of combination use of Thai herb and spice extracts with in-house crude bacteriocins and with high hydrostatic pressure on

decontamination of meat cuts could be also obtained and be alternatively selected as choices for fresh meat decontamination.

3) Some parts of the outcome findings were expected to be published in international journals

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CHAPTER II

LITERATURE REVIEWS

2.1 Antimicrobial activity of plant

Much attention lately has been focused on the extracts from herbs and spices which have been used traditionally to improve the sensory characteristics and extend the shelf-life of foods (Botsoglou et al., 2003). Essential oils are regarded as ‘‘natural’’ alternatives to chemical preservatives and their use in foods meets the demand of consumers for minimally processed products (Nychas, 1995). Traditional Thai food has a very distinctive character because of the special combination uses of herb and spices in preparation. These herb and spice ingredients are more than just good taste but they make Thai food more healthly. Recently, the demand for medicinal herb products has begun to grow and gain popularity. Natural antimicrobial agent development from Thai food ingredients may respond to the national drug policy and “Thai Herbs for Health” promotion (Thiengburanathum, 1996; Liangmaneethep, 1997). Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against foodborne pathogens (Deans and Ritchie, 1987). In modern food industries mild processes are applied in order to obtain safe products which have a natural or ‘‘green’’ image (Burt, 2004).Herbs are plants that are used fresh, or dry, to add flavor, aroma or piquancy to food and drinks (McGee, 2004). Several studies have indicated that biological functions as a result of ingesting herbs

have significant health benefits (Cheah and Abu-Hasim, 2000; Jayasinghe, Gotoh, Aoki, and Wada, 2003). Thailand is a tropical country with a wide diversity of herbs, including acacia, ginger, galangal, garlic, shallot and chili. These have been found to be the good sources of phytochemicals. Various parts of many Thai indigenous plants are used as food, beverage, medicine, or for chewing and these are a potential source of new natural antioxidants. Plants contain a variety of substances called ‘‘phytochemicals” that come from naturally occurring components present in plants. The phytochemical preparations with dual functionalities in preventing lipid oxidation and antimicrobial properties have tremendous potential for extending the shelf life of food products.

Although it remains unclear which of the compounds of plants are the active ones, essential oils and phenolics recently have received increasing attention because of some interesting new findings regarding their biological activities, and are widely distributed in edible plants (Bakkali et al., 2008; Kamel et al., 2007; Archana et al., 2005). Phytochemicals (divided into two groups; primary and secondary metabolite), which are naturally occurring biochemicals in plants that give plants their color, flavor, smell and texture. Plant secondary metabolite differ from ubiquitous primary metabolite (e.g. carbohydrate, proteins, fats, nucleic acid) (Bako and Aguh, 2007).The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, anthraquinone, other phenolic compounds and essential oils(EOs) (Kisangau et al., 2007). Plants synthesize, by a secondary metabolism many compounds with complex molecular structures and some of them have been related with antimicrobial properties found in plant and their derivatives. Among these secondary metabolites are alkaloids, flavonoids, isoflavonoids, tanins, cumarins,

glycosides, terpens and phenolic compounds (Simões et al., 1999). Originally added to change or improve taste, spices and herbs can also enhance shelf-life because of their antimicrobial nature. Some of these same substances are also known to contribute to the self-defence of plants against infectious organisms (Deans and Ritchie, 1987; Kim et al., 2001). Systematic screening for biological interactions between microorganisms and plant products has been valuable source of new and effective antimicrobial substances, which could have different action ways on/in the microbial cell when compared to other conventional antimicrobials. Generally, the susceptibility of bacteria to the antimicrobial effect of essential oil also appears to increase with a decrease in pH of food, storage temperature and amount of oxygen within the packaging. The physical structure of a food may limit the antibacterial activity of EO (Skandamis et al., 2000). Essential oils and extracts of plants are of growing interest in the industry and scientific research because of their antioxidants, antibacterial, antifungal, antiviral and anti-parasitical activities that make them useful as natural additives in foods, cosmetic and pharmaceutical industries (Bakkali et al., 2008; Kamel et al., 2007; Mabberley, 1997). Antimicrobial activity against a range of bacterial pathogens of importance is also found in EOs, non-toxic compounds, and some organic acids.

2.2 Herbs and spices

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against foodborne pathogens (Deans and Ritchie, 1987). Consumer demand for less use of synthetic preservatives has led to research and use of ‘‘naturally derived’’

antimicrobials. Leafy green vegetables (LGV) are widely consumed in East Asia and are prepared using various cooking methods. These vegetables have been eaten for centuries and are classified as GRAS (Generally Recognized As Safe).An abundance of research has shown that fresh LGV contain important functional food components, such as ß-carotene, ascorbic acid, riboflavin, and folic acid, as well as minerals (Grusak and DellaPenna, 1999). In particular, these foods contain a large amount of polyphenols (e.g., phenolic acids, flavonoids, and aromatic compounds), the most abundant phytochemicals in the human diet. LGV are also known for their characteristic color, flavor and therapeutic value (Faller and Fialho, 2009; Gupta et al., 2005). Their bioactive substances and phytonutrients have a wide range of biological functions, including antioxidant and antimicrobial activities (Burt, 2004; Gutierrez, Barry-Ryan, and Bourke, 2008). Naturally occurring antimicrobial compounds have good potential to be applied as food preservatives. Antimicrobial substances from natural sources like plants have been investigated to achieve higher levels of food safety. In addition for centuries, indigenous plants have been used in herbal medicine for curing various diseases, including enteritis (Otshudi et al., 1999; Essawi and Srour, 2000).

2.2.1 Mechanism of antimicrobial action

Plant essential oils have been shown to have activity against *Aeromonas hydrophila, Listeria monocytogenes, Clostridium botulinum, Enterococcus faecalis, Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Enterobacteriaceae, Campylobacter jejuni, Vibrio parahaemolyticus, Pseudomonas fluorescens, Bacillus cereus, Shigella* spp., *Salmonella enterica Typhimurium* and *Enteritidis*, and *Escherichia coli* as well as yeasts and moulds (*Saccharomyces cervisiae, Aspergillus*

flavus, Aspergillus parasiticus) (Karapinar and Aktug, 1987; Moleyar and Narasimham, 1992; Hao et al., 1998a, b; Smith-Palmer et al., 1998; Bagamboula et al., 2003). The mechanism of action for the antimicrobial activity of natural preservatives is not fully understood, however, membrane disruption by terpenoids and phenolics; metal chelation by phenols and flavonoids; and effect on genetic material by coumarin and alkaloids are thought to inhibit growth of microorganisms (Cowan, 1999). The exact target(s) for natural antimicrobials are often not known or well defined, as it is difficult to identify a specific action site where many interacting reactions take place simultaneously.

It is reported that membrane-disrupting compounds can also cause leakage of cellular content, interference with active transport or metabolic enzymes, or dissipate cellular energy in ATP form (Davidson, 2001). Different studies have demonstrated the effectiveness of antimicrobials and their effective compounds to control or inhibit the growth of pathogenic and spoilage microorganisms. Degradation of the cell wall, damage to cytoplasmic membrane and membrane proteins, leakage of intracellular contents, coagulation of cytoplasm and depletion of proton motif force can cause cell death (Burt, 2004; Nychas et al., 2003).

The effectiveness of antimicrobial compound depends on pH of the food, type and number of contaminating microorganisms, type and concentration of antimicrobial. Storage temperature may also influence the effectiveness of antimicrobial as diffusibility of compounds is related to the temperature (Friedman et al., 2004). The loss of the differential permeability character of the cytoplasmic membrane is frequently identified as the cause of cell death. Some workers have explored this further, reasoning that loss of membrane function is only part of the explanation for

antimicrobial activity (Walsh et al., 2003). Certainly, the ability of phenolics to interfere with cellular metabolism through a number of mechanisms (substrate complexing, membrane disruption, enzyme inactivation and metal chelation) is well known (Cowan, 1999). It is also evident that their ability to preferentially partition from water to membrane structures and penetrate the membrane are important factors which have a bearing on the sensitivity or resistance of exposed cells (Griffin et al., 1999; Cox et al., 2000). Shelef (1983) noted that while much of the early in vitro work with essential oils and their components showed they had substantial activity, when used in food systems amounts required were high (1–3%) and these levels were often higher than would normally be organoleptically acceptable (Lis-Balchin et al., 1998a). The presence of carbohydrate, protein, salt and pH reaction influence the effectiveness of these agents in foods. Their antimicrobial potency is also reduced in foods with lower water activity. Some examples from in vitro work also show these effects are varied.

The antibacterial activity is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents (Ikigai et al., 1993; Otake et al., 1991). Although numerous studies have been done in-vitro to evaluate the antimicrobial activity of plant extracts, very few studies are available for food products. Normally, crude plant extracts are use in food products and reduction of effectiveness may occur due to interaction of flavonoid in the Eos and other components such as sugar and proteins. (Kapoor et al., 2007; Parvathy et al., 2009; Rhee et al., 1994, Glass and Johnson, 2004), causing protection of bacteria from antimicrobial effects of EOs (Pandit and Shelef, 1994). However, carbohydrates in foods do not appear to protect bacteria from

the antimicrobial effects of EOs (Shelef et al., 1984). Gill et al. (2002) suggested that the greater availability of nutrients in foods compared with laboratory media may enable bacteria to repair damaged cells faster. In this respect not only the intrinsic properties of the food are important but also extrinsic determinants, such as temperature or characteristics of bacteria, can affect bacterial sensitivity (Burt, 2004).

Antimicrobial activities of phenolic compounds may involve multiple modes of action. The type of the microorganism and its cell wall structure are thought to play an important role (Shan et al., 2007). Phenolic compounds can denature enzymes (Furneri et al., 2002) and they can also bind to substrates such as minerals, vitamins and carbohydrates making them unavailable for microorganisms (Stern et al., 1996; Shahidi and Naczk, 2004). Extracts of garlic, cinnamon, curry, mustard, basil, ginger and other herbs exhibit antimicrobial properties (Arora and Kaur, 1999; Marino et al., 1999). Furthermore, EOs of many aromatic plants, such as those belonging to the Labiatae family, had been shown to possess antimicrobial activities (Cosentino et al., 1999; Elgayyar et al., 2001). Extracts from Chinese chives and cassia reduced the count of Escherichia coli and other bacteria during storage of juices, milk and meat (Mau et al., 2001).

2.2.2 Use of natural preservatives for microbial decontamination of meat and meat products

Refrigeration storage is usually the most common preservative method of fresh meat and meat products. In order to extend refrigerated storage time, antimicrobial and antioxidant additives especially of synthetic origin, are added to muscle foods. Shan et al. (2009) found that grape seed extract reduced the final microbial load and inhibited lipid oxidation in raw pork during storage at an ambient

temperature for up to 9 days. Survival and growth of both susceptible and antibiotic-resistant *Campylobacter* strains have been inhibited effectively on agar plates and in contaminated ground beef by application of roselle (*Hibiscus sabdariffa* L.) (Yano, Satomi, and Oikawa, 2006; Yin and Chao, 2008). Over et al. (2009) reported that green tea at 3000, 6000, and 9000 ppm resulted in a significant antimicrobial effect against foodborne pathogens when combined with organic acids in a broth culture. Glass and Sindelar (2010) reported that several commercially available ingredient mixtures including a 1.5% blend of lemon/cherry/vinegar powder, 2.0% buffered vinegar and 3.0% cultured sugar/vinegar blend inhibited the growth of *Listeria monocytogenes* in ham, beef and turkey.

Combination of different plant extracts showed better preservative effects on meat as rosemary extracts and dry powders of orange and lemon applied to beef meatballs were found to be effective in controlling bacterial spoilage during 12 days storage period at 8°C (Fernandez-Lopez et al., 2005). Mixtures of Scutellaria, honeysuckle, Forsythia and cinnamon or cinnamon, rosemary and clove oil showed 1.81 to 2.32 log reductions in microbial counts as compared with control in vacuum-packaged fresh pork during 28 days storage (Kong et al., 2007). Winter savory (*Satureja montana*) EOs in combination with other preservation methods such as reduced temperature, pulsed light, high pressure, pulsed electric and magnetic fields, irradiation, or packaging under a modified atmosphere can be utilized as economical natural antibacterial substance to control growth of foodborne bacteria and improve quality of minced pork (Carraminana et al., 2008). The application of plant EOs for control of food-borne pathogens and food spoilage bacteria requires the evaluation of a number of aspects; the effects on organoleptic properties, evaluation of the range of activity

against the organisms of concern to a particular product as well as food compositional effects on activity. Optimal application in real food systems depends on these factors, therefore, the application of EOs in food should be incorporated in the studies to determine and quantify the effect of food ingredients on their antimicrobial activity (Gutierrez., Barry-Ryan., and Bourke, 2008).

Biopreservation has gained increasing attention as means of naturally controlling the shelf life and safety of meat products. Lactic acid bacteria (LAB) have a major potential for use in biopreservation because they are safe for consumption and, during storage, they naturally dominate the microbiota of many foods. LAB are ‘‘generally recognized as safe’’ (GRAS), due to their typical association with food fermentations and their long tradition as food-grade bacteria. In addition, antimicrobial peptides produced by LAB can be easily broken down by digestive proteases, so they will not produce gut microbiota disturbance. LAB can exert a bioprotective or inhibitory effect against other microorganisms as a result of the competition for nutrients and/or of the production of bacteriocins or other antagonistic compounds such as organic acids, hydrogen peroxide and enzymes (Castellano, Belfiore, Fadda and Vignolo, 2008). Bacteriocins are ribosomally-produced antimicrobial polypeptides or proteins that produce, in their mature form, an antibacterial effect against a narrow spectrum of closely related bacteria (Jack et al., 1995). Bacteriocins due to their proteinaceous nature are probably inactivated by proteases in the gastrointestinal tract. Most of the bacteriocins known so far are cationic molecules up to 60 aminoacids residues and thermostable. Bacteriocins produced by LAB are a heterogeneous group of peptides and proteins. The latest revised classification scheme divides them into two main categories: the lanthionine-containing lantibiotics (class I) and the nonlanthionine-

containing bacteriocins (class II) while the large; heat-labile murein hydrolases (formerly class III bacteriocins) constitute a separate group called bacteriolysins (Cotter, Hill, and Ross, 2005).

Bacteriocins usually have low molecular weight (rarely over 10 kDa) they undergo posttranslational modification and can be easily degraded by proteolytic enzymes especially by the proteases of the mammalian gastrointestinal tract, which makes them safe for human consumption. Bacteriocins are in general cationic, amphipathic molecules as they contain an excess of lysyl and arginyl residues (Rodriguez et al., 2000). They are usually unstructured when they are incorporated in aqueous solutions but when exposed to structure promoting solvents such as triofluroethanol or mixed with anionic phospholipids membranes they form a helical structure (Moll et al., 1999).

Nisin is the most widely exploited and applied bacteriocin. It is active against Gram (+) positive bacteria including highly pathogenic and food spoilage microorganisms including *Stapphylococcus aureus* and *L. monocytogernes*. Nisin can be effective at nanomolar concentrations depending on the target strain. Nisin is ribosomally synthesized as a precursor peptide that is later enzymatically modified. This prepeptide is biologically inactive and contains a C-terminal prepeptide domain, following a variety of posttranslational modification reactions and, is cleaved from the N-terminal leader sequence to yield the mature antimicrobial compound. It is an auto regulatory two component system which can be activated fully by nisin in very low sub toxic amounts (ng/ml) (Mierau, 2005). Nisin is heat stable at 121ºC but for prolonged heating, becomes less heat stable, especially between pH 5 to 7. Nisin is sensitive to α-chymotrypsin but resistant to trypsin, elastase, carboxyl peptidase,

pepsin, and erepsin. Nisin is utilized as a food additive, is commercially produced and is assigned under the number E234 (ECCU 1983 EEC Commission Directive 8314631EEC) (Mierau, 2005; Mierau and Lei, 2005). Gram positive (+) bacteria are characterized by a high content of anionic lipids in the membrane nisin binds rapidly to anionic liposome's and this interaction is strong since nisin has been able to diffuse slowly to other liposome's. Fragments of nisin have been used to identify the regions that are involved in membrane interaction (Daw and Falkiner, 1996).

2.3 High hydrostatic pressure in the food processing

2.3.1 Basic chemical and thermal principles of high pressure processing

The basic principles that determine the behavior of foods under pressure are:

- **Le Chatelier's principle**: any reaction, conformational change, phase transition, accompanied by a decrease in volume is enhanced by pressure (Cheftel, 1995; Frakas and Hoover, 2000; Yordanov and Angelova, 2010);

- **Principle of microscopic ordering**: at constant temperature, an increase in pressure increases the degrees of ordering of molecules of a given substance. Therefore pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny and Masson,1993).

- **Isostatic principle**: the principle of isostatic processing is presented in Figure 2.1. The food products are compressed by uniform pressure from every direction and then returned to their original shape when the pressure is released (Olsson, 1995). The products are compressed independently of the product size and geometry because transmission of pressure to the core is not mass/time dependant thus the process is minimized (Cheftel, 1995; Frakas and Hoover, 2000; Ledward,

1995). If a food product contains sufficient moisture, pressure will not damage the product at the macroscopic levels as long as the pressure is applied uniformly in all directions (Crawford et al., 1996).

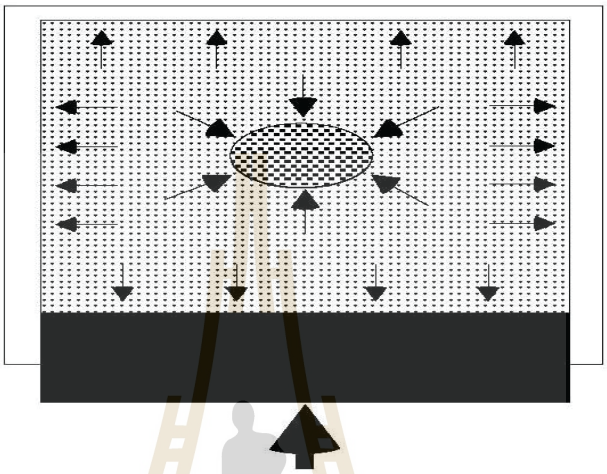

Figure 2.1 The principle of isostatic processing (Olsson, 1995).

2.3.2 The effect of high pressure on meat

The application of HHP to meat and meat products results in a modification of quality parameters such as color, texture and water holding capacity. Alterations have no negative impact on the nutritional value. In addition, pressure can also be applied to meat in the form of hydrodynamic pressure treatments in order to induce mechanical tissue disintegration and therefore tenderize the meat. The effectiveness of the HHP is known to depend on technological parameters, such as pressure, holding time and temperature, as well as the type and the physiological state of microorganisms (Hugas et al., 2002; Gao et al., 2006).

2.3.2.1 The effect of high pressure on microbial inactivation

The effectiveness of HHP for microbial control depends on factors

such as the process parameters, pressure level, temperature and exposure time, as well as by intrinsic factors of the food itself, such as pH, strain, growth stage of micro-organisms and food matrix (Hugas et al., 2002; Garriga et al., 2004). Increasing pressure and treatment holding time resulted in an increase in microbial inactivation. Microbial inactivation may be higher when HHP is applied above or below 20ºC (Ritz et al., 2000; Patterson, 2005). It is also known that food intrinsic factors can also affect the microbial resistance to high pressure. Moreover, the ability of bacteria to survive HHP can be greatly increased when treated in nutritionally rich media, e.g. meat, containing substances like proteins, fat and carbohydrates (Simpson and Gilmour, 1997; Erkmen and Dogan, 2004). Moderate level of pressure of about 10-50 MPa decreases the rate of growth and reproduction, whereas higher level of pressure causes inactivation (Rademacher, 2006).Pressure levels applied for the pasteurization of meats and meat products, range in an area of 400–600 MPa with short processing times of 3-7 min and at room temperature. These treatments lead in most cases to an inactivation of more than four log units for the most common vegetative pathogenic and spoilage microorganisms resulting in an increased shelf-life and improved safety (Bajovic, Bolumar and Heinz, 2012). HHP renders food more stable due to its ability to reduce the number of spoilage and pathogenic micro-organisms, and to inactivate certain food enzymes (Patterson, 2005).

The cell membrane is the primary target of pressure damage, mainly through altering its permeability as a consequence of phospholipid crystallization. Other cellular functions sensitive to pressure include: ion exchange modifications, fatty acid composition, ribosome morphology, cell morphology, protein denaturation and inhibition of enzyme activity, destabilization of DNA replication complex, vacuole

formation, etc. In several microorganisms, it has been shown that the sub lethal damage is initiated by membrane phase transitions affecting mainly transport proteins (Vogelet al., 2001). This can be a general mechanism of cell death. In general, cell death increases with pressure but it does not follow a first order kinetics (logarithmic death rate), since there is a tailing off in inactivation (Kalchayanand et al., 1998a).

The basic principles of HHP microbial inactivation are based on protein denaturation which results in enzyme inactivation (Barbosa-Canovas, Pothakamury, and Swanson, 1995), and the agglomeration of cellular proteins (Farr,1990). The change of the permeability of the cell membrane results from the crystallization of fatty acids from phospholipids (Cheftel and Culioli, 1997). The partial inactivation of enzyme systems by high pressure leads to a breakdown of metabolic actions in biological systems (Knorr and Heinz, 2001). Further, the inactivation could be linked to protein denaturation resulting in the dissolution of membrane bound enzymes (Hoover et al., 1989; Smelt, 1998). The protein denaturation depends on such external factors as pH, salt content, water activity (Aw) and the presence of other ingredients like sugars (Molina-Höppner et al., 2004; Smelt, 1998). Inactivation depends on a number of factors related to the Gram type, physiological state and strain particularities (Jofré et al., 2010). The different chemical composition and structural properties of the cell membrane in Gram-positive and Gram-negative microorganisms result in differences in resistance to HHP (Russell, 2002). Gram-positive bacteria are generally more resistant compared to Gram-negative (Shigehisa et al., 1991).Apparently, the double-layered phospholipids which are present in the lipid membranes are packed tightly during the compression phase, promoting the transition towards a gel state (Hazel and Williams, 1990), and during decompression the dual

layer structure is lost, with pore formation and cytoplasmic material leaking (Hoover et al., 1989; Shimada et al., 1993).

2.3.2.2 The effect of high pressure on meat color

Myosin is relatively sensitive to pressure and depending on the species will denature at around 180–300 MPa to give an opaque appearance, similar to that seen in cooked meat (Bak et al., 2012), increasing the pressure above 300 MPa leads to little further increase in lightness. Carlez et al. (1995) reported that meat discolouration in pressure-processed meat was due to the following: (1) a whitening effect due to myoglobin denaturation and/or to heme displacement or release and (2) oxidation of the ferrous myoglobin to ferric myoglobin above 400 MPa. Cheftel and Culioli (1997) suggested that pressure processing of fresh red meat causes drastic changes, especially in redness, and thus cannot be suitable of practical applications. Color changes in meat products after HHP treatment have been reported. Increase in lightness and a decrease in redness in meat have been reported after high pressure treatment. In minced beef, an increase in lightness values (L*) began at 200 MPa and became stabilized at around 300 to 400 MPa (Carlez et al., 1995). Shigehisa et al. (1991) found that the color of pressure processed pork slurries became paler above 200 MPa and gray-brown above 400 MPa. Carlez, Veciana-Nogues, and Cheftel (1995) evaluated the effect of HHP on minced beef and concluded that L* color values increased significantly at the pressure range 200–350 MPa, giving the meat a pink color, while a* values decreased at 400–500 MPa, resulting in a gray-brown color. Szerman et al. (2011) studied the application of HHP on beef carpaccio at three different levels (400, 500 and 600 MPa) with low temperatures (0–5°C) and at room temperature (20°C) and observed that the negative effect on chromatic parameters was

reduced under frozen conditions and also on the water holding capacity indicating a minimization of the denaturation of the sarcoplasmic and myofibrillar proteins but showing a lower inactivation on microorganisms.

2.3.2.3 The effect of high pressure on texture

Pressure-induced texture modifications have been used to affect myofibrillar proteins and their gel-forming properties, raising the possibility of the development of processed muscle-based food. As previously stated, high pressure can affect molecular interactions (hydrogen bonds, hydrophobic interactions and electrostatic bonds) and protein conformation, leading to protein denaturation, aggregation or gelation (Messens, Van Camp and Huyghebaert, 1997).

In fresh meat, the application of low pressure levels can be used to improve the functional and rheological properties of turkey meat with low pH or pale soft exudative (PSE) meat (Chan, Omana, and Betti, 2011). In general, low pressures (200 MPa) can tenderize pre-rigor meat, whereas tenderization post-rigor with HHP can only be achieved by higher temperatures (Sun and Holley, 2010). The influence of HHP on the meat tenderness is depending on the rigor stage, pressure and temperature level applied and their combination (Sun and Holley, 2010). Meat tenderization by HHP is likely caused by lysosome breakdown and subsequent proteolytic activity release to the medium (Hugas, Garriga, and Monfort, 2002).

2.3.2.4 Combination effects of HHP and natural antimicrobial

The combination of HHP with biopreservation to enhance bacterial inactivation and reduce the recovery of sublethally injured cells during product storage, has been demonstrated in cooked meat products (Aymerich et al., 2005; Jofré et al., 2007; Marcos et al., 2008).Morgan et al. (2000) observed a synergistic killing

effect of HHP in the presence of the bacteriocin lacticin 3147. HHP treatment at 250 MPa caused a 2.2-log reduction of *Staphylococcus aureus* in reconstituted skim milk, while 10,000 AU/ml lacticin 3147 alone caused a 1-log cycle reduction. Combination of both means resulted in destruction of more than 6-log cycles of the pathogen. Lacticin 3147 exerts a bactericidal effect by disturbing the selective permeability of the membrane of sensitive cells, and the authors found that as pressure increased, the effect of lacticin 3147 became more pronounced.

The high levels of inactivation of antimicrobial agents and HHP hurdles are believed to be due to the combined factor destabilization of membrane structure or function although their specific modes of action are different (Kalchayanand et al., 1994; Masschalck et al., 2001a; Ray, 2001). High-pressure membrane damage can increase the cell penetrability and activity of antimicrobial agents, while, conversely, treatment with cell wall weakening agents sensitizes pressure-resistant bacteria to HHP (Earnshaw et al., 1995). In addition, residual bacteriocins in the food matrix would continue to exert their inhibitory effect after treatment with the non thermal processing hurdle, thereby inactivating and preventing the recovery of any sub lethally injured cells (Morgan et al., 2000).The method of application of an HHP treatment can also influence the bactericidal efficiency of added antimicrobials compounds. The addition of naturally occurring antimicrobials has proven to be an effective hurdle when combined with non thermal processing techniques.

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CHAPTER III

SCREENING OF LOCAL THAI HERB EXTRACTS FOR ANTIBACTERIAL ACTIVITY

3.1 Abstract

Antibacterial activity of ethanolic extracts of 15 local Thai herbs was carried against twelve selected indicator bacteria. Twelve indicator bacteria were used namely *Enterobacter aerogenes* (TISTR1540), *Staphylococcus aureus* (TISTR029), *Pseudomonas fluorescens* (TISTR358), *Enterococcus faecalis* (TISTR379), *Micrococcus luteus* (TISTR745), *Listeria monocytogenes* (TISTR17303), *Escherichia coli* (TISTR887), and dominant bacterial culture screened and selected from spoiled raw pork chops, aerobically and vacuum packed, and kept at refrigeration temperatures, which were identified as *Serratia liquefaciens*, *Klebsiella oxytoca*, *Enterobacter* sp, *Acinetobacter calcoaceticus and Hafnia alvei.* The antimicrobial efficacy was determined using paper disk diffusion method, with the concentration of 20, 60 and 100 ppm (in 1% DMSO) of loaded extract solution. Seven of herb extracts were found to have antibacterial activity. The most active antibacterial extracts found against all Gram-positive and Gram-negative bacteria were from *Spondias pinnata* (L.f.) Kurz while no activities were not found for the extracts from *Momordica charantia*, *Leucaena leucocephala* (Lamk.) de Wit, *Sauropus androgynus*, *Moringa oleifera* Lam, *Centella asiatica*, *Feroniella lucida* (Scheff.), *Acanthus ebracteatus* Vahl and *Sesban sesbania*. Five ethanolic extracts with only high concentration from

Senna siamea Lam, *Schinus terebinthifolius*, *Anacardium occidentale*, *Careya sphaerica* Roxb, Gaertn and *Syzygium gratum* (Wight) S.N.Mitra *var. gratum* could effective to bacteria indicator. Ethanolic extract from *Garcinia mangostana* Linn. with all concentrations used showed antibacterial activity to *Staphylococcus aureus*, *Enterococcus faecalis*, *Micrococcus luteus* and *Listeria monocytogenes*.

Keywords: Thai herbs, Antibacterial activity, paper disc diffusion, ethanolic extract

3.2 Introduction

For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana et al., 2007). Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. Thai plants have been used as medicines for many centuries because they contain active phytochemicals including phenolic compounds. These components function as antibiotics, help to make cell walls impermeable to gas and water, act as structural materials to give plants stability and provide protection against ultraviolet (UV) light. Hence, plants in the tropical zone including Thailand contain a high concentration of phenolic compounds formed as secondary metabolites in plants (Shahidi and Naczk, 2003). *Momordica charantia* L. possesses many uses, including antidiabetic, carminative, antihelmintic, antimalaral and antimicrobial, antiviral, anticancerigenous, contraceptive, immunostimulant, laxative, antioxidant, insecticidal, and skin treatments (eczema, acne, mycoses, scabies, hemorrhoid and furuncules (Yesilada et al., 1999; Grover and Yadav, 2004 and Basch et al., 2003). According to

Omoregbe et al. (1996) aqueous, ethanolic and methanolic extracts of *M. charantia* leaves presented antimicrobial activity against *Escherichia coli*, *Salmonella paratyphi*, *Shigella dysenterae*, *Streptomyces griseus* and *Mycobacterium tuberculosis*. *Senna siamea* Lam. (Irwim and Barneby, *Cassia siamea* Lam) belongs to the sub-family Fabaceae (Caesalpinioideae) of the family Leguminosae (Fowler, 2006). Young leaves of this plant have been used as vegetables in Thailand (Otimenyin et al., 2010) and as an antimalarial (Odugbemi et al., 2007). Presence of alkaloids, tannins, saponins, glycosides, steroids, phenolic compounds and flavonoids in the extracts confirmed the presence of rich bioactive principles in the leaf. Tannins, steroids and glycosides had been reported in ethanol extract of the leaf of *S. siamea* (Bukar et al., 2009 and Mohammed, 2012) Secondary metabolites are mostly produced by plant during adverse condition for protection against herbivores (Chitra, 2009). *Sauropus androgynus* (L.) Merr. which also used as vegetable, belongs to family Euphorbiaceae is commonly used as an effective medicinal herb in the treatment of diabetics, cancer, inflammation, microbial infection, cholesterol and allergy due to its antioxidant effect (Paul, 2011). *Schinus terebinthifolius* also known as Brazilian pepper, Aroeira, Florida holly, Rose pepper, or Christmas berry belongs to the Anacardiaceae family (Manrique et al., 2008). *S. terebinthifolius* is well known for its medicinal properties (de Lima et al., 2006) Brazilian peppertree is reported to have astringent, antibacterial, diuretic, digestive stimulant, tonic, antiviral and wound healing properties. (Molina-Salinas et al., 2006). *Momordica charantia* (Family *Cucurbitaceae*) are used in the Amazon, Brazil and parts of Asia, among its many uses, for treatment of skin infections. The fruits and leaves contain alkaloids, glycoside, saponin-like substances, resin, an aromatic volatile oil and mucilage.

Previous studies had also demonstrated that *M. charantia* is very rich in triterpenes, proteins, and steroids. Those of major interest include momordin, alpha- and betamomorcharin, cucurbitacin B1 and oleanolic acid (Oliff, 2007; Kohlert, 2000; Trombetta, 2005).

It is speculated that the antimicrobial activities of triterpenes depend on interactions between their lipid components with the net surface charge of microbial membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity (Trombetta, 2005). *Anacardium occidentale* is a thick tortuous trunk and woody branches tree of about 15 m in height longing to the family Anacardiaceae and, is native to Brazil and are found to have a great economic and medicinal value (Paramashivappa et al., 2001; Rajseh et al., 2009). It has also been reported to posses anti-diabetic, anti-inflammatory and anti-ulcerogenic properties (Akinpelu, 2001). Omojasola and Awe (2004) reported the antimicrobial activity of the leaf extract of *A. occidentale* and *Gossypium hirsutum* against *E. coli*, *Shigella dysenteriae S.* typhimurium. *Centella* is in the family Apiaciae or Umbelliferae, genus Centella and species Asiatica. It has been used for centuries as traditional medicine in India and oriental countries for treatment of mental fatigue, anxiety, epidermal wound, eczema and leprosy (Guo et al., 2004). It also inhibits growth of *Staphylococcus* spp. and reduces inflammation (Saralamp et al., 1996). The most prominent group of biologically active compounds is the triterpenes (Wijeweera et al., 2006) which consist of asiatic acid, madecassic acid and asiaticoside. *Barringtonia acutangula* (L.) Gaertn belonging to family Barringtoniaceae is a medium size glabrous tree found throughout India in decidous and evergreen forests, mostly along the bank of rivers

and streams (Warrier ,1994). It is used in the folklore in vitiated conditions of *kapha* and *pitta*, leprosy, arthralgia, dysmenorrhea, plumbago, skin diseases, diarrhea, inflammation, flatulence, hemorrhoids and (Satapathy, 1994). The plant has been reported to have antiimplantation activity in female albino rats (Prakasg et al., 1985). Spondias pinnata (Linn. F.) kurz (Family Anacardiaceae) is a deciduous tree distributed in India, Sri Lanka and South-East Asian countries. The genus Spondias Linn.includes 17 species, 7 of which are native to the neotropics and about 10 are native to tropical Asia. The phytochemistry of this plant has been studied and it is found that this plant contains, sterols, flavonoids and gums (Tandon and Rastogi, 1976). The gum exudates of the species contained acidic polysaccharides. Previously isolated compounds are ß-amyrin, oleanolic acid and amino acids (alanine, leucine) (Rastogi and Mehtrotra, 1993).

Garcinia mangostana Linn. commonly known as "mangosteen", is a tropical evergreen tree and is an emerging category of novel functional foods sometimes called "superfruits" presumed to have a combination of appealing subjective characteristics, such as taste, fragrance and visual qualities, nutrient richness, antioxidant strength (Moongkarndi, 2004) and potential impact for lowering risk of human diseases (Pedraza-Chaverri, 2008). Mangosteen is one of the most famous fruits in Thailand (Palakawong et al., 2010) and the pericarps of *G. mangostana* have been widely used as a traditional medicine for the treatment of diarrhea, skin infection and chronic wounds in South East Asia for many years (Mahabusarakam, 1987). Extract from its pericarp has been demonstrated the antimicrobial activity against a wide variety of microorganisms (Iinuma et al., 1996; Sundaram et al., 1983; Mahabusarakum et al., 1983; Mahabusarakam et al., 1986; Suksamrarn et al., 2003;

Sakagami et al., 2005 and Chomnawang, 2005). Previous studies have shown that the extracts from various parts contain varieties of secondary metabolites such as prenylated and oxygenated xanthones. Xanthones or xanthen-9H-ones is a secondary metabolite found in some higher plant that involves mangosteen (Peres, 2000). Xanthones could be isolated from peel, whole fruit, bark, and leaves of mangosteen. Several studies have shown that obtained xanthones from mangosteen have remarkable biological activities such as antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal, and antiviral activities (Suksamrarn et al., 2006 and Pedraza-Chaverri, 2008).

Sesbania sesban Linn. is a soft, slightly woody, 1-6 m tall perennial nitrogen fixing small tree. The leaves are compound 12-18 cm long made up of 6-27 pairs of leaflets. The raceme has 2-20 flowers which are yellow with purple or brown streaks on the corolla. Pods are subcylindrical, straight or slightly curved up to 30 cm long and 5 mm wide containing 10-50 seeds. The plant is used as carminative, anthelmintic, astringent, anti-inflammatory, antimicrobial, antifertility, demulcent and purgative. It is also given as a medicine against fever, ulcers and etc (Sheikh Sajid R et al., 2012).

This study designed to evaluate and screen antimicrobial efficiency of 15 local Thai herbs ethanolic extracts by testing against 12 pathogenic and spoilage bacteria by the paper disc diffusion technique.

3.3 Materials and methods

3.3.1 Preparation of herb and spice extracts

Fifteen indigenous herbs used for this study as shown in Table 3.1 were

purchased from local market places in Sakon Nakhon Provinces and Thai herbs collection division of Sakon Nakhon Campus, Rajamangala University of Technology Isan. The herbs were cut into small pieces, freeze dried (Heto FD8 Thermo Electron Corporation, USA), finely ground (IKA-Werke GmbH & Co., Germany) and stored at –20ºC for further application.

Table 3.1 List of collected Thai indigenous herbs used for the study

Common name/Thai name	Scientific name	Parts of herbs used
Bitter cucumber	*Momordica charantia*	Leaves / branches
White popinac, Wild tamarind	*Leucaena leucocephala (*Lamk.) de Wit	Leaves / branches
Thai copper pod, Cassod tree	*Senna siamea* Lam	Leaves / branches
Pak-wan Tree	*Sauropus androgynus*	Leaves / branches
Brazillian Pepper-tree	*Schinus terebinthifolius*	Leaves / branches
Horse radish tree	*Moringa oleifera* Lam	Leaves / branches
Cashew nut tree, Cashew apple	*Anacardium occidentale*	Leaves / branches
Tiger Herbal, Bua-bok	*Centella asiatica*	Leaves / branches
Wood apple	*Feroniella lucida (*Scheff.*)*	Leaves / branches
Hog Plum, Ma kok nam	*Spondias pinnata* (L.f.) Kurz	Leaves / branches
Mangosteen/mungkoot	*Garcinia mangostana* Linn	Peel or pericarp
Patana oak/gra doan	*Careya sphaerica* Roxb	Leaves / branches
Sea Holly/ngeak pla mhoo	*Acanthus ebracteatus* Vahl	Leaves / branches
Phuk mek	*Syzygium gratum (*Wight*)* S.N.Mitra var. gratum	Leaves / branches
Sesban/khae baan	*Sesban sesbania*	Leaves / branches

Ethanolic extraction of dried herbs were done by mixing 50 g each of dried herbs with 400 ml of 95% (v/v) ethanol and constantly shaken in a shaker (New Brunswick Scientific, UK) at 100 rpm for 12 hr. then, filtered through a Whatman No. 1. The residue was re-extracted with 200 ml of 95% ethanol and the filtrates were combined, concentrated on a rotary evaporator (Rotary evaporator rotavapor R-114, Buchi,

Switzerland) at 40ºC. The extract was freeze dried (Heto FD8, Thermo Electron Corporation, USA), stored in a sealed bottle and kept at -20ºC until use.

The dried extract powder was solubilized in 1% dimethyl sulfoxide (DMSO) to prepare test solutions of 20, 60 and 100 ppm for testing antimicrobial activity against indicator bacteria.

3.3.2 Dominant bacterial strains for indicator bacteria from fresh pork

Fresh pork chop samples were obtained from Fresh Meat Company, Nakhon Chai Si, Nakhon Prathom Provinces and store in the refrigeration for 3 days. Collection of bacteria was performed briefly 10 g pork sample was homogenized in 90 ml (0.85% NaCl), and aliquots were plated out directly at 1:10 dilution in 0.85% NaCl. After serially diluting each sample in sterile 0.85% NaCl, 0.1 ml portions were separately plated onto plate count agar (PCA) and de Man, Rogosa and Sharpe (MRS) agar and incubated at 37°C for 24 h. Microbial colonies were counted and expressed as $\log_{10}$cfu/g sample. Cell morphology and cell arrangement were observed by Gram staining. Biochemical tests were conducted according to Bergey's Manual of Determinative Bacterology (Holt et al., 1994). Identification of dominant bacteria by using test kit in API kit at Thailand Institute Science and Technology Research (TISTR).

3.3.3 Determination of antimicrobial activity: Paper disc diffusion assay

Selected indicator bacteria obtained from Thailand Institute of Scientific and Technological Research (TISTR) were used for testing the antibacterial of herbs extracts. Twelve bacterial strains, namely *Pseudomonas fluorescens* TISTR 358, *Staphylococcus aureus* TISTR 029, *Escherichia coli* TISTR 887, *Acinetobacter calcoaceticus* TISTR 1264, *Enterobacter aerogenes* TISTR 1540, *Enterococcus*

faecalis TISTR 379, *Micrococcus luteus* TISTR745, *Listeria monocytogenes* TISTR17303 and dominant spoilage bacteria isolated from fresh pork; PM 20, MT 35, PM 23, MT4 and MT 5 were used for antibacterial testing. Stock bacterial cultures were maintained at 4ºC on slant agar. The cultivation/assay medium for *L. monocytogenes* and *S. aureus* was tryptone soy broth or agar (TSB, TSA, Oxoid, Hampshire, UK), for other strains was nutrient broth or agar (Muller Hilton). Active culture for the experiment was prepared by streaking a loopfull of bacterial culture on petri dishes agar and then, incubated at 30 or 37°C for 24 hr. The antibacterial action of the extract was tested on different bacterial strains using the paper disk diffusion method (NARMS, 2002). For the cultivation/assay, the medium used were the same as above. Bacterial cultures for antimicrobial testing were prepared by picking a colony from 24-h-old TSA/NA plates and suspended in an appropriate medium. The cultures were grown aerobically for 18-20 h and centrifuged at 9,000 rpm at 4°C. The bacterial suspension was adjusted by diluting the inoculated bacteria with sterile 0.85% NaCl until it reached the desirable level of about 10^6-10^8 cfu/ml), then transferred into a flask containing 25 ml sterile nutrient agar at 43–45 ºC, and poured into Petri dish plate (8 cm diameter). Ten sterile paper disks (6 mm in diameter; Becton, Dickinson & Co.) were placed on the surface of each agar plate and were impregnated with extract solution for antibacterial activity test. The plates were incubated at 37ºC for 24 h. A paper disks impregnated with 1% DMSO was served as negative control and a disk with 200 ppm of nisin served as a positive control. Replicate at each concentration was performed. The inhibition zone diameter was measured (including the filter paper disc, 6 mm in diameter) using vernier caliper and expressed a clear zone in millimetres.

3.4 Results and Discussion

3.4.1 Isolation and identification of bacteria from fresh pork chop

Bacterial cultures were collected from pork chop, isolated and screened prior to be identified by TISTR Laboratory. Numbers of bacteria counts of each storage conditions were in the range of 5.7-6.1 log cfu/g as shown in Table 3.2.

Table 3.2 Microorganism counts in fresh pork after 72 hr storage at 4°C

Sample	Total viable counts (log cfu/g)	
	PCA	MRS
Aerobic pack	6.11	5.70
Vacuum pack	5.30	5.85

Thirty-seven different bacterial isolates obtained from the spoiled pork chop packed in both conditions, were consisting of 29 isolates of short rod, and 8 isolates of cocci. Morphologically, most of the isolates were Gram-negative bacteria only 2 isolate were Gram-positive. The different morphological characteristics of 37 isolates are shown in Table 3.3.The highest bacteria populations in aerobically packed counted were PT 1, PT 2, PT 3, PT 4, PT 5, PM 2, PM 3, PM 4, PM 7, PM 8, PM 9, PM 10, PM 12, PM 13, PM 14, PM 15, PM 16, PM 17, PM 18, PM 19, PM 21, PM 22, PM 23, PB 1 and PB 2 (96.15%) and PM 20 (3.85%) and in vacuum packed were MT 1, MT 3, MT 4, MT 6, MT 7, MT 8, MT 9, MT 20, MT 21 and MT 35 (90.91%) and MT 5 (9.09%). All isolates obtained were divided into seven groups according to Gram strain and biochemical properties, i.e motility, oxidase, catalase, oxidation-fermentation, sugar (glucose, sucrose and lactose) test. Seven dominant isolates were further identified, consisting of PM 20, PM 23, PM 3, PT 3, MT 5, MT 4 and MT 35 from aerobically and vacuum condition, respectively.

Table 3.3 Morphological characteristic and basic biochemistry of bacterial isolates obtained from aerobically and vacuum packed pork chop

Bacterial isolate code	Cell shape	Gram stain	Oxidase	Catalase	Oxidation/ Fermentation test
PT1	Short Rods	-	-	+	+/+
PT2	Short Rods	-	-	+	+/+
PT3	Short Rods	-	-	+	+/+
PT4	Short Rods	-	-	+	+/+
PT5	Short Rods	-	-	+	+/+
PM2	Cocci	-	-	+	+/+
PM3	Cocci	-	-	+	+/+
PM4	Cocci	-	-	+	+/+
PM7	Cocci	-	-	+	+/+
PM10	Cocci	-	-	+	+/+
PM14	Cocci	-	-	+	+/+
PM16	Cocci	-	-	+	+/+
PM19	Cocci	-	-	+	+/+
PM8	Short Rods	-	-	+	+/+
PM9	Short Rods	-	-	+	+/+
PM12	Short Rods	-	-	+	+/+
PM13	Short Rods	-	-	+	+/+
PM15	Short Rods	-	-	+	+/+
PM17	Short Rods	-	-	+	+/+
PM18	Short Rods	-	-	+	+/+
PM20	Short Rods	+	-	+	+/+
PM21	Cocci	-	-	+	+/+
PM22	Short Rods	-	-	+	+/+
PM23	Short Rods	-	-	+	+/+
PB1	Short Rods	-	-	+	+/+
PB2	Short Rods	-	-	+	+/+
MT1	Short Rods	-	-	-	+/+
MT3	Short Rods	-	-	-	+/+
MT6	Short Rods	-	-	-	+/+
MT35	Short Rods	-	-	-	+/+
MT4	Short Rods	-	-	+	+/+
MT7	Short Rods	-	-	+	+/+
MT8	Short Rods	-	-	+	+/+
MT9	Short Rods	-	-	+	+/+
MT20	Short Rods	-	-	+	+/+
MT21	Short Rods	-	-	+	+/+
MT5	Short Rods	+	-	+	+/+

Note; PT, PM and PB isolates were collected from aerobically packed; MT, isolates were collected from vacuum packed

3.4.2 Identification of bacteria species

Representative dominant bacterial floras above were identified according to their morphological characteristic and basic biochemistry as shown in Table 3.3. Twenty-eight were short rod and the rest were cocci. PM 23 and MT 1, MT 3, MT 6 and MT 35 were not able to produce catalase and oxidase. Morphological characteristics of bacteria isolated from spoiled pork showed the difference in details as shown in Table 3.3. From biochemical test of all six isolates (Table 3.4) only PM 3 and PT 3 showed similar results while only MT 5 (Table 3.3) was Gram positive among selected bacteria from spoiled pork. By using test kit in API kit, the PM 20, PM 3 and PT 3 isolates were similar to *Serratia liquefaciens* with 96.0%, PM 23 showed similar 99.0% to *Enterobacter* sp, MT 5 showed 99.0 % similar to *Staphylococcus* sp, MT 4 showed 99.9 % similar to *Hafnia alvei* and MT 35 showed 97.4 % similar to *Klebsiella oxytoca*. Species of the genera *Serratia*, *Enterobacter*, *Proteus*, *Leclercia*, and *Hafnia* have frequently been isolated from spoiled hams. *Serratia* is reported to be proteolytic and non-pathogenic (Losantos et al., 2000). Moreover, its genera most commonly present on working surfaces in the meat processing industry (Stiles, 1981). *S. liquefaciens* is a major spoilage agent in fresh, cooked or cured meat. Its proteolytic activity causes the production of off odors and off flavors. Moreover, its ability to produce biogenic amines (e.g. putrescine and cadaverine) in meat products has been reported (Silla-Santos, 1996). *H. alvei* and *S. liquefaciens* have also been implicated in meat spoilage, and reported to cause various defects such as objectionable odors (Dainty et al., 1989; Garcia-Lopez et al.,1998; Stanbridge and Davies, 1998), gaseous distension of vacuum packages (Brightwell et al., 2007; Chaves et al., 2012), and green discoloration (Dainty et al., 1989;

Stanbridge and Davies, 1998).

3.4.3 Inhibition of selected herbs and spices extracts against indicator bacteria

Preliminary screening of the antimicrobial activity of the fifteen local Thai herbs against 12 pathogens microorganisms using filter paper disc agar diffusion technique was shown in Table 3.5. Results from clear zone showed that only *Spondias pinnata* (L.f.) *Kurz* extract could inhibit all of 12 indicator bacteria while *Momordica charantia*, *Leucaena leucocephala* (Lamk.) de Wit, *Sauropus androgynus*, *Moringa oleifera* Lam, *Centella asiatica*, *Feroniella lucida* (Scheff.), *Acanthus ebracteatus* Vahl and *Sesban sesbania* could not. Four ethanolic extracts from *Senna siamea* Lam, *Schinus terebinthifolius*, *Anacardium occidentale* and *Garcinia mangostana* Linn could inhibit the same species of bacteria both Gram-positive and Gram-negative, i.e., *E. faecalis*, *M. luteus*. At high concentration of ethanolic extracts from *Barringtonia Acutangula* (Linn.) Gaertn and *Syzygium gratum* (Wight) S.N.Mitra var. gratum could inhibit the same species of bacteria (*M. luteus)*. The ethanolic extracts of *S. terebinthifolius*, *S. pinnata* (L.f.) Kurz and *G. mangostana* Linn had greater potential as an antimicrobial agent against Gram-positive and Gram- negative bacterias. These results were in agreement with some previous studied. Bukar et al. (2009) reported the ethanolic extract of *S. siamea* Lam against *Pseudomonas aeruginosa* at high concentration levels of 500 µg/disc and 1000 µg/disc had zones of inhibition of 10 mm and 16 mm respectively. Torrungruang et al. (2007) showed the antibacterial activity of mangosteen Pericarp extract against cariogenic *Streptococcus mutans*. From the studied of de Lima et al. (2007) using extracts of *S. terebinfolius* showed activity against *Staphylococcus aureus* but, were not active against *Escherichia coli*.

These activities may be attributed to the presence of m-cymene, 1-ß-pinene, α-pinene, α-terpinene, γ-terpinene and camphene found in *S. terebinthifolius* essential oil. It has been demonstrated that α-pinene and ß-pinene are able to destroy cellular integrity and thereby inhibit respiration and ion transport processes. They also increase the membrane permeability in yeast cells and isolated mitochondria (Andrews et al., 1980). Our results were in agreement with the finding of Mayachiew and Devahastin, 2008 who reported good antimicrobial activity of local Thai herb and spices (*Phyllanthus emblica* Linn (Makham Pom) and *Alpinia galangal* (Kha)). The main reason for the differences in bacterial susceptibility could be the outer membrane surrounding the cell wall in Gram-negative bacteria, which restricts diffusion of compounds through its lipopolysaccharide covering (Vaara, 1992). In addition, the periplasmatic space contains enzymes which are capable of breaking down foreign molecules introduced from the outside (Vaara, 1992). Our results show remarkable antimicrobial activity for the ethanol extract of *Spondias pinnata* (L.f.) *Kurz* against all microorganisms tested, both Gram-positive and Gram- negative bacteria.

Table 3.4 Morphological characteristic and some biochemical test of selected bacteria

Test	PM 23	PM 20	PM 3	PT 3	MT 4	MT 35	MT 5
Color	White-yellow	White-yellow	White-yellow	White-yellow	White-yellow	White	White
Form	Round	Round	Round	Round	Irregular and spreading	Round	Round
Size	0.5mm	0.5-1.0mm	0.5mm	0.5mm	0.5-1.0mm	0.5mm	0.5mm
Margin	Smooth	Smooth	Wavy	Smooth	lobate	smooth	smooth
Shape	Short rod	Cocci	Cocci	Short rod	Short rod	Rod	Short rod
Gram strain	-	-	-	-	-	-	+
Motility	-	-	-	-	+	-	-
Oxidase	-	-	-	-	-	-	-
Catalase	-	+	+	+	+	-	+
ß-galactosidase(ortho-nitro-phenyl-ßD-galactopyranoside	+	+	+	+	+	+	
Arginine dihydrolase	+	+	+	+	-	-	
Lysine decarboxylase	+	-	+	+	+	+	
Ornithine decarboxylase	+	-	+	+	+	-	
Citrate utilization	+	+	+	+	+	+	
H_2S production	-	-	-	-	-	-	
Urease	-	-	-	-	-	+	
Tryptophane deaminase	-	-	-	-	-	-	
Indole production	-	-	-	-	-	+	
Acetoin production of sodium pyruvate(Voges Proskauer)	+	+	+	+	+	+	
Gelatinase	+	-	+	+	-	-	
Fermentation or oxidation of:							
D-glucose	+	+	+	+	+	+	+
D-manital	+	+	+	+	+	+	

Note; +, Positive test; -, Negative test; ND, Not detected

Table 3.4 (continued)

Test	PM 23	PM 20	PM 3	PT 3	MT 4	MT 35	MT 5
Inositol	+	-	+	+	-	+	
D-sorbitol	+	-	+	+	-	+	
D-rhamnose	-	+	-	-	+	+	
D-saccharose(sucrose)	+	+	+	+	-	+	+
D-melibiose	+	+	+	+	-	+	-
Amygdalin	+	+	+	+	-	+	
L-arabinose	+	+	+	+	+	+	
Cytochrome oxidase	-	-	-	-	-	-	-
D-fructose	ND	ND	ND	ND	ND	ND	+
D-manose	ND	ND	ND	ND	ND	ND	+
D-lactose(bovine origin	ND	ND	ND	ND	ND	ND	+
D-trehalose	ND	ND	ND	ND	ND	ND	+
D-manitol	ND	ND	ND	ND	ND	ND	-
Xylitol	ND	ND	ND	ND	ND	ND	-
D-melibose	ND	ND	ND	ND	ND	ND	-
D-raffinose	ND	ND	ND	ND	ND	ND	-
D-xylose	ND	ND	ND	ND	ND	ND	-
D-sacharose(sucrose)	ND	ND	ND	ND	ND	ND	+
Methyl-αD-glucopyranodside	ND	ND	ND	ND	ND	ND	-
N-acetyl-glucosamine	ND	ND	ND	ND	ND	ND	+
Reduction of nitrate to nitrites	ND	ND	ND	ND	ND	ND	
Alkaline phosphatase	ND	ND	ND	ND	ND	ND	-
Acetyl-methyl-carbinol production of sodium pyruvate (voges proskauer)	ND	ND	ND	ND	ND	ND	+
Argine dihydrolase	ND	ND	ND	ND	ND	ND	-
Urease	ND	ND	ND	ND	ND	ND	+
Catalase test	ND	ND	ND	ND	ND	ND	+

Note; +, Positive test; -, Negative test; ND, Not detected

Table 3.5 Antimicrobial activity of local Thai herbs extracts by paper disc diffusion test

Local thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	*S.aureus*	*P.fluorescens*	*E. faecalis*	*M. luteus*	*L.monocytogenes*	*E. coli*	*S.liquefaciens*	*K.oxytoca*	*Enterobacter sp.*	*A.calcoaceticus*	*H. alvei*
Momordica charantia	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Leucaena leucocephala (Lamk.) de Wit	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Senna siamea Lam	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	+	-	-	+	++	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	
Sauropus androgynus	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Schinus terebinthifolius	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-	-	-	-
	100	-	++	++	++	++	+	-	-	-	-	-	-

Note; -, No inhibition; +, <10 mm.; ++, 10-15 mm.; +++, >15 mm.; Nisin 200 ppm for positive control; 1%DMSO for negative control

Table 3.5 (continued)

Local thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	***S.aureus***	***P.fluorescens***	***E. faecalis***	***M. luteus***	***L.monocytogenes***	***E. coli***	***S.liquefaciens***	***K.oxytoca***	***Enterobacter sp.***	***A.calcoaceticus***	***H. alvei***
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Moringa oleifera Lam	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Anacardium occidentale	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-	-	-	-
	100	-	+	-	+	+	+	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Centella asiatica	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Feroniella lucida (Scheff.)	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Spondias pinnata (L.f.) Kurz	20	-	++	-	+	+++	-	-	-	-	+	-	-
	60	+	++	++	+++	+++	-	++	++	+	++	++	-
	100	++	+++	+++	+++	+++	+	++	++	++	+++	+++	++

Note; -, No inhibition; +, <10 mm.; ++, 10-15 mm.; +++, >15 mm.; Nisin 200 ppm for positive control; 1% DMSO for negative control.

Table 3.5 (continued)

Local thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	*S.aureus*	*P.fluorescens*	*E. faecalis*	*M. luteus*	*L.monocytogenes*	*E. coli*	*S.liquefaciens*	*K.oxytoca*	*Enterobacter sp.*	*A.calcoaceticus*	*H. alvei*
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Garcinia mangostana Linn.	20	-	+	-	+	++	-	-	-	-	-	-	-
	60	-	++	-	++	++	-	-	-	-	-	-	-
	100	-	++	-	++	+++	+	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
BarringtoniaAcutangula(Linn.)Gaertn	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	+	-	-	-	-	-	-	-	-	-	-
	100	-	+	-	-	++	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Acanthus ebracteatus Vahl	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Syzygium gratum (Wight) S.N.Mitra var. gratum	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	+++	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++
Sesban Sesbania	20	-	-	-	-	-	-	-	-	-	-	-	-
	60	-	-	-	-	-	-	-	-	-	-	-	-

Note; -, No inhibition; +, <10 mm.; ++, 10-15 mm.; +++, >15 mm.; Nisin 200 ppm for positive control; 1% DMSO for negative control

Table 3.5 (continued)

Local thai herbs	Ethanolic Extract (ppm)	Zone of inhibitions (mm)											
		E.aerogenes	***S.aureus***	***P.fluorescens***	***E. faecalis***	***M. luteus***	***L.monocytogenes***	***E. coli***	***S.liquefaciens***	***K.oxytoca***	***Enterobacter sp.***	***A.calcoaceticus***	***H. alvei***
Sesban Sesbania	60	-	-	-	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-	-	-	-	-	-
Nisin	200	-	+	-	-	+	-	-	-	+	+	-	++

Note; -, No inhibition; +, <10 mm.; ++, 10-15 mm.; +++, >15 mm.; Nisin 200 ppm for positive control; 1% DMSO for negative control

3.5 Conclusions

Out of the 15 local Thai herbs, seven of them showed antibacterial activity. The crude ethanol extract of herbs having highly effective activity against indicator bacteria were found for *Spondias pinnata* (L.f.) *Kurz, Senna siamea* Lam, *Schinus terebinthifolius, Anacardium occidentale* , *Barringtonia Acutangula* (Linn.) *Gaertn, Syzygium gratum* (Wight) S.N.Mitra *var*. gratum. The extract from *S. pinnata* (L.f.) Kurz showed the most effective activity against all dominant floras obtained from spoiled pork chops, aerobically and vacuum packed, and kept at refrigeration temperatures. Further studies were suggested on identification of active compounds and suitable purification of these herb extracts for further used as biopreservative agents in meat and products.

3.6 References

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CHAPTER IV

EFFECTS OF SELECTED THAI HERB EXTRACTS ON MICROBIAL AND PHYSICOCHEMICAL QUALITY OF SLICED PORK LOINS

4.1 Abstract

Fresh sliced pork loins were dipped in 0.2% (w/v) crude bacteriocins (B), 0.2% (w/v), nisin (N), 10 % (w/v) each of extract from, *Spondias pinnata* (L.f.) Kurz, (E1), *Garcinia mangostana* Linn (E2) and *Schinus terebinthifolius* (E3). The treated pork samples were aerobically and vacuum packed in plastic bags and stored at 4ºC for 8 days. Microbial counts and eating qualities of the treated pork samples were determined. Total plate counts (TPCs) of all aerobically packed pork samples were not significantly different ($p>0.05$) although E2 extract exhibited higher antimicrobial activity than other extracts and N solution exhibited lower antimicrobial activity. For vacuum packed condition, TPCs of all samples were not significantly different ($p>0.05$) from day 2 through the end of storage. The total coliform counts of aerobically packed pork samples were significantly different ($p<0.05$) only in day 0 and day 2.The E2 treated samples had the lowest count of 0.35 log cfu/cm^2 while the E3 treated samples had higher total counts of 2.0 log cfu/cm^2. There was no significant difference ($p>0.05$) in total coliform counts of all vacuum packed sample during storage time and E2 and E1 treated samples had lower counts than E3 treated

one which had similar count to that of control, B treated and N treated samples at the end of storage period. Total acidity of control sample was not different ($p>0.05$) compared with B, N, E2 and E3 treated ones while difference ($p<0.05$) was found with E1 treated one in aerobically packed condition. In vacuum packed condition, total acidity of all extract treated and N treated samples were higher ($p<0.05$) than C and B treated ones at the end of storage. The pH values of pork samples raged 5.5-5.8 in both packaging conditions ($p<0.05$). Similar metmyoglobin contents were observed for all pork samples with the highest amount was observed in day 4 and gradually decreased toward the end of storage in both packaging conditions. Hardness of N, E1, E2 and E3 treated and aerobically packed pork samples were different ($p<0.05$) throughout storage period while of which B treated and control pork ones were not differed ($p<0.05$). The lightness (L*) of all aerobically samples were not significantly different ($p>0.05$) and similar results were found for yellowness (b*) except for E2 treated samples ($p<0.05$). Redness (a*) of all samples tended to increase throughout storage period. Lightness of vacuum packed pork samples from all treatments was not different ($p>0.05$) while more intense redness (a*) was found for E1 and E2 treated pork samples than other treated samples ($p<0.05$). Similarly, no difference ($p>0.05$) of yellowness (b*) was not found for all pork samples from all treatments. Purge loss of treated pork samples packed in both conditions were not differed ($p>0.05$) except for that of E3 treated in vacuum packed condition was slightly differed ($p<0.05$). Cooking yields were slightly different ($p<0.05$) among E1, E2 and E3 treated pork samples packed in both conditions. Overall acceptance of aerobically packed samples, both sliced raw and cooked pork, was not different while it was observed only among raw pork samples packed in vacuum condition from all treatments ($p<0.05$).

4.2 Introduction

For the assurance of food safety nowadays consumers are demanding food products with natural ingredients alternatives to chemical additives with increased safety, quality and shelf-life. Antimicrobials are used in food to control and to prevent growth of spoilage and pathogenic microorganisms, including pathogenic microorganisms (Tajkarimi, Ibrahim, and Cliver, 2010). Certain plants and their extracts used as flavoring agents are known to possess antimicrobial activity offering a potential alternative to synthetic preservatives (Gould, 1996). Much attention has been focused on the extracts from herbs and spices which have been traditionally used to improve the sensory, odor or pigment characteristics and extend the shelf-life of foods (Gibbons, 2008). Plant-derived ingredients possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural. Essential oils (EOs) and other extracts from plants, herbs and spices and some of their constituents, have shown antimicrobial activity against different food pathogens and spoilage microorganisms (Bakkali et al., 2008; Burt, 2004; Holley and Patel, 2005).

Fresh meat and meat products can be easily contaminated with microorganisms and, if not properly handled and preserved, support growth of spoilage and pathogen bacteria, leading to loss of quality and potential public health problems (Vernozy-Rozand et al., 2002). In recent years, naturally occurring antimicrobial and antioxidant compounds derived from plant sources have been preferably employed in meat products because of their potential health benefits and safety compared with synthetic preservatives (García-Iñiguez de Ciriano et al., 2009; Sebranek et al., 2005). The use of natural preservatives to increase the shelf-life of meat products is a promising

technology since many plant-derived substances have antioxidant and antimicrobial properties. It has been shown that plant extracts are useful for reduction of pathogens associated with meat products. However, some authors have recorded low antimicrobial effects against pathogens in contaminated meat products (Grosso et al., 2008).

The use of chemical preservatives to prevent food spoilage and inhibit food-borne pathogens is being questioned due to lack of consumer acceptance and potential health risks. Since consumers are becoming increasingly health aware, the application of bacteriocins as a natural preservative in food has recently received considerable attention (Papagianni and Anastasiadou, 2009). Bacteriocins are ribosomally synthesized anti-microbial compounds that are produced by many different bacterial species including many members of the lactic acid bacteria (LAB) (Klaenhammer, 1988; Jack, Tagg and Ray, 1995). Some bacteriocins produced by LAB, such as nisin, inhibit not only closely related species but are also effective against foodborne pathogens such as *Listeria monocytogenes* and many other Gram-positive spoilage microorganisms (Tagg, Dajani and Wannamaker, 1976). Bacteria which produce broad host range bacteriocins such as nisin would be desirable, however, the use of nisin as a preservative in meat has been extensively examined but has found little success to date. Nisin appears to be unstable in meat, particularly at ambient temperatures which may be due to binding of the bacteriocin to sulphydryl groups or meat particles (Chung, Dickson and Crouse, 1989). It also interacts strongly with phospholipids which limits its activity in meat with a high fat content (Henning, Metz and Hammes, 1986).

Antimicrobial activity against a range of bacterial pathogens of importance is also

found in other EOs, non-toxic compounds, and some organic acids. These natural occurring antimicrobial substances have been widely tested for their inhibitory effectiveness against foodborne pathogens. Developing an effective of natural antimicrobials against food spoilage microorganisms could result in new sanitization or preservation methods to control microbial growth on fresh meat, and to extend the shelf-life of fresh meat. Therefore, this study was conducted to investigate inhibitory effects of three selected herb extracts obtained from the previous experiment (Chapter III) on decontamination of fresh meat in comparing with known activity of nisin and crude bacteriocins produced from *Lactococcus lactis* TISTR 1401 (Sriwira, 2008). After being treated, the meat samples were aerobically and vacuum packed and kept in a cooling room at 4ºC. Microbial contents and eating quality of treated meat were investigated during storage for the period of 8 days.

4.3 Materials and Methods

4.3.1 Preparation of herb extracts

Three selected Thai herbs consisting of hog plum (*Spondias pinnata* (L.f.) Kurz: leaves (E1), mangosteen (*Garcinia mangostana* Linn) skin (E2) and Brazillian pepper-tree (*Schinus terebinthifolius*) leaves (E3) were screened and selected from 15 local Thai herbs according to their ability to inhibit the growth of indicator bacteria and predominant bacteria obtained from fresh pork samples. The freeze-dried herb powder was extracted using ethanol as a solvent according to item 3.3.1 in Chapter III.

4.3.2 Preparation of freeze dried crude bacteriocins preparation

Crude bacteriocins were prepared from the fermentate produced by *Lactococcus lactis* TISTR 1401 according to the method of Intarapichet *et al* (2008).

Brifely, fermentation of *L. lactis* TISTR 1401 was done anaerobically in 10% MRS broth with the addition of 2% (w/w) yeast extracts, 2%(w/w) beef extract and 2% (w/w) glucose 2% w/w) and incubated at 37^{o}C for 24 h. The fermentate was centrifuged at 12000×g at 4^{o}C for 20 min (Thermo scientific SORVALL Legend Mach 1.6 R centrifuge). The supernatant was adjusted to pH 6.5 and then, catalase was added to final concentration of 1 mg/ml, incubated at 25^{o}C for 30 min and filtered through 0.22 micron cellulose acetate membrane filter. The filtrate was freeze-dried (GEA/LYOVAC GT 2-S), stored in a vaccum bag and kept at 4^{o}C until use. Crude bacteriocins powder and commercial nisin were prepared in 0.2% solution just before being used.

4.3.3 Samples preparation and treatment

Slices of pork chops about 2.54 cm thick, were obtained from Fresh Meat Company, Nakhon Chai Si, Nakhon Prathom Province within 8 h after slaughtering. The pork samples were randomly divided into 6 groups for dipping in 6 treatments solutions; hog plum (*Spondias pinnata* (L.f.) Kurz: leaves (E1), mangosteen (*Garcinia mangostana* Linn) skin (E2) and Brazillian pepper-tree (*Schinus terebinthifolius*) leaves (E3) extract solutions (10% w/v), crude bacteriocins (0.2% w/v) (B), nisin (0.2% w/v) (N) and DMSO (1% v/v) (C). The pork samples were dipped in each solution for 5 min, drained for 10 min and aerobically or vacuum packed in plastic bag (PA/PE), and stored at 4°C. The pork samples were randomly taken for analysis every 2 days of the storage period of 8 days.

4.3.4 Microbial enumeration

Aerobic microbial contents in term of total plate counts of the surface of pork samples was determined by swabbing procedure using a sterilized template with

known area. Series of dilution were made using 0.85% NaCl solution and plated out on 3M petrifilm (Aerobic count plate), placed a dry-film aerobic count plate on flat surface, lifted the top of the film and inoculated 1 ml test suspension onto center of film base then, carefully placed the top of the film down on inoculum. The test suspension was distributed over the pre-scribed growth area with down ward pressure in the center of plastic spreader de vice (recessed side down). The plate was left undisturbed for 1 min to permit gel to solidify and then, incubated at 35±1°C for 48 h. For total coliforms 3M *E.coli* was used and incubated at 35±1°C for 48 h. The numbers of bacterial counts were expressed as $\log_{10}$ cfu/cm^2.

4.3.5 Total acidity and pH

Total acidity of the pork samples was determined according to AOAC (1990). Total acidity was analyzed by titrating with NaOH, using phenolphthalein (1%) as indicator and determined as lactic acid. pH of the pork samples were measured using a meat pH meter HI 99163, HANNA, probe FC 232 D, stainless steel blade FC 095.

4.3.6 Purge loss

Initial weight of pork was weighed after dipping in treatment solution. At time of sampling, the pork sample was slightly blotted and weighed. Percentage of purge loss was calculated from difference of initial and final weights (Sutton et al., 1997).

4.3.7 Cooking yield

Percent cooking yield was determined by calculating weight differences of samples before and after cooking the pork sample to an internal temperature 72°C (Murphy, Criner and Grey, 1975) and calculated as followed.

$$Cooking\ Yield\ (\%) = \frac{[Cooked\ meat\ sample\ weight]}{[Raw\ meat\ sample\ weight]} \times 100$$

4.3.8 Moisture analysis

Moisture content of raw pork sliced was measured following AOAC (1990) method.

4.3.9 Analysis of metmyoglobin content

The method of Faustman and Phillips (2001) was used for metmyoglobin analysis of the pork samples. The meat samples (25 g) was homogenized in 10 ml ice-cold 40 mM phosphate buffer (pH 6.8) for 45 s using an Ultra-Turrax T25 tissue macerator (13,500 rpm). The homogenate was allowed to stand at 4ºC for 1 h and centrifuged at 4,500 rpm at 4ºC for 30 min. The supernatant was filtered through a 0.40 µm and absorbance of the filtrate of 1.0 ml was measured at 572, 565, 545 and 525 nm using a Spectrophotometer UV (Biochrom, Libra S226). The percentage of metmyoglobin was determined as described by Krzywicki (1982) using the following formula:

%MetMb ={ 2:51(A572/A525) + 0:777(A565/A525) +0:8(A545/A525) + 1:098}*100

4.3.10 Color measurement

Color of the meat sample was measured in terms of CIE L*, a*, b* values using colorimeter (Hunter lab). Before measurement, the apparatus was standardized against a white plate.

4.3.11 Texture analysis

Texture of meat samples was measured using the texture analyzer: (TA.XT.Plus, Texture Technologies Crop., Scardals, USA) The Compression force was measured by using spherical probes (P/ 0.5 s, 1.2 cm diameter ball probe) rate of probe move=2 mm/sec distance press of prob=10 mm, 10 point per each size, read as hardness in Newton(N)

4.3.12 Sensory evaluation

Sensory evaluation of raw and cooked pork samples was performed using the intensity scores (1 to 5) by 8 trained panelists. The pork sample was cooked in a plastic bag to an internal temperature of 72°C, cut into a bite size and served warm. Sensory attributes evaluated consisted of color, flavor, texture and overall acceptance. For raw pork samples, intensity scores were assigned as follows: Color score (1=dark brownish red, 2=moderately brownish red, 3=brownish, 4=dull reddish pink and 5=very bright reddish pink), Flavor score (1=putrid, 2=strongly sour, 3=sour, 4=slightly sour and 5=raw pork flavor), Texture score (1=soft and exude, 2=very slightly tender, 3=tender like, 4=harsh and dry and 5=firm and dense), Overall acceptance; 1=reject, 2=dislike, 3=neither nor like, 4=slightly accepted and 5=accepted). For cooked pork samples, intensity scores were assigned as follows: Color (1=very dark grey, 2=dark grey, 3=medium brown grey, 4=brownish grey and 5=very light brownish grey), Flavor (1=unpleasant, 2=painty, rancid, 3=sour pork flavor, 4=sweet pork flavor and 5=cooked pork flavor), Texture (1=very tough, 2=slightly tough, 3=slightly tender, 4=moderately tender, and 5=very tender), Overall acceptance (1=rejected, 2=slightly dislike, 3=neither nor like, 4=slightly accepted and 5=accepted).

4.4 Statistical analysis

Statistical analysis was evaluated by randomized complete block design. Analysis of variance was analyzed and comparison of means was done by Duncan's Multiple Range Test. Significant difference was defined at $p<0.05$. Two replications of the experiment were performed

4.5 Results and discussion

4.5.1 Microbial enumeration

4.5.1.1 Total plate counts

Microbial contents of the pork samples during 8-days of storage period are presented in Figure 4.1-4.4. Among treatments at each day of taking the samples, the total plate counts (TPC) of all aerobically packed samples (Figure 4.1A) were not significant ($p>0.05$) along storage period although samples treated with mangosteen extract (E2) had lower TPC than other samples. The initial count at day 0 of control sample was higher (1.76 log cfu/cm^2) than other treated samples. However, the samples treated with hog plum extract (E1) and E2 had the lowest counts (0.99 and 0.91 log cfu/cm^2, respectively). From day 4 to the end of storage, the sample treated with nisin had higher TPC than other samples. However, TPCs of all samples gradually increased while the E2 treated one still had lower counts than other samples.

For vacuum packed samples (Figure 4.1B), the numbers of TPCs was similar to those of aerobic packaged ones except for day 0 that, the E2 treated samples had lower counts than the other samples ($p<0.05$). From day 2 to the end of storage, TPCs counts of all samples were non significantly ($p>0.05$) and the E2 treated one still showed lower while nisin treated samples showed the higher counts than other samples.

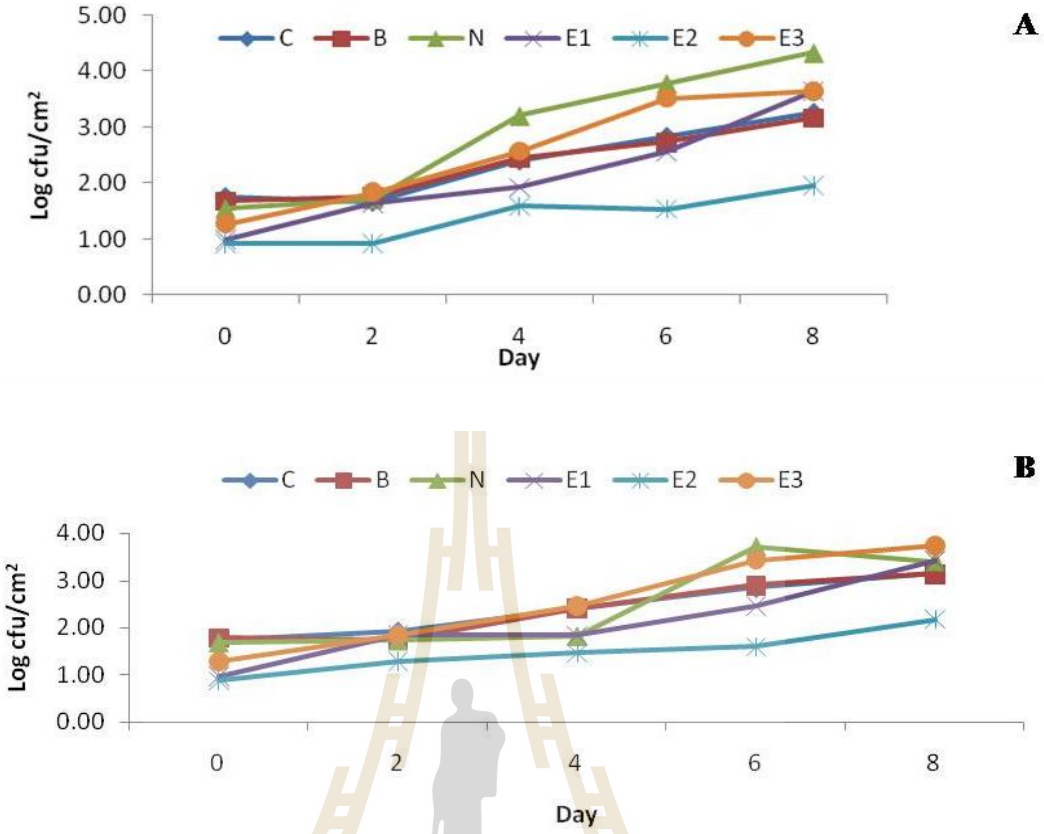

Figure 4.1 Total plate counts of pork chops treated with antimicrobial agents, aerobically packed (A) and vacuum packed (B) stored at 4°C; (n=4). C=control, B= 0.2% crude bacteriocins, N= 0.2% nisin. E1= 10% *Spondias pinnata* extract , E2= 10% *Garcinia mangostana* Linn extract and E3=10% *Schinus terebinthifolius* extract.

For total coliform counts of aerobically packed samples significances were found in day 0 and 2 of storage ($p<0.05$) (Figure 4.2A) due to the addition of those five natural antimicrobials especially the E2 extract (0.35 log cfu/cm^2) which gave the lowest count while no differences were found among C, B, N and E1. Although the coliform counts of the samples were not found from day 4 through the end of storage; however, E2 treated sample showed lower counts while nisin still had

higher counts. Meanwhile, there was no significant difference ($p>0.05$) of total coliform counts in vacuum packed samples (Figure 4.2B) along storage although the E2 and E1 treated samples still had lower count than E3 treated one which had similar count to C, B and, N treated one at the end of storage. Treatment with E2 could reduced microbial better than other treatments. From other study about *Garcinia*

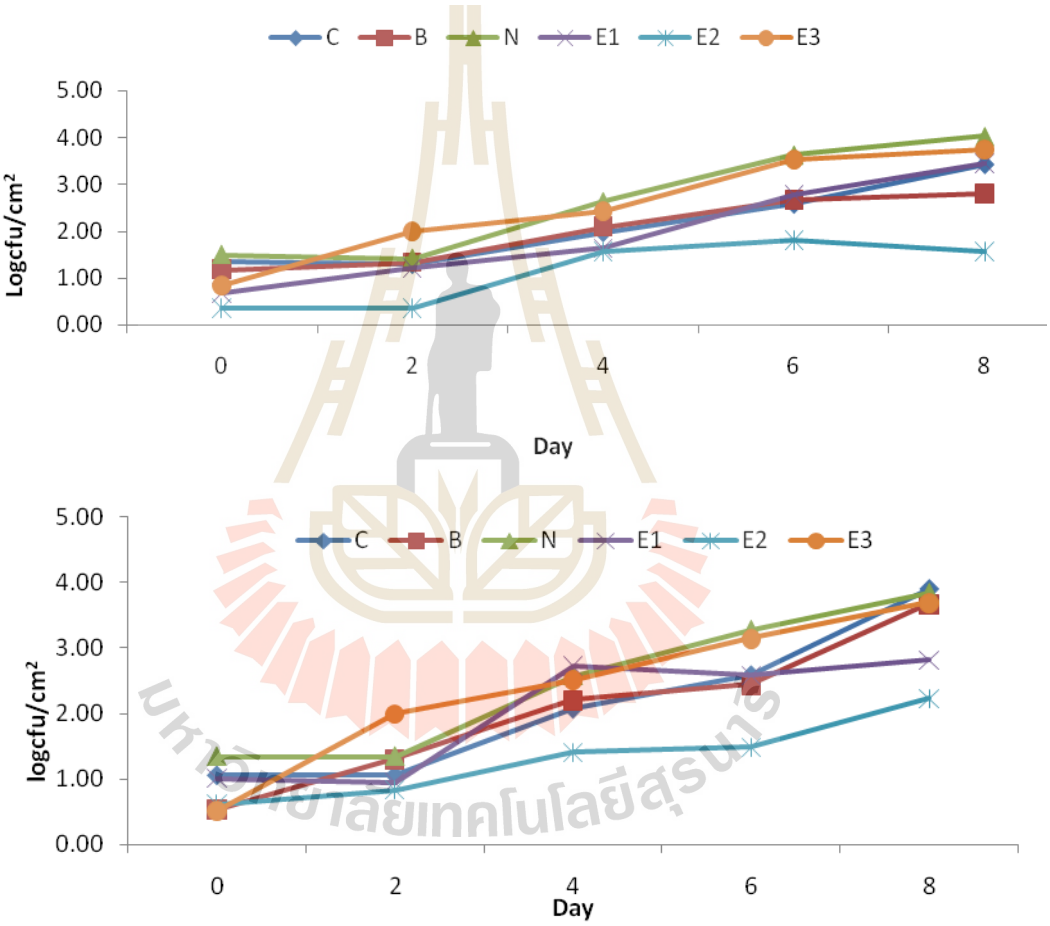

Figure 4.2 Total coliform counts of pork chops treated with antimicrobial agents, aerobically packed (A) and vacuum packed (B) stored at 4 °C; (n=4); C=control; B= 0.2% crude bacteriocins ; N= 0.2% nisin ; E1= 10% *Spondias pinnata* extract ; E2= 10% *Garcinia mangostana* Linn extract and E3= 10% *Schinus terebinthifolius* extract.

mangostana Linn, Munekazu et al. (1996) found that Xanthone and its derivatives have activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus.* Moreover, Alpha-mangostin, rubraxanthone, and xanthochymol isolated from G. mangostana, G. diocia and G. subelliptica, respectively, showed strong antibacterial activity against both methicillin-resistant and methicillin-sensitive *S. aureus* (Iinuma et al., 1996a, 1996b). The antibacterial activity is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents (Ikigai et al., 1993; Otake et al., 1991), and the generation of hydroperoxides from polyphenols (Akagawa et al., 2003).

Although numerous studies have been done in vitro to evaluate the antimicrobial activity of plant extracts, very few studies are available for food products, probably because plant extracts did not produce as marked inhibition as many of the pure compounds in foods. The reduced effectiveness may be attributed to the use of crude extracts in most studies. This could be due to the crude extracts generally contain flavonoids in glycosidic form, where the sugar present in them decreases the effectiveness against some bacteria (Kapoor et al., 2007; Parvathy et al., 2009; Rhee et al., 1994). In this study of both package conditions, the result showed no significant differences in total microbial counts of all treatments which being explained by Uhart et al. (2006) who reported that spices inactivate *Salmonella typhimurium* DT 104 in in vitro condition, but the activity decreased considerably when added to a complex food system such as ground beef. In addition, Kim et al. (2004) observed that ground beef samples did not show significant difference in *Listeria monocytogenes* and *Staphycoccus Aureus*, and total bacterial counts after

being treated with green and jasmine tea as compared with untreated samples. However, a slight reduction in viable count of *S. enteric, Serotype Enteritidis* and *L. monocytogenes* in ground beef by water soluble arrowroot tea extract (up to 6% w/w) was reported (Kim and Fung, 2004).

The cause of lower antimicrobial activity of nisin according to nisin appears to be unstable in meat, particularly at ambient temperatures which may be due to the binding of bacteriocins to sulphydryl groups or meat particles (Chung, 1989) and the strong interaction with phospholipids in meat with a high fat content (Henning, 1986). In addition, nisin loses activity in fresh meat during storage (Cutter and Siragusa, 1994; 1996a, b; 1997; El-Khateib et al., 1993) probably due to an enzymatic reaction with glutathione as postulated by Rose et al. (1999).

4.5.2 Physicochemical properties of treated pork

4.5.2.1 Total acidity of treated pork chops

Total acidity of treated pork samples both aerobically and vacuum packed are presented in Table 4.1-4.2. In aerobically packed (Table 4.1) there were significant differences ($p<0.05$) among E1 treated and C, N and E3 treated samples while no significant differences ($p>0.05$) among E1, E2 and B treated ones observed along the period of storage. For vacuum packed storage (Table 4.2) only samples treated with B and E3 were found significantly differed ($p<0.05$) and tended to increase in total acidity towards the end of storage. There were no significant difference ($p>0.05$) among N, E1 and E2 treated samples in day 0 and day 2 while control samples showed significant difference ($p<0.05$) compared with B and E3 treated ones. In addition, control samples showed significant difference ($p<0.05$) compared with other samples in the last day of storage.

Table 4.1 Total acidity of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

Day	Total acidity (%)					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	5.39 ± 0.01^{BC}	6.05 ± 0.22^{ABC}	6.17 ± 0.00^{BC}	6.89 ± 0.12^{A}	6.19 ± 0.97^{AB}	5.40 ± 0.00^{C}
2	5.40 ± 0.31^{BC}	5.87 ± 0.04^{ABC}	6.09 ± 0.10^{BC}	6.38 ± 0.38^{A}	5.93 ± 0.97^{AB}	5.41 ± 0.03^{C}
4	6.39 ± 0.00^{BC}	6.36 ± 0.21^{ABC}	6.24 ± 0.10^{BC}	6.00 ± 0.42^{A}	5.83 ± 0.82^{AB}	5.89 ± 0.71^{C}
6	5.80 ± 0.56^{BC}	6.62 ± 0.32^{ABC}	5.34 ± 0.36^{BC}	6.62 ± 0.49^{A}	6.86 ± 0.29^{AB}	5.97 ± 0.28^{C}
8	6.60 ± 0.32^{BC}	5.99 ± 0.86^{ABC}	5.48 ± 0.25^{BC}	6.87 ± 0.15^{A}	6.55 ± 0.40^{AB}	6.24 ± 0.11^{C}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$); (n=4)

Table 4.2 Total acidity of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

Day	Total acidity (%)					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	5.65 ± 0.08^{B}	4.71 ± 0.54^{aC}	6.47 ± 0.00^{A}	7.03 ± 0.36^{A}	7.01 ± 0.33^{A}	5.27 ± 0.18^{bBC}
2	5.69 ± 0.20^{B}	5.11 ± 0.05^{abC}	6.70 ± 0.31^{A}	6.70 ± 0.04^{A}	6.58 ± 0.18^{A}	5.10 ± 0.37^{bC}
4	6.11 ± 0.45^{A}	4.13 ± 0.10^{abB}	5.55 ± 1.29^{A}	6.42 ± 0.00^{A}	6.40 ± 0.23^{A}	6.17 ± 0.29^{aA}
6	6.12 ± 0.12^{BC}	5.59 ± 0.06^{bcC}	5.63 ± 0.32^{C}	6.84 ± 0.39^{AB}	7.25 ± 0.33^{A}	6.48 ± 0.33^{aB}
8	5.58 ± 0.27^{C}	5.77 ± 0.54^{cBC}	6.23 ± 0.08^{AB}	7.02 ± 0.45^{A}	7.06 ± 0.25^{A}	6.55 ± 0.11^{aA}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$); (n=4)

4.5.2.2 pH of treated pork chops

The pH of all pork samples are presented in Table 4.3 and 4.4. The pH values ranged from 5.4 to 5.9 during storage and were not significantly different ($p>0.05$) for all samples packed in both conditions. These pH results were in agreement with those reported by Park and Jin (2007) who found that the pHs of raw

pork patties were not affected by storage time. Price and Schweigert (1987) also reported that meat with a pH greater than 5.8 may be more conducive to spoilage resulting in decreasing in shelf-life. Moreover, Rousset and Renerre (1991) and Rey et al. (1976) concluded that higher pH products had greater microbial counts and their shelf-life decreased. This could be observed when the meat were packed either under vacuum or wrapped with an oxygen permeable film.

Table 4.3 pH of aerobically packed pork chops treated with antimicrobial agents (Mean ± SD); (n=6)

Day	pH					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	5.42±0.09	5.52±0.04	5.40±0.08	5.40±0.09	4.793±0.86	5.45±0.09
2	5.36±0.03	5.44±0.04	5.43±0.11	5.38±0.06	5.26±0.01	5.52±0.20
4	5.48±0.25	5.57±0.18	5.50±0.21	5.52±0.21	5.43±0.17	5.53±0.09
6	5.52±0.16	5.56±0.12	5.83±0.41	5.53±0.23	5.45±0.19	5.53±0.09
8	5.59±0.29	5.65±0.14	5.61±0.08	5.52±0.19	5.51±0.38	5.62±0.23

Table 4.4 pH of vacuum packed pork chops treated with antimicrobial agents (Mean±SD) (n=6)

Day	pH					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	5.43±0.05	5.51±0.10	5.46±0.01	5.52±0.27	5.33±0.10	5.42±0.14
2	5.46±0.11	5.42±0.19	5.41±0.13	5.47±0.01	5.39±0.02	5.40±0.03
4	5.47±0.16	5.65±0.14	5.64±0.37	5.80±0.47	5.49±0.25	5.51±0.22
6	5.54±0.09	5.55±0.18	5.92±0.55	5.51±0.07	5.39±0.09	5.76±0.33
8	5.57±0.24	5.61±0.24	5.68±0.15	5.56±0.18	5.49±0.33	5.64±0.21

4.5.2.3 Metmyoglobin content of treated pork chops

For aerobically packed pork samples, it was observed that percentage of metmyoglobin (MetMb) contents were highest in day 4 of storage (Table 4.4). Decreasing in MetMb content was observed toward the day 8 of storage except control samples. The MetMb contents of pork samples treated with all three herb extracts were lower ($p<0.05$) than those treated with nisin and crude bacteriocins at day 4 and day 6 of storage time. However, there were no differences among herb extract treated pork samples and control samples in day 2 till day 6. In addition, it was noticed that during storage the MetMb contents of control pork samples tended to be higher than treated samples despite of no significant difference observed. Higher levels of MetMb in aged pork cold be due to loss of MetMb reducing activity (Zhu and Brewer, 1998a), a combination of depletion of required substrates and co-factors (Giddings, 1974) and loss of mitochondrial structure (Cheah and Cheah, 1971; Tang et al, 2005). The accumulation of MetMb on the surface of pork upon further storage affects redness, which decreases with storage time (Rosenvold and Andersen, 2003a; Lindahl et al., 2005a).

MetMb contents of all samples from all treatments packed in vacuum condition were similar to those packed in aerobic condition as shown in Table 4.5. The MetMb contents of the treated samples were highest from day 4 and decreased towards the last day of storage. MetMb contents of E1, E2 treated and control samples decreased significantly($p<0.05$) from day 4 to day 8. Some researchers found that MetMb reduction occurred only under anaerobic conditions (Watts, Kendrick, Zisper, Hutchins, and Saleh, 1966) or increased compared with aerobic conditions (Al-Shibani, Price, and Brown, 1977; O'Keefe and Hood, 1982; Shimizu and Matsuura,

1968; Yamanaka, Takamizawa, and Amano, 1973). In contrast, Ledward (1985) believes that MetMb reductase is oxygen-dependent and Echevarne et al. (1990) found no significant differences between aerobic and anaerobic MetMb-reducing activities. Moreover, Hagler, Coopes and Herman (1979) showed that the rates of metmyoglobin reduction, measured using purified enzyme either aerobically or anaerobically, were identical. The decreasing of metmyoglobin, aerobically packed in addition to changes in microbial proliferation over aging, physical changes that affect

Table 4.5 Metmyoglobin content of aerobically packed pork chops treated with antimicrobial agents (Mean± D)

Day	Metmyoglobin (%)					
	C	B	N	E1	E2	E3
0	0.14 ± 0.02^{dBC}	0.11 ± 0.03^{cCD}	0.19 ± 0.01^{aA}	0.19 ± 0.01^{bA}	0.08 ± 0.02^{dD}	0.15 ± 0.01^{dAB}
2	0.21 ± 0.02^{bcA}	$0.13\pm0.00^{bcAB(}$	0.08 ± 0.06^{bBC}	0.16 ± 0.06^{bAB}	0.13 ± 0.00^{cABC}	0.04 ± 0.03^{cC}
4	0.30 ± 0.00^{a}	0.29 ± 0.04^{a}	0.22 ± 0.04^{a}	0.27 ± 0.02^{a}	0.30 ± 0.02^{a}	0.23 ± 0.08^{a}
6	0.18 ± 0.00^{cC}	0.25 ± 0.01^{aA}	0.21 ± 0.00^{aB}	0.19 ± 0.01^{bC}	0.19 ± 0.00^{bC}	$0.192\pm^{bC}$
8	0.23 ± 0.01^{bA}	0.17 ± 0.01^{bC}	0.15 ± 0.00^{abCD}	0.13 ± 0.01^{bC}	0.17 ± 0.01^{bCD}	0.20 ± 0.00^{bB}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=4)

Table 4.6 Metmyoglobin content of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

Day	Metmyoglobin (%)					
	C	B	N	E1	E2	E3
0	0.15 ± 0.01^{cC}	0.20 ± 0.01^{bB}	0.10 ± 0.01^{D}	0.17 ± 0.02^{BC}	0.23 ± 0.02^{bA}	0.19 ± 0.01^{bB}
2	0.13 ± 0.00^{c}	0.12 ± 0.02^{c}	0.09 ± 0.07^{b}	0.12 ± 0.03^{cd}	0.10 ± 0.05^{c}	0.08 ± 0.05^{b}
4	0.26 ± 0.02^{a}	0.29 ± 0.01^{a}	0.27 ± 0.07^{a}	0.26 ± 0.04^{a}	0.32 ± 0.01^{a}	0.21 ± 0.00^{a}
6	0.18 ± 0.00^{bE}	0.26 ± 0.00^{aB}	0.28 ± 0.00^{aA}	0.20 ± 0.00^{bC}	0.19 ± 0.00^{bD}	0.13 ± 0.00^{aF}
8	0.12 ± 0.01^{cBC}	0.27 ± 0.01^{aA}	0.16 ± 0.10^{bBC}	0.09 ± 0.01^{dBC}	0.07 ± 0.02^{cC}	0.19 ± 0.00^{aAB}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=4)

color can also occur. In addition, Echevarne, Renerre and Labas (1990) observed that there was an increase or no change in the metmyoglobin-reducing activity during the storage of beef muscles over a 9-day post-mortem period.

4.5.2.4 Moisture contents of treated pork chops

The moisture contents of both aerobically and vacuum packed pork samples are shown in Table 4.7-4.8. For aerobically packed samples (Table 4.7) moisture contents of all treated pork samples were not different ($p>0.05$) except at day 4 which slightly higher than control sample. The moisture contents of E1 treated and control sample did not change during storage while other treated samples tended reduced ($p<0.05$) from day 4 till the end of storage. In vacuum packed samples (Table 4.8), similar results were observed but there was difference ($p<0.05$) among samples treated with natural antimicrobials.

Table 4.7 Moisture of aerobically packed pork chops treated with antimicrobial agents for (Mean±SD)

Day	Moisture (%)					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	72.06±1.7	71.81±0.3^{bc}	72.02±0.7^{c}	71.94±0.1	71.91±0.5^{ab}	71.98±0.6^{bc}
2	70.79±0.5	73.41±0.5^{ab}	72.14±0.2^{bc}	70.13±2.0	73.4±4.1^{a}	73.88±1.1^{ab}
4	70.92±4.3^{C}	74.78±0.5^{aA}	73.72±0.3^{aAB}	72.56±0.8^{B}	74.58±0.8^{aA}	74.61±0.5^{aA}
6	73.78±0.5	72.95±0.3^{b}	73.35±0.7^{ab}	72.14±1.5	70.18±1.7^{b}	73.05±0.1^{abc}
8	70.75±0.8	70.92±1.3^{c}	70.07±0.1^{d}	71.51±2.2	69.91±0.1^{b}	70.82±1.4^{c}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$) (n=4)

Table 4.8 Moisture of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

Day	Moisture (%)					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	72.17±1.1^{a}	72.23±0.9^{a}	71.71±0.5^{a}	72.42±0.6^{b}	72.09±0.2^{a}	72.13±1.0
2	73.69±0.3^{a}	73.58±0.5^{a}	73.48±0.0^{ab}	73.63±0.5^{ab}	72.52±0.4^{a}	72.18±0.8
4	75.67±0.0^{aA}	72.94±0.0^{aBC}	72.20±0.0^{bcC}	75.39±0.0^{aA}	74.85±1.7^{aAB}	71.81±0.8^{C}
6	73.22±0.8^{a}	73.45±0.0^{a}	73.00±0.4^{c}	73.42±0.2^{ab}	73.13±0.1^{a}	71.64±2.2
8	63.87±0.0^{b}	68.13±1.8^{b}	67.00±0.2^{d}	68.77±1.9^{c}	66.48±1.6^{b}	71.28±2.0

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$) (n=4)

4.5.2.5 Hardness of treated pork chops

The hardness of aerobically and vacuum packed pork samples are shown in Table 4.9-4.10. The hardness among aerobically packed samples (Table 4.9) were significantly different ($p<0.05$) along storage. In day 0, control and samples treated with bacteriocins had higher hardness and differed from herb extract treated samples ($p<0.05$). Meanwhile in the last two day of storage, extract treated samples had higher hardness than control and samples treated with bacteriocins. Comparing among herb extract treated treatments, the hardness of all samples treated had the same trend and increased ($p<0.05$) while no difference was not found for other samples ($p>0.05$). The hardness value of vacuum packed pork are shown in Table 4.10. Significant differences were found among control and treated samples ($p<0.05$) from day 2 to the last day of storage. Among extract treatments, hardness of vacuum packed samples showed the similar trend as aerobically packed samples vacuum packed.

Table 4.9 Hardness of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

Day	Hardness (N)					
	C	B	N	E1	E2	E3
0	52.74 ± 4.15^{A}	45.21 ± 9.76^{AB}	36.84 ± 4.22^{bBC}	34.45 ± 5.79^{cC}	32.45 ± 8.66^{bC}	37.77 ± 12.11^{bBC}
2	48.80 ± 9.65^{A}	44.22 ± 4.30^{AB}	48.36 ± 8.91^{aA}	40.92 ± 7.63^{bcAB}	38.38 ± 4.40^{bAB}	37.11 ± 4.56^{bB}
4	51.36 ± 8.12^{B}	45.11 ± 8.68^{B}	45.85 ± 9.03^{aB}	64.29 ± 7.82^{aA}	39.24 ± 10.03^{bB}	45.21 ± 6.26^{abB}
6	47.07 ± 12.86^{ABC}	34.24 ± 9.39^{C}	38.13 ± 4.87^{bBC}	52.33 ± 13.36^{abAB}	58.45 ± 7.04^{aA}	35.82 ± 8.18^{bBC}
8	38.33 ± 6.77^{CD}	46.06 ± 6.87^{BC}	30.78 ± 4.09^{bD}	52.62 ± 10.39^{abB}	66.55 ± 7.39^{aA}	53.29 ± 10.36^{aB}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$) (n=20)

Table 4.10 Hardness of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

Day	Hardness(N)					
	C	B	N	E1	E2	E3
0	65.48 ± 12.34^{aA}	40.73 ± 9.31^{aB}	26.35 ± 7.54^{bBC}	63.54 ± 10.53^{aA}	37.3 ± 8.13^{bBC}	24.9 ± 3.55^{cC}
2	34.8 ± 9.82^{a}	40.8 ± 5.23^{a}	27.55 ± 5.05^{b}	27.12 ± 5.56^{b}	34.21 ± 8.71^{b}	36.16 ± 4.93^{ab}
4	76.16 ± 6.43^{aA}	36.63 ± 2.64^{abD}	42.87 ± 7.45^{aCD}	63.44 ± 13.2^{aB}	47.69 ± 7.01^{aC}	37.67 ± 4.23^{abCD}
6	72.73 ± 12.12^{aA}	31.37 ± 5.06^{bC}	37.67 ± 8.13^{abBC}	33.07 ± 8.97^{bC}	45.86 ± 3.70^{aB}	38.53 ± 9.03^{aBC}
8	45.83 ± 10.42^{bAB}	34.29 ± 4.23^{abC}	37.38 ± 7.54^{abBC}	53.68 ± 6.44^{aA}	31.17 ± 7.38^{bC}	27.2 ± 6.13^{bcC}

Upper case letters are significant difference in the same row ($p<0.05$). Lower case letters are significan difference in the same column ($p<0.05$) (n=20)

4.5.2.6 Color of treated pork chops

Color evaluation (CIE L*, a* and b*) of aerobically packed pork samples are presented in Table 4.11-4.13. The lightness (L*) of samples from all treatments were not significantly different ($p>0.05$) and similarity was found for yellowness (b*) except only samples treated with E2 extract ($p<0.05$) which differed from others. Redness (a*) of most samples tended to increase along storage except samples treated with E3 extract. When compared among treatments, sample treated with E2 extract

had more redness than other samples.Yellowness (b*) of all treatments were not significantly different ($p>0.05$) along storage. When compared among treatments, sample treated with E2 had more yellowness than other samples.

Table 4.11 Color L* value of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

	L* value					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	53.2±3.0	56.1±2.1	56.4±0.5	56.7±1.1	59.9±2.8	55.8±0.3
2	53.5±3.3	53.4±0.8	54.8±0.3	53.9±0.7	58.8±4.4	55.3±1.2
4	54.2±0.2	54.7±0.7	55.2±0.0	56.4±2.9	57.4±5.1	56.2±0.1
6	54.9±0.9	56.0±0.3	58.0±0.0	57.1±0.0	56.1±2.2	61.1±3.2
8	52.9±0.4	54.2±2.3	57.0±0.4	59.0±4.4	56.0±0.4	59.6±1.0

(n=20)

Table 4.12 Color a* value of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

	a* value					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	-2.28±^{b}	-2.03±	-2.63±^{b}	-1.98±^{b}	-2.24±^{c}	-1.65±^{ab}
2	-1.92±^{abAB}	-2.92±^{C}	-2.34±^{bC}	-1.09±^{bA}	-1.86±^{bAB}	-2.15±^{bcB}
4	-1.56±^{abB}	-1.99±^{BC}	-1.01±^{aA}	-2.18±^{aC}	-0.72±^{aA}	-1.61±^{abB}
6	-1.54±^{abC}	-1.76±^{C}	-1.30$^{\pm B}$	-1.00±^{aB}	-0.57±^{aA}	-2.61±^{cD}
8	-1.20±^{aAB}	-1.71±^{C}	-0.89±^{aAB}	-0.94±^{aAB}	-0.64±^{aA}	-1.32±^{aB}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$)

(n=20)

Table 4.13 Color b* value of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

Day	b* value					
	C	**B**	**N**	**E1**	**E2**	**E3**
0	6.65±0.3^{B}	6.81±1.6^{B}	5.77±0.3^{B}	4.87±1.2^{B}	8.40±0.3^{A}	4.58±0.6^{B}
2	6.45±0.2^{B}	6.10±0.2^{B}	6.64±0.3^{B}	6.18±1.8^{B}	10.25±4.5^{A}	4.81±1.7^{B}
4	6.63±o.9^{B}	6.05±0.5^{B}	6.58±1.6^{B}	6.97±2.6^{B}	9.48±4.5^{A}	5.30±1.2^{B}
6	6.57±0.2^{B}	6.53±0.9^{B}	6.93±0.2^{B}	7.93±2.7^{B}	8.11±0.2^{A}	8.23±4.5^{B}
8	7.48±0.9^{B}	6.54±0.0^{B}	7.13±1.1^{B}	7.78±1.2^{B}	8.21±0.9^{A}	7.78±1.2^{B}

Upper case letters are significantly different in the same row ($p<0.05$). (n=20)

Color evaluation in term of CIE L*, a* and b* of vacuum packed pork samples are presented in Table 4.14-4.16. Samples treated with E2 had higher lightness (L*) than others samples and tended to decrease ($p<0.05$) along storage while most samples were not different except samples treated with bacteriocins which slightly increased. Samples treated with E1 and E2 extracts had higher redness (a*) than samples from other treatments ($p<0.05$). Samples treated with bacteriocins, nisin and E3 extract were not different in redness while control and samples treated with E1 and E2 extracts showed increasing in redness from day 4.

The yellowness (b*) of all samples treated with antimicrobial agents was not different ($p>0.05$) along storage. Comparing among treatments only samples from day 2 and day 4 were significantly different ($p<0.05$) and no differenced observed till the end of storage. In vacuum package, myoglobin is preserved in its reduced deoxygenated form, Mb, when meat is chilled and stored without exposure to air. The color does not change during storage as long as the package is intact, and oxygen is totally excluded. However, storage time under anaerobic conditions influences the color when the meat is exposed to air. Ageing increases the ability of pork to bloom,

resulting in increased lightness, redness and yellowness (Apple et al., 2001; Zhu et al., 2001; Lindahl et al., 2005a, 2005b, 2005c).

Table 4.14 Color L* value f of vacuum packed pork chops treated with antimicrobial agents (Mean ±SD)

	L* value					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	51.4±1.3^{D}	52.7±0.7^{bCD}	56.0±0.8^{BC}	59.8±1.1^{AB}	60.7±2.8^{aA}	56.4±1.5^{BC}
2	52.8±0.5^{C}	53.9±1.1^{bBC}	56.7±2.0^{AB}	56.1±1.2^{B}	59.1±1.2^{abA}	55.9±0.4^{B}
4	55.0±1.3	54.5±0.5^{ab}	57.7±2.2	59.1±5.2	53.4±2.0^{c}	55.1±1.1
6	54.1±0.7	54.5±0.4^{ab}	56.6±1.2	58.2±1.0	56.2±1.3^{bc}	58.4±0.1
8	54.0±3.6	56.0±0.5^{a}	56.6±1.0	56.6±5.0.	55.0±3.0^{c}	57.3±0.1

Upper case letters are significantly t different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=20)

Table 4.15 Color a* value of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

	a* value					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	-2.85±1.5^{abB}	-2.99±0.4^{BC}	-2.66±0.9^{B}	-3.59±0.0^{cC}	-2.88±0.0^{cB}	-1.77±0.1^{A}
2	-2.31±1.0^{bB}	-3.01±0.9^{B}	-2.86±0.3^{AB}	-2.16±0.6^{bA}	-2.19±0.0^{bA}	-2.70±0.0^{A}
4	-2.66±0.0^{aB}	-2.78±0.5^{B}	-2.34±0.3^{B}	-0.97±0.4^{aA}	-1.17±0.0^{aA}	-2.71±0.3^{B}
6	-2.78±0.0^{abC}	-2.64±1.2^{C}	-2.70±0.4^{C}	-0.99±1.3^{aAB}	-0.37±1.1^{aA}	-1.96±0.2^{BC}
8	-2.52±0.5^{aBC}	-2.78±0.8^{C}	-2.44±0.4^{BC}	-0.25±1.0^{aA}	-0.49±0.4^{aAB}	-2.53±0.6^{BC}

Upper case letters are significantly t different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=20)

Table 4.16 Color b* value of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

	b* value					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	6.33±0.3	6.02±0.1	6.66±0.8	6.25±3.4	10.36±4.1	4.67±1.1
2	6.53 ± 0.1^{AB}	6.61 ± 0.4^{AB}	7.35 ± 0.2^{A}	6.35 ± 1.4^{AB}	10.26 ± 3.1^{A}	4.62 ± 0.5^{B}
4	6.89 ± 0.0^{ABC}	6.23 ± 1.0^{C}	7.30 ± 1.5^{AB}	7.80 ± 0.0^{A}	6.74 ± 1.0^{BC}	5.43 ± 0.7^{C}
6	7.51±0.1	6.72±1.0	7.01±1.1	8.05±0.8	8.05±0.3	6.23±1.1
8	6.54±0.5	7.45±1.3	7.19±0.7	8.58±0.3	7.56±1.7	6.46±2.0

Upper case letters are significantly different in the same row ($p<0.05$) (n=20)

4.5.2.7 Purge loss of treated pork chops

Purge loss of pork samples packed in both packed conditions (Table 4.17-4.18) was similar ($p<0.05$) except for samples treated E3 extract in vacuum packed condition. There were some differences among aerobically packed samples of all treatments in day 0 and 8 and vacuum packed ones in day 0 and day 4 and similar tend of percentage of purge loss was observed. There is some processing factors concern about purge loss. Some of these factors include storage time, physical disruption of the product and storage condition. In general little drip loss occurs in pre-rigor meat. At later times, after slaughtering, the muscle has little drip loss in pre-rigor meat. However, at later times, after the muscle has gone into rigor, the drip losses tend to increase (Jolley et al., 1980; 1981). The importance of lowering the carcass temperature as quickly as possible has been emphasized. However, it is also important to maintain the temperature of fresh meat as low as possible (without freezing) to maintain water-holding capacity. For example, increasing the storage temperature from 0 to 4°C can cause a significant increase in drip loss (Sayre et al., 1964)

Table 4.17 Purge loss value of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

	Purge loss (%)					
day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	2.91±0.13^{C}	4.83±0.01^{AB}	4.20±0.28^{B}	4.41±0.85^{B}	5.54±0.16^{A}	5.58±0.27^{A}
2	4.53±0.75	5.30±1.19	3.94±0.49	5.48±0.05	5.69±1.33	7.20±0.05
4	4.66±1.69	5.23±1.57	5.17±1.13	6.76±1.64	5.47±1.40	7.46±0.44
6	4.85±0.31	4.18±0.60	4.19±1.05	6.12±1.87	6.81±0.03	5.84±0.10
8	4.82±0.45^{B}	4.55±0.98^{B}	6.36±0.55^{AB}	6.31±0.16^{AB}	7.77±1.23^{A}	7.42±1.11^{A}

Upper case letters are significantly different in the same row ($p<0.05$) (n=4)

Table 4.18 Purge loss value of vacuum packed pork chops treated with antimicrobial agents (Mean±SD)

	Purge loss (%)					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	2.47±0.80^{B}	2.67±0.24^{B}	5.10±0.47^{A}	4.75±0.18^{A}	5.60±0.59^{A}	5.20±0.12^{cA}
2	3.25±1.09	5.10±2.49	4.20±0.99	6.99±2.05	6.22±1.61	6.04±0.46^{bc}
4	3.23±0.11^{D}	4.16±0.62^{CD}	5.59±0.32^{BC}	7.36±0.70^{AB}	4.52±1.86^{CD}	8.12±0.44^{aA}
6	5.83±0.18	4.32±0.07	5.37±0.51	7.41±0.86	6.69±1.77	6.67±0.36^{b}
8	5.24±1.75	5.33±1.28	4.00±0.41	8.70±0.41	7.46±2.41	6.26±0.36^{b}

Note; Upper case letters are significant difference in the same row ($p<0.05$) (n=4)

4.5.2.8 Cooking yield of treated pork

For cooking yield (Table 4.19-4.20) both aerobically and vacuum packed there were no significant differences ($p>0.05$) between all samples. For aerobically packed only samples treated bacteriocins and E2 had slightly significant difference ($p<0.05$) in the initial storage. Meanwhile for vacuum packed there had only samples treated extracts had slightly difference ($p<0.05$) in the initial storage.

Table 4.19 Cooking yield of aerobically packed pork chops treated with antimicrobial agents (Mean±SD)

	Cooking yield (%)					
Day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	42.13±1.57	44.02±2.70^{a}	46.01±6.12	41.21±3.20	45.23±3.13^{a}	43.80±6.66
2	31.62±2.53	33.10±1.05^{b}	40.30±7.22	33.96±0.91	31.27±1.59^{b}	32.84±1.96
4	37.08±8.38	36.66±3.18^{b}	35.78±5.12	36.43±4.61	36.32±5.77^{b}	36.62±4.82
6	29.02±1.84	33.63±2.96^{b}	32.60±4.29	34.28±4.25	30.47±0.61^{b}	34.11±2.48
8	31.69±1.99	31.55±2.93^{b}	32.62±2.86	32.59±0.86	30.33±0.62^{b}	31.42±4.95

Lower case letters are significantly different in the same column ($p<0.05$) (n=4)

Table 4.20 Cooking yield vacuum packed of pork chops treated with antimicrobial agents (Mean±SD)

	Cooking yield (%)					
day	**C**	**B**	**N**	**E1**	**E2**	**E3**
0	35.19±1.48	40.03±9.80	42.48±0.74	46.14±3.88^{a}	51.50±3.22^{a}	44.42±2.98^{a}
2	25.68±0.34	33.46±4.12	32.71±3.07	34.09±4.71^{b}	30.52±3.47^{b}	34.13±3.50^{b}
4	32.60±5.41	32.78±2.55	33.57±6.78	34.92±6.61^{b}	31.28±7.89^{b}	25.89±1.61^{c}
6	28.50±2.67	31.06±5.70	32.69±3.45	28.71±1.45^{b}	30.46±6.09^{b}	29.31±4.11^{bc}
8	28.07±5.14	31.59±5.78	33.15±0.65	28.46±4.49^{b}	31.20±2.98^{b}	30.16±2.36^{bc}

Lower case letters are significantly different in the same column ($p<0.05$) (n=4)

4.5.2.9 Sensory evaluation of treated pork chops

4.5.2.9.1 Aerobically packed raw pork. Sensory assessment of aerobically packed raw pork samples are presented in Table 4.21.

Color: The color of pork treated with three herb extracts, aerobically packed in PE bag showed no significant difference from control, nisin and crude bacteriocins treated samples ($p>0.05$).

Flavor: About 4 days of storage, the flavor of all treated samples changed from raw pork flavor to sour flavor and increasing was observed as storage time progressed.

Texture: There was no significant impact on meat texture ($p<0.05$) of all treated pork samples during storage except for sample treated with mangosteen extract was found to soften than other treatments ($p<0.05$) at day 2 of storage.

Overall acceptance: The overall acceptance of all samples was acceptable in the day 0 and found to slightly unacceptable in day 8 different ($p<0.05$). However, it was found that acceptance of all samples treated with three selected extracts was found to be lower than those samples from other treatments including control one in day 2.

Table 4.21 Sensory evaluation of aerobically packed raw pork chops treated with antimicrobial agents.

Sensory attribute	day	Score					
		C	B	N	E1	E2	E3
Color	0	4.6	3.8	4.0	3.1	3.3	4.1
	2	4.0	2.9	3.4	2.9	3.0	3.6
	4	3.9	3.8	3.4	3.5	2.4	3.6
	6	2.6	3.0	3.3	3.7	2.9	2.4
	8	3.3	3.4	2.4	2.7	2.8	3.1
Flavor	0	4.9^{a}	4.9	4.9^{a}	4.7	4.5^{a}	4.6^{a}
	2	4.8^{a}	4.4	4.6^{a}	4.4	4.3^{a}	4.4^{ab}
	4	3.6^{b}	3.8	3.8^{a}	3.8	3.8^{ab}	4.1^{b}
	6	3.8^{b}	4.1	4.1^{a}	3.9	3.7^{ab}	4.0^{b}
	8	3.1^{b}	2.5	3.1^{b}	2.7	3.2^{b}	3.0^{c}
Texture	0	4.3	3.7	3.6	3.6	3.2	3.8
	2	4.4^{A}	4.3^{BC}	3.9^{AB}	4.0^{ABC}	3.1^{D}	3.6^{C}
	4	4.4	3.7	3.8	3.8	3.3	3.9
	6	3.9	3.1	4.1	3.0	3.3	3.7
	8	3.6	3.8	3.8	3.9	3.9	3.3
Overall acceptance	0	4.9^{a}	4.8	4.8^{a}	4.4	4.1	4.3^{a}
	2	4.9^{aA}	4.7^{B}	4.6^{aAB}	4.3^{C}	4.2^{C}	4.2^{aC}
	4	3.6^{b}	3.7	3.6^{ab}	3.7	3.3	3.7^{a}
	6	3.5^{b}	3.4	3.8^{ab}	3.6	3.4	3.6^{a}
	8	2.8^{b}	2.2	2.9^{b}	2.3	2.7	2.6^{b}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=16)

4.5.2.9.2 Aerobically packed cooked pork. Sensory assessment of aerobically packed cooked pork samples are presented in Table 4.22.

Color**:** All samples showed no significance in color attribute ($p<0.05$) in the day 0 with dull reddish pink (of 3.8-4.4 score), indicating no effect of concentrations of herb extracts, crude bacteriocins and nicin used on this parameter. After eight days of storage, the color of all samples was dark (of 2.7-4.2 score). The sample treated with mangosteen extract was found to be dark compared with meats from other treatments ($p<0.05$).

Flavor and Texture: The flavor and texture of all samples after storage for eight days had no significant different ($p<0.05$) compared with those samples in attributes in day 0. The flavor of cooked pork of all treated (samples) was not changed according to storage time with the score of, 3.8-4.6 for day 0 and, 3.9-4.2 for day 8.

Table 4.22 Sensory evaluation of aerobically packed cooked pork chops treated with antimicrobial agents.

Sensory attribute	day	Score					
		C	**B**	**N**	**E1**	**E2**	**E3**
Color	0	4.1	4.2	4.4	3.8	3.8	4.4
	2	3.6	4.5	3.8	3.9	3.5	3.9
	4	3.6^{A}	3.9^{A}	4.2^{A}	2.8^{B}	2.8^{B}	4.2^{A}
	6	3.5	4.1	4.3	2.6	2.9	3.0
	8	3.2^{B}	3.9^{A}	4.0^{A}	3.4^{B}	2.7^{C}	3.4^{B}
Flavor	0	4.4	4.4	4.3	4.6	3.8	4.3
	2	4.4	4.3	4.3	4.2	4.0	4.4
	4	4.4	4.4	4.4	4.3	4.1	4.4
	6	4.5	4.4	4.6	4.1	4.6	4.4
	8	4.2	4.4	4.2	3.9	3.9	4.2
Texture	0	3.8	3.9	3.4	3.2	3.3	3.9
	2	2.1	3.2	3.8	3.5	2.9	3.8
	4	2.3	3.2	3.6	2.5	2.6	3.3
	6	2.4	3.8	2.9	2.4	2.4	2.5
	8	2.4	3.3	2.8	2.6	2.4	2.8
Overall acceptance	0	4.8	4.9	4.9	4.5^{a}	4.0	4.7
	2	4.2	4.8	4.9	4.5^{a}	4.0	4.7
	4	4.4	4.6	4.8	4.5^{a}	3.6	4.7
	6	4.0	4.4	4.4	3.4^{b}	3.8	3.9
	8	3.8	4.2	4.0	3.6^{b}	3.1	3.9

Upper case letters are significantly different in the same row ($p<0.05$) (n=8)

Overall acceptance: The overall acceptance of all samples decreased from acceptable and slightly accept (4.0-4.9) to neither nor like and slightly dislike (2.2-2.9) with no significant difference between all treated.

4.5.2.9.3 Vacuum packed raw pork. Sensory assessment of vacuum packed raw pork samples are presented in Table 4.23.

Color: Color attribute of all samples except the one treated with crude bacteriocins had no significant difference ($p<0.05$) throughout eight days storage time. However, E2 treated sample was darker than others. Brownish red was observed in day 0 and moderately brownish red was observed in day 8.

Flavor: Panelists could detect the difference of flavor between samples among all treatments in the beginning of storage. All meat samples treated with three selected herb extracts had slightly sour flavor which was significantly differ from control, B and N treated ones ($p<0.05$). However, different flavor was observed among samples from all treatments during day 2 to day 8 of storage.

Texture: Herb extracts, B and N treatments showed no significant impact on texture ($p<0.05$) of meat samples compared with control throughout storage time.

Overall acceptance: After eight days storage, there were no significant different ($p<0.05$) in overall acceptance of among from all treatments, although sample treated with mangosteen skin extract had lower acceptance score than others.

Table 4.23 Sensory evaluation of vacuum packed raw pork chops treated with antimicrobial agents.

Sensory attribute	day	Score					
		C	**B**	**N**	**E1**	**E2**	**E3**
Color	0	4.1	4.1^{a}	3.1	3.3	2.8	3.7
	2	3.6	3.2^{b}	2.9	3.7	2.8	3.0
	4	2.9	2.8^{bc}	2.9	3.0	2.4	3.1
	6	2.4	2.5^{c}	2.9	2.9	2.8	2.8
	8	2.8	3.0^{b}	2.25	3.3	2.0	2.8
Flavor	0	4.8^{A}	4.9^{A}	4.9^{aA}	4.4^{CD}	4.1^{D}	4.6^{BC}
	2	4.6	4.6	4.6^{ab}	4.1	4.3	4.4
	6	4.0	4.3	3.8^{c}	3.9	3.8	4.1
	8	3.8	3.8	3.6^{c}	3.6	3.0	3.6
Texture	0	4.6	3.5	3.6	3.5	3.7	4.3
	2	4.2	4.4	3.6	3.8	3.7	4.6
	4	3.6	3.6	4.1	4.8	3.3	3.9
	6	4.5	3.1	3.4	2.9	4.3	3.1
	8	3.8	3.4	3.3	3.4	3.1	3.8
Overall acceptance	0	4.9	4.8	4.6^{a}	4.0	4.1	4.6^{a}
	2	4.6^{A}	4.4^{AB}	4.1^{abC}	4.2^{B}	4.2^{B}	4.3^{abB}
	4	3.7	4.0	3.5^{bc}	3.8	3.6	4.0^{ab}
	6	3.8	3.9	3.6^{bc}	3.6	3.6	3.8^{bc}
	8	3.5	3.8	3.1^{c}	3.4	2.6	3.3^{c}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=16)

4.5.2.9.4 Vacuum packed cooked pork. Sensory assessment of vacuum packed cooked pork samples are presented in Table 4.24.

Color: Throughout storage time, the color of cooked pork among treatment had no significant difference ($p<0.05$). The color score was between 2.4-3.6, indicating medium brown grey to dark grey.

Flavor: In general the flavor of cooked pork of all treatment had no significant difference ($p<0.05$) throughout storage time.

Texture: Significant difference in texture of meat samples among all treatments was not observed ($p<0.05$) throughout storage time except for samples treated crude bacteriocins.

Overall acceptance: There were no significant different ($p<0.05$) in

overall acceptance among samples from all treatments throughout storage time, demonstrating that all treatment did not affect this attribute of the pork samples kept in vacuum pack condition. However, storage time of the meat samples within each treatment was found to affected the overall acceptance ($p<0.05$) when kept in vacuum condition. In general, the acceptant score of meat samples from all treatments were in the good range of neither like nor like to slightly accepted when stored up to eight days.

Table 4.24 Sensory evaluation of vacuum packed cooked pork chops treated with antimicrobial agents.

Sensory attribute	day	Score					
		C	B	N	E1	E2	E3
Color	0	4.6^{a}	4.4	4.4	3.5	3.9	4.1
	2	4.3^{ab}	4.3	4.6	3.2	3.8	4.0
	4	3.7^{bc}	4.2	3.7	3.2	2.9	3.5
	6	3.2^{c}	4.1	4.1	3.6	3.3	3.3
	8	3.6^{bc}	3.4	3.4	2.6	2.4	3.6
Flavor	0	4.3	4.5	4.4	4.5	4.3	4.6
	2	4.3^{ABC}	4.5^{A}	4.4^{AB}	4.4^{AB}	3.9^{C}	4.1^{BC}
	4	4.3	4.4	4.4	4.4	4.1	4.4
	6	4.6	4.6	4.6	4.3	4.3	4.4
	8	3.9	4.3	4.3	4.0	4.0	4.3
Texture	0	3.4	3.6^{a}	3.8	3.1	3.6	3.1
	2	3.6	3.4^{ab}	3.4	2.4	3.3	3.8
	4	3.4	3.2^{ab}	2.7	2.5	3.3	3.5
	6	2.3	2.7^{bc}	2.9	3.1	3.1	2.7
	8	2.9	2.2^{c}	2.6	2.2	2.5	3.3
Overall acceptance	0	4.6^{a}	4.9^{a}	4.8^{a}	4.8^{a}	4.4^{a}	4.8^{a}
	2	4.7^{a}	4.8^{ab}	4.6^{a}	4.2^{a}	3.7^{b}	4.4^{a}
	4	4.4^{a}	4.6^{ab}	4.3^{a}	4.1^{a}	3.6^{b}	4.6^{a}
	6	3.8^{b}	4.4^{b}	4.3^{a}	4.1^{a}	3.6^{b}	3.9^{b}
	8	3.7^{b}	3.8^{c}	3.9^{b}	3.1^{b}	2.9^{c}	3.8^{b}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=16)

4.6 Conclusions

The data presented in this study showed that the extract of *Garcinia mangostana* Linn (E2) was better on the proliferation of total plate count and total coliforms than

Spondias pinnata (L.f.) Kurz, K (E1) and *Schinus terebinthifolius* (E3) which showed no differences as freeze dried crude bacteriocins (B), nisin (N) and control (C). Almost of treated samples were quite the same tended in result of physicochemical evaluation both aerobic and vacuum packed such as there were no differences in pH value which affected to % purge loss,% cooking yield, lightness and overall acceptance in sensory evaluation. The sensory assessment with overall acceptant of trained panelists could not show the difference response between treated treatments in both aerobic and vacuum packed. For further study the synthesis of purified compounds from three Thai herbs will be the benefit to use in meat and meat products.

4.7 References

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CHAPTER V

EFFECTS OF SELECTED HERB EXTRACTS IN COMBINATION WITH HIGH PRESSURE TREATMENT ON MICROBIAL AND PHYSICOCHEMICAL QUALITIES OF FRESH PORK

5.1 Abstract

This study evaluated the impact of high pressure processing (HHP at 200 and 300 MPa) and in combination with bacteriocins (B) and Thai herb extracts; hog plum (*Spondias pinnata* (L.f.) Kurz) (E1) and Brazillian pepper tree (*Schinus terebinthifolius*) (E3) on microbial, physicochemical quality and shelf life of aerobically and vacuum packed pork loins, stored at 4°C. Microbial contents and pH, Aw, weight loss, hardness and color of treated pork samples were analyzed during storage at 4°C for 9 days. At 200 MPa pressure and aerobic packing condition, total plate counts (TPCs) among samples from all treatments did not differ ($p>0.05$) throughout storage time although HHP+E3 sample had lower counts than those from other treatments. However, in day 6 HHP+E1 sample had higher TPC ($p<0.05$) than those from other treatments. At 300 MPa, TPCs of pork samples from all treatments were different ($p<0.05$) during day 0 to day 3 but not HHP+B sample ($p>0.05$). In day 6 TPCs of samples among all treatments did not differ. However, TPCs of samples

among treatments were different ($p<0.05$) in day 9 while HHP+E3 sample had lower counts than those from other but not HHP+B sample.

For vacuum packing condition and at 200 MPa, TPCs of samples from all treatments in day 0, 6 and 9 there were no different. However, different count of HHP sample from others was found in day 3. In addition, TPC of HHP+E1 sample was higher ($p<0.05$) than those of HHP+ E3 and HHP+ B. At 300 MPa pressure, sample from all treatments had TPC lower than 10 cfu/cm^2. Increasing in pressure for HHP had no impact on pH, Aw and weight loss (%) of pork samples of all treatment. In aerobic packing condition, all pork samples became more tender while the hardness values of HHP+B and HHP+E3 samples at 300 MPa slightly increased with storage time. Lightness (L*) of pork samples increased with an increase of the pressure applied. Redness (a*) of pork samples decreased while their yellowness (b*) was not affected. In general, the HHP+B treatment gave lighter color of samples than that other treatments. In vacuum packed condition, L* and a* value of all treated samples increased while b* value slightly decreased and then constant. Application of HHP and HHP+B gave lighter color than HHP+E1 and HHP+E3 treatment. Pressurization of pork at 200 MPa, the HHP and HHP+B treated samples had a* value significantly lower ($p<0.05$) than HHP+E1 and HHP+E3 treated ones throughout storage time. At 300 MPa pressure, a* values of all treated samples remained almost constant but slightly higher than those treated at 200 MPa. Pork samples from all treatments became tender ($p<0.05$) as storage time progressed each day. In addition all tested pork samples pressurized at both pressure at 10°C and packed in both aerobic and vacuum conditions, contained lactic acid bacteria and *Enterobacteriaceae* below 10 cfu/cm^2.

5.2 Introduction

High hydrostatic pressure (HHP) is a non-thermal technology consisting of submitting the foods to pressures above 100 MPa (Cruz et al., 2010). This technology preserves the quality without significant alterations of the food matrix, with the advantage of efficiently eliminating microorganisms, providing microbiological safety and increased shelf life (Mathias et al., 2010). Adverse effects of high pressure are alterations in color and texture of food, due to structural changes in macromolecules such as proteins. The covalent protein bonds are little affected by high pressure, but hydrophobic and electrostatic bonds can be affected, causing significant conformational changes, and affecting functionality, and frequently irreversibly, depending on the nature of the protein and the pressure applied (Lamballerie-Anton, Taylor, and Culioli, 2002).

The process is isostatic, i.e., the pressure is transmitted uniformly, instantly, and adiabatic, which means that no matter the food shape or size. There is little variation in temperature with increasing pressure (the temperature increases approximately 3ºC per 100 MPa, depending on the composition of the food (Smelt, 1998; Wilson et al., 2008). Pressure processing is usually carried out in a steel cylinder containing a liquid pressure-transmitting medium such as water, with the sample being protected from direct contact by using sealed flexible packaging. Maintaining the sample under pressure for an extended period of time does not require any additional energy apart from that required to maintain the chosen temperature (Cheftel and Culioli, 1997).

HHP processing is carried out with intense pressure in the range of 100-1000 MPa, with or without heat, allowing most foods to be preserved with minimal effect on taste, texture or nutritional characteristics. The main advantage of HHP processing,

compared with thermal sterilization and pasteurization, is maintenance of sensory and nutritional characteristic of treated food products (Balasubramaniam et al., 2008). During the last two decades, HHP technology has achieved a greater industrial application in comparison to others non-thermal preservation technologies. HHP technology has been successfully applied for processing of cured meat products - cooked or dried- and cooked ready to-eat meats (Campus, 2010). However, it has been reported that HHP can impact structural, physiochemical, morphological and textural characteristics of the meat, and can cause partial discolouration of fresh red meat (Cheftel, 1995; Kim et al, 2007).

Application of combined hurdles together with HHP has been proposed to increase the microbial effect of low pressure processes in order to minimize the unwanted changes induced by ultra-high hydrostatic pressures (above 400 MPa) in meat and meat products. Synergistic effects with HHP have been described with antimicrobials, low pH, carbon dioxide, vacuum packaging and chilled storage (Garriga and Aymerich, 2009). Moreover, additional hurdles or processes are useful to avoid the recovery of sub lethal injured cells (Jofré et al., 2010; Liu et al., 2012). The ability of bacteria to survive HHP increases when they are treated in nutritionally rich media such as meat or meat products, which contain carbohydrates, proteins and fat. Therefore, experimentation with real food matrices is strongly recommended (Garriga et al., 2004). To further increase the shelf life and safety of pressurized products, HHP has been investigated in combination with other technologies such as bacteriocins or other natural antimicrobials (Rastogi et al., 2007). Therefore, the objectives of this study were to investigate the effectiveness of HHP in combination with herb extract and bacteriocins on inhibition growth of normal bacterial flora on fresh pork and to

evaluate physicochemical properties of treated pork packed under aerobically and vacuum conditions and stored at 4^{o} C.

5.3 Material and methods

5.3.1 Preparation of herb extracts and bacteriocins

Two Thai herbs; hog plum or ma gok paa (*Spondias pinnata* (L.f.) Kurz) (E1) and Brazillian pepper tree or ma toom khaek (*Schinus terebinthifolius)* (E3) were screened and selected from 15 local Thai herbs according to their ability to inhibit growth of indicator bacteria and predominant bacteria obtained from fresh pork samples. The herbs were freeze-dried, extracted using ethanol, and the herb extract was freeze-dried and resolubilized in 1% DMSO for using. Extract solution of 1% w/v in 1% DMSO was shaken at 150 rpm, 20 min, 40ºC, centrifuged at 9000 rpm, at 4ºC for 15 min then filtered through a Whatman No 1 filter paper.The filtrate was again filtered through a sterile cellulose acetate filter (0.45 micron) and the final filtrate was kept at 4ºC before use. Freeze-dried powder of crude bacteriocins (B) was prepared from the fermentate of *Lactococcus lactis* TISTR 1401 according the method of Intarapichet et al (2008) as described in item 4.3.2 in Chapter IV.

5.3.2 Sample preparation and high pressure processing

Fresh postmortem pork loins (*Longissimus dorsi*) were obtained from Max Rubner-Institut, Kulmbach, Germany. Visible fat was trimmed off and the loin was transversally cut into slices of about 2.54 cm thick per slice. The pork slice was randomly dipped in 0.2% crude bacteriocins or 1.0% of each extract solution, then aerobically and vacuum packaged in plastic bag (PA/PE: O_2 30 cm^3 per m^2 and CO_2 150 cm^3) and kept in a freezing room. The sample package was shrunken in hot water

just before being pressurized at 200 or 300 MPa at 10ºC for 10 min. The HHP treatment was carried out in an industrial hydrostatic pressurization unit (EPSI N.V. Belgium). The HHP conditions were set as following; meat temp=0ºC, temperature medium=5ºC-10ºC, pressure medium: H_2O:Glycol,50:50 and finish temp=10ºC. The treated pork samples were stored at 4±1ºC for 9 days and sampled for aerobic plate counts, weight loss, tenderness, pH, Aw and color measurement every 3 days.

5.3.3 Microbial enumeration

At each selected sampling time, microbial enumeration was performed using swabbing method with a sterilized template with known area. The cotton swab was placed in a sterile plastic bag containing 1ml of sterile 0.85% NaCl solution, shaken and serial dilutions were made with 9 mL of 0.85% NaCl solution. After homogenization for 90 s in a stomacher dilutions were made to determine the microbial content. Microbial enumeration was made by pour plate technique, using plate count agar (PCA, Biokar) and incubated at 30ºC for 72 h. For anaerobic counts, Anaerocult A (Merck, Germany) was used, the plates were incubated in anaerobic jars at 30°C for 72 h. Lactic acid bacteria (LAB) were grown on de Man Rogosa Sharp (MRS) agar (Biokar,pH=5.7) at 30°C for 72 h. Double-layered violet red bile glucose (VRBG) agar (Biokar) was used to enumerate *Enterobacteriaceae* after incubation at 37°C for 24 h. The plates were prepared in duplicate for each dilution. The microbial count was expressed in log cfu/cm^2. Microbial enumeration was performed every 3 days during storage (9 day).

5.3.4 Physicochemical quality

5.3.4.1 Percent weight loss was determined

Percentage of weight loss was determined by calculating weight

difference of the pork sample before and after cooking to an internal temperature of 72^{o}C.

5.3.4.2 Color (L*, a*, b*)

Color of sample was measured in terms of CIE L*, a*, b* values using Minolta Chromameter CR 300 (Konica Minolta, Munich, Germany).

5.3.4.3 Texture

Texture of the sample was measured for Hardness by determining the Warner-Bratzler shear force using a texture analyzer (3369 Instron, Pfungstadt, Germany).

5.3.4.4 pH values

The pH value were determined by Knick Portamess 912 pH; Electrode SE 104 C (Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany). The pH meter was calibrated before and during the readings using standard phosphate buffers (pH value of calibration buffers was 7.02 and 4.00 at 20ºC) and adjusted to the expected temperature of measured muscles).

5.3.4.5 Water activity

Water activity was measured using the following device: Type SE aw Lab (company: SE-Schulz Electronic, Höhenkirchen, Germany).

5.3.4.6 Statistical analysis

Statistical analysis was evaluated by randomized complete block design. Analysis of variance was analyzed and comparison of means was done by Duncan's Multiple Range Test. Significant difference was defined at $p<0.05$. Two replications of the experiment were performed

5.4 Results and discussion

5.4.1 Microbial counts of aerobically packed pork

It was found that strength of pressure input in the treatment had an effect on microbial reduction of the pork samples as shown in Figure 5.1. Throughout the storage period, all pork samples pressurized at 300 MPa, with and without crude bacteriocins and herb extracts (HHP+B, HHP+E1, HHP+E3 and HHP) had the lower total plate counts (TPCs) compared with those treated at 200 MPa. Four of control samples were prepared and used for each treatment, i.e., fresh meat dipped either in crude bacteriocins, hog plum extract (E1), Brazillian pepper leave extract (E3) or in 1% DMSO for microbial comparision. It was found that throughout storage period all samples without HHP treatments contained higher numbers of TPCs compared with those HHP treated samples and those from treated in combination with HHP. It was obvious that at the last day of storage (day 9) the TPCs of all control samples treated with B, E1 and E3 alone were 2-fold higher than the samples treated and with HHP at 200 MPa and 4-fold higher that those ones treated and with HHP at 300 MPa. The TPCs of pork sample from each group were ~3.6-4.3 log cfu/cm^2, ~1.8-2.2 log cfu/cm^2 and ~0.3-1.3 log cfu/cm^2, respectively.

The TPCs of meat samples from all treatments gradually increased according to an increasing of storage time.

Comparing among pork samples pressurized at 200 MPa, higher microbial reduction was found for those treated with bacteriocins combined with HHP (HHP+B 200MPa) and matoom khaek extract combined with HHP (HHP+E3 200 MPa) and (pressurized pork samples (HHP 200 MPa) compared with those treated with crude bacteriocins and both herb extracts combined with HHP (HHP+E1 200 MPa, and

HHP+E3 200MPa)

At the pressure of 300 MPa, TPCs of all samples treated with HHP and without HHP treatment combination were similar during day 3 to day 9 of storage although lower TPC was found for HHP+B sample at day 0. Pressure at 300 MPa gave better microbial reduction compared with pressure at 200 MPa for all combination treatments when treated samples were kept throughout storage time. When compared TPCs of the samples within each pressure applied, pressurization at 200 MPa TPCs of the samples from all treatments were not significantly different ($p>0.05$) throughout 9 days of storage although HHP+E3 had lower counts than other treatments except in day 6 and HHP+E1 had higher total counts ($p<0.05$) than other treatments. When pressurized at 300 Mpa, TPCs among samples from all treatments in day 0 to day 3 were found to be significant differences ($p<0.05$). However, only TPCs of samples from the treatment only treatment of HHP+B was not significant difference ($p>0.05$). In day 6, there was no significant difference of TPCs between samples while in day 9 significant difference ($P<0.05$) was found between samples and HHP+E3 sample had lower counts than others.

5.4.2 Physicochemical quality of aerobically packed pork

Water activity (Aw), pH and weight loss (%) of pressurized sliced pork loin in aerobically packed during 9 days of storage at 4 °C are shown in Table 5.1-5.3. It was observed that no significant differences were found among all treatments ($p>0.05$). Normally, pH of meat muscle decreases from 7.0 upon slaughter to approximately 5.3-5.8. In extreme cases, this decline can take only 1 h. The typical decline for pork is in the range 6-12 h, and beef usually completes its pH decline in 18-40 h (Smulders et al, 1992).

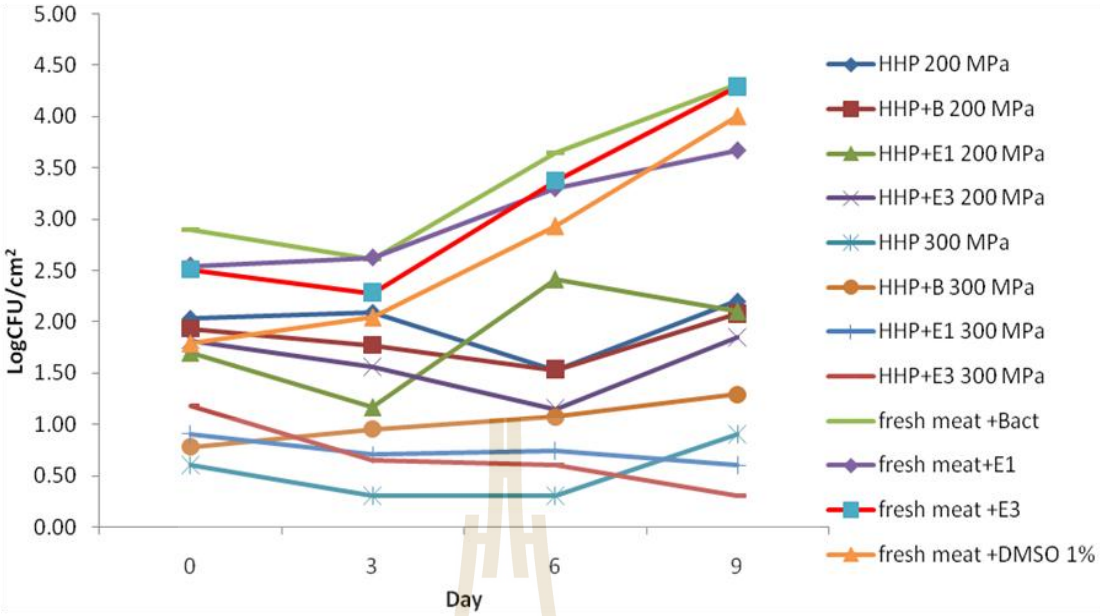

Figure 5.1 Total plate count of pork chops aerobically packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), crude bacteriocins (B) and control samples (n= 4).

During storage, all pork samples became more tender as the hardness values decreased ($p<0.05$) for both HHP conditions and all combination treatments while the hardness values of HHP+B and HHP+E2 samples at 300 MPa slightly increased with storage time as shown in Table 5.4. This was in agreement with the results of Iwasaki et al, (2006) who confirmed that moderate pressure at 100 to 300 MPa caused denaturation of protein (swelling, aggregation, gelation), resulting in an increase in hardness of the whole meat. In general, low pressure below 200 MPa can tenderize pre-rigor meat, whereas tenderization of post-rigor meat with HPP can only be achieved by higher temperatures (Sun and Holley, 2010).

Table 5.1 Water activity of aerobically packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD) (n=4)

Pressure	Day	Aw			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	97.8±0.3	96.8±0.3	96.7±0.6	96.9±0.6
	3	96.9±0.4	97.1±0.1	97.2±0.4	97.1±0.2
	6	96.7±0.4	96.8±0.6	96.8±0.2	96.5±0.9
	9	97.7±0.4	96.6±0.2	96.8±0.2	96.9±0.2
300 MPa	0	96.6±0.8	97.2±0.4	97.3±0.1	97.0±0.0
	3	96.6±0.3	96.7±0.2	97.2±1.0	97.1±0.1
	6	97.0±0.1	96.5±0.6	96.9±0.3	97.0±0.5
	9	97.0±0.3	97.1±0.3	97.0±0.9	97.5±0.4

Table 5.2 pH of aerobically packed pork chops treated by HHP (200 and 300 MPa) and in combination with extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD) (n=6)

Pressure	Day of storage	pH			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	5.8±0.2	5.7±0.2	5.7±0.1	5.6±0.2
	3	5.7±0.1	5.7±0.1	5.7±0.2	5.7±0.1
	6	5.8±0.4	5.8±0.2	5.7±0.1	5.8±0.2
	9	5.9±0.4	5.7±0.3	5.7±0.1	5.9±0.3
300 MPa	0	5.7±0.2	5.8±0.1	5.8±0.1	5.8±0.1
	3	5.8±0.2	5.8±0.1	5.8±0.1	5.9±0.2
	6	5.8±0.1	5.8±0.2	5.8±0.1	5.8±0.1
	9	5.8±0.1	5.8±0.2	6.0±0.3	5.9±0.2

Table 5.3 Weight Loss (%) of aerobically packed pork chops treated by HHP (200 and 300 MPa) and in combination with extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD) (n=4)

Pressure	Day of storage	Weight loss (%)			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	30.0±1.4	31.1±1.7	28.4±4.2	29.7±1.7
	3	27.9±0.4	30.1±2.1	29.7±1.6	29.8±2.8
	6	29.7±0.1	29.4±4.0	25.3±2.3	25.7±3.3
	9	23.0±1.8	25.3±0.5	26.8±4.4	24.2±4.0
300 MPa	0	29.1±1.41	31.8±4.8	26.5±4.5	24.1±9.7
	3	27.7±4.7	26.5±1.1	27.2±7.4	27.2±1.0
	6	29.9±0.8	29.5±5.7	27.1±4.4	27.0±1.5
	9	28.5±0.9	28.0±0.6	25.4±0.4	25.8±0.8

The influence of HHP on the meat tenderness is depending on the rigor stage, pressure and temperature level applied, and their combination (Sun and Holley, 2010). Meat tenderization by HHP is likely caused by lysosome breakdown and subsequent proteolytic activity release to the medium (Hugas, Garriga, and Monfort, 2002). Generally at lower pressure levels, (<150 MPa) binding strength and texture parameters are improved (Macfarlane et al., 1984; Crehan et al., 2000). However, it has also been shown that as pressure level increases some textural properties are decreased (Yuste et al., 1999; Crehan et al., 2000; Mor-Mur and Yuste, 2003). Cheftel and Culioli (1997) believed that textural changes were result of high pressure induced disruption of protein functionality, particularly myosin heavy chains, ultimately causing textural changes. Lamballerie-Anton et al. (2002) argued that increased tenderness post-rigor meat was not caused by high pressure but is a result of the increased lysosomal enzyme activity caused by high pressure.

Table 5.4 Hardness (N) of aerobically packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (mean±SD) (n=20)

Pressure	Day of storage	Hardness (N)			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	51.0 ± 6.2^{aA}	49.44 ± 4.0^{aA}	37.6 ± 1.6^{aB}	44.8 ± 2.4^{aC}
	3	35.4 ± 2.7^{cB}	26.8 ± 2.0^{dA}	38.4 ± 3.9^{aA}	38.6 ± 3.8^{bA}
	6	39.9 ± 4.3^{bA}	33.0 ± 5.6^{cB}	$37.4.2\pm2.8^{aAB}$	35.4 ± 1.8^{bB}
	9	36.2 ± 3.2^{cBC}	41.0 ± 5.3^{bA}	33.5 ± 5.2^{bC}	37.4 ± 3.8^{bB}
300 MPa	0	53.7 ± 3.5^{aA}	32.3 ± 2.9^{B}	46.9 ± 3.3^{aB}	37.3 ± 4.8^{bC}
	3	38.3 ± 4.7^{b}	38.3 ± 6.8	36.7 ± 4.6^{c}	41.2 ± 4.9^{a}
	6	40.6 ± 6.4^{bA}	36.9 ± 3.7^{B}	38.9 ± 3.1^{cB}	41.5 ± 4.8^{aA}
	9	39.1 ± 3.6^{b}	37.1 ± 3.8	40.8 ± 3.5^{b}	37.8 ± 3.2^{b}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$).

Changes in colors of meat samples as measured by using color meter are shown in Table 5.5-5.7. Application of increasing pressure to meat could cause a lightening effect on the meat surface. It was observed that higher HHP at 300 MPa caused lighter and less redness of pork color as shown in Table 5.5 and 5.6 for L* and a* values, respectively. When crude bacteriocins were combined with HHP the color of pork samples was lighter than that from other treatments ($p<0.05$) and redness was less intense while yellowness (b*) of pork samples was not affected (Table 5.7). The effect of HHP on pork color in our experiment was in agreement with the results of Carlez et al (1995) who reported that L* values increased when the pressure increased from 200 to 350 MPa and a* values decreased with increasing pressure, especially above 400 MPa, while b* values remained constant for minced beef. In addition, Carlez et al (1995) also concluded that pressure at or above 200 MPa caused a

“whitening” effect to the meat and total myoglobin content was less for samples pressurized in a range of 200-300 MPa. Moreover, Carlez et al (1995) evaluated the effect of HHP on minced beef and concluded that L* color values increased significantly at the pressure range 200–350MPa, giving the meat a pink color, while a* values decreased at 400–500MPa, resulting in a gray-brown color. In addition at the range 200-350MPa, lightness increased as a consequence of the denaturation of globin and/or displacement or loss of heme iron. Besides, at the range 300-600 MPa redness decreased, probably due to the oxidation of ferrous myoglobin to metmyoglobin. It was proposed that HHP processing would diminish meat redness as a consequence of an oxidation increase (from ferrous myoglobin to metmyoglobin). The decrease in a* value could be related to the increase in metmyoglobin ($Fe3^{+}$), resulting in a brown coloration of meat which is undesirable and responsible for the meat to be rejected by consumers (Renerre, 1990). Overall, high pressure causes dramatic changes in the color of fresh meat and thus makes difficulty for the commercialization of HHP fresh meats since they lack the typical color of fresh meat from the consumer's perspective (Cheftel and Culioli, 1997).

Total plate counts of pork chops in vacuum packed treated by HHP at 200 MPa and in combination of herb extracts and crude bacteriocins and control samples (without HHP) are shown in Figure 5.2. At pressure 200 MPa, compared with control without high pressure at the beginning, it was observed that only HHP could reduce total counts of 0.74 log cfu/cm^2 which better than all combined treatments. From day 3 to 6, HHP combined with B and with E1 and E3 gave the same results for total counts reduction. (0.37- 0.95 log cfu/cm^2). In the last day of storage, HHP,HHP combined with E1 and E3 gave no difference TPCs (1.53 ,1.39,1.26 log cfu/ cm^2,

respectively) and better reduction in TPC than the treatment of HHP combine with B (0.5 log cfu/cm^2). When compared within combined treatments, at day 0, 6 and 9 there was no significant difference ($p>0.05$) of TPCs except for day 3 and treatment of HHP and, HHP combined with E1 showed higher TPCs ($p<0.05$) than HHP combined with E3 and HHP combined with B.

In the last day of storage, treatment of HHP combined with E3 showed lower counts than other treatments. In addition, the number of Lactic acid bacteria (LAB) and *Enterobacteriaceae* counts were lower below 10 cfu/cm^2(data not shown).

Regarding chromatic parameters (Table 5.8), for samples treated at 200 MPa HHP, it could be seen that L* increased significantly ($p<0.05$) in HHP sample compared with other treated samples.

Table 5.5 Color L* of aerobically packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), crude bacteriocins (B) (Mean ±SD)

Pressure	Day of storage	L* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	68.9±2.1	68.8±0.1^{b}	66.8±2.9^{a}	61.5±2.7
	3	66.6±2.7^{B}	72.7±1.9^{aA}	64.0±2.0^{abC}	67.2±1.8^{B}
	6	66.2±2.1	71.2±2.0^{a}	67.3±1.9^{a}	63.7±1.5
	9	67.9±2.3	65.3±3.0^{b}	60.9±3.5^{b}	63.9±0.7
300 MPa	0	69.0±2.9^{B}	73.7±2.5^{A}	70.3±1.6^{aB}	73.8±2.4^{aA}
	3	70.0±2.2^{A}	70.4±3.2^{A}	71.2±2.4^{aA}	64.8±3.0^{bB}
	6	72.5±2.6	72.7±1.9	69.2±2.3^{ab}	66.2±2.6^{b}
	9	71.7±1.9^{A}	71.7±3.3^{A}	65.8±2.2^{bC}	68.5±1.9^{bB}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=20).

Table 5.6 Color a* aerobically packed of pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD) (n=20)

Pressure	Day of storage	a* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	10.20±1.3	8.59±1.7^{b}	9.26±1.0	10.08±1.3
	3	10.27±1.4^{B}	8.19±0.9^{aC}	11.54±0.8^{A}	8.12±1.0^{C}
	6	9.67±1.1	8.89±1.1^{b}	9.44±0.8	9.14±1.3
	9	8.71±1.1	9.36±0.9^{ab}	10.92±1.7	10.27±1.5
300 MPa	0	8.45±1.4^{B}	5.93±0.7^{C}	8.29±1.3^{A}	7.25±1.2^{B}
	3	8.16±1.1^{B}	7.09±1.5^{C}	9.45±1.0^{A}	7.90±0.6^{B}
	6	6.93±1.5^{B}	6.43±1.2^{C}	9.21±1.1^{A}	8.53±1.8^{B}
	9	7.73±1.9^{B}	4.77±1.9^{C}	10.66±1.5^{A}	8.85±0.9^{B}

Table 5.7 Color b* aerobically packed of pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD) (n=20)

Pressure	Day of storage	b* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	8.67±1.1	8.44±1.5	9.12±1.2	7.44±1.3
	3	7.8±1.5	8.65±1.6	8.45±1.8	9.22±1.6
	6	6.37±2.1	8.16±1.8	8.19±1.0	7.02±1.6
	9	6.78±2.1	6.31±1.5	8.49±2.6	8.85±2.0
300 MPa	0	9.79±0.8	9.14±0.4	9.88±0.9	9.33±0.5
	3	7.55±1.5	8.44±1.8	9.64±1.5	9.55±1.2
	6	7.86±1.5	8.38±1.6	8.57±1.1	6.46±1.2
	9	8.59±1.2	5.31±1.2	8.67±1.0	8.84±1.3

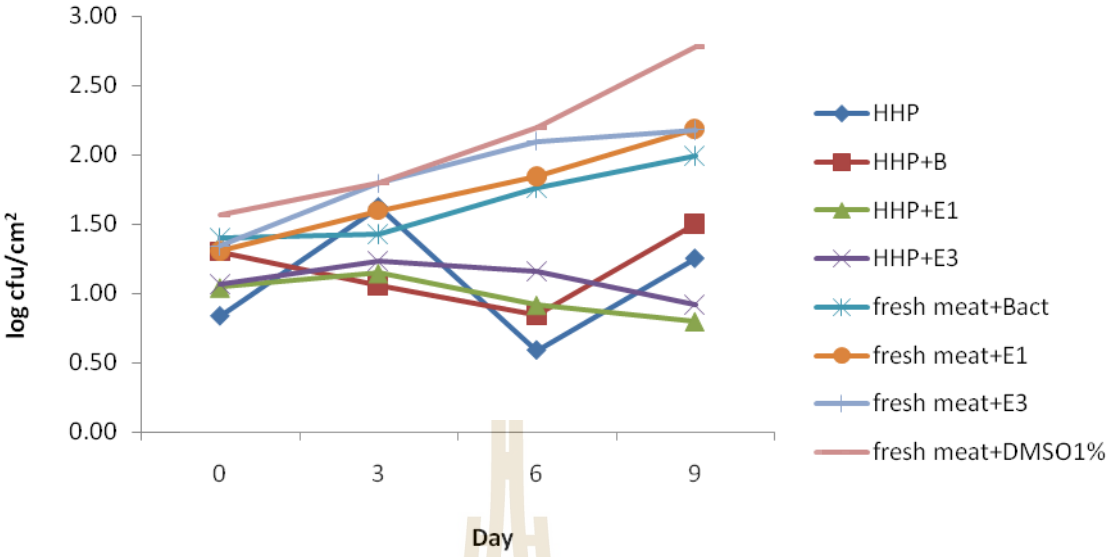

Figure 5.2 Total plate count of vacuum packed pork chops treated by HHP (200 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (n=4).

Table 5.8 Color L* at 200 and 300 MPa of pork chops in vacuum packed treated by combined HHP (200 and 300 MPa) with extracts (E1, E3), crude bacteriocins (B) (Mean ±SD)

Pressure	Day of storage	L* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	69.11±0.7^{A}	68.30±1.9^{bcA}	68.57±2.0^{aA}	66.12±0.2^{aB}
	3	67.39±1.2^{B}	71.70±4.0^{aA}	67.64±4.2^{aB}	62.34±6.8^{aB}
	6	67.39±2.7^{A}	69.68±0.7^{bA}	64.87±1.8^{bB}	56.70±4.1^{bC}
	9	70.36±6.4^{A}	66.68±2.0^{cB}	66.705±1.8^{abB}	67.15±2.8^{aB}
300 MPa	0	71.49±3.1^{aB}	74.76±2.5^{aA}	73.36±0.3^{aAB}	72.01±4.7^{aB}
	3	68.51±1.2^{bB}	69.30±2.7^{bB}	68.94±5.6^{bB}	71.80±1.2^{aA}
	6	68.14±1.4^{bB}	70.43±2.3^{bA}	66.13±1.0^{cC}	64.73±1.7^{cC}
	9	71.61±5.1^{aA}	71.23±5.4^{bA}	63.29±3.4^{cB}	67.79±2.1^{bB}

Upper case letters are significantly different in the same row ($p<0.05$).

Lower case letters are significantly different in the same column ($p<0.05$) (n=20).

Applying pressure at 300 MPa for all samples it could be seen that L* increased in comparision with lower pressure. The increment of L* value could be associated with globin denaturation and/or with heme displacement at pressures above 300 MPa (Carlez, 1995).

These results was in agreement with those of Cheftel &Culioli, (1997) who reported the increase in L* values or "whitening/brightening" effect of pressure which attributed to globin denaturation, heme displacement or release, and ferrous ion oxidation .The increase in L* values begins from 200MPa and becomes stabilized for pressures around 300–400MPa, as shown by Carlez, Veciana-Nogues, and Cheftel (1995) in beef mince and Shigehisa et al (1991) in pork slurries. The lighter appearance of meat could be due to globin denaturation and heme displacement or release (Carlez, 1994).

Pressurization of pork at 200 MPa, the HHP and HHP+B treated samples had a* value significantly lower values ($p<0.05$) than HHP+E1 and HHP+E3 treated ones throughout storage time (Table 5.9). When the pressure at 300 MPa was used, a*values of all treated samples remained almost constant but slightly higher than all samples treated at 200 MPa. Increasing in redness of the pork samples correlated with a lower accumulation of metmyoglobin during pressurized sample storage (Cheah and Ledward, 1997). At pressures up to 300 MPa, with a pressurization liquid of 10°C, the production of metmyoglobin was decreased leading to an increase in a* value (Jung et al., 2003).

Table 5.9 Color a* of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean±SD)

Pressure	Day of storage	a* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	7.80 ± 0.7^{C}	9.97 ± 0.9^{aA}	8.63 ± 1.3^{cBC}	9.49 ± 0.3^{bAB}
	3	8.48 ± 0.3^{B}	9.92 ± 0.9^{aA}	9.52 ± 1.2^{bA}	9.23 ± 1.6^{abA}
	6	9.01 ± 1.5^{BC}	9.55 ± 0.4^{aB}	11.60 ± 0.5^{aA}	8.50 ± 0.8^{cC}
	9	7.93 ± 1.3^{C}	8.08 ± 0.7^{bC}	10.83 ± 0.1^{aA}	9.86 ± 0.7^{aB}
300 MPa	0	10.55 ± 0.1^{bA}	8.35 ± 0.3^{bC}	9.56 ± 1.1^{cB}	9.87 ± 0.8^{B}
	3	12.62 ± 1.5^{aA}	11.08 ± 0.5^{B}	11.39 ± 0.9^{bB}	11.10 ± 0.7^{B}
	6	10.39 ± 1.4^{b}	11.47 ± 1.2^{a}	11.51 ± 0.7^{b}	11.11 ± 0.7
	9	10.82 ± 0.2^{b}	10.74 ± 0.1^{a}	12.55 ± 0.7^{a}	12.70 ± 1.7

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) ($n=20$).

For modification of b* parameter (Table 5.10), pressurized at 200 and 300 MPa of HHP, HHP+E3 and HHP+E1 tended to constant while HHP+B gradually decreased along storage time. It could be seen that application of higher pressure made a slightly increased of b*.The increase in b* values could be due to HHP caused the formation of metmyoglobin (Carlez et al., 1995).

Hardness of pork samples of all treatments pressurized at 200 MPa HHP tended to reduce except HHP+E1 (Table 5.11). In addition when compared among treatments, hardness of HHP only was much higher than other treatments but not difference from HHP+E1 in day 6 of storage. Application of pressured at 300 MPa HHP, HHP+B and HHP+E3, the hardness of pork samples increased in 3 days of storage but was not significantly different through the end of storage while hardness

of HHP+E1 reduced continuously. Normally, the pressure range from 100 to 300 MPa, the changes are normally reversible, whereas at higher pressures they are normally non-reversible (Rastogi et al, 2007).

Table 5.10 Color b* of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD)

Pressure	Day of storage	b* value			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	6.50 ± 0.5^{aB}	7.43 ± 0.7^{aA}	7.22 ± 1.2^{A}	$5.630.7^{aC}$
	3	5.44 ± 0.9^{bC}	6.65 ± 0.4^{bAB}	7.06 ± 0.3^{A}	4.50 ± 2.2^{aC}
	6	5.30 ± 1.2^{bB}	5.88 ± 0.5^{cB}	7.28 ± 0.5^{A}	3.17 ± 1.1^{bC}
	9	6.07 ± 2.0^{abB}	5.36 ± 0.4^{dC}	7.49 ± 1.0^{A}	6.29 ± 0.6^{aB}
300 MPa	0	7.39 ± 1.0^{aB}	7.15 ± 0.9^{aBC}	8.14 ± 0.3^{aA}	6.55 ± 1.4^{aC}
	3	6.19 ± 0.9^{bB}	6.56 ± 0.1^{bB}	7.48 ± 2.1^{aA}	7.26 ± 0.3^{aA}
	6	4.61 ± 0.5^{cC}	6.22 ± 0.5^{bA}	6.17 ± 0.9^{bA}	5.54 ± 0.5^{bB}
	9	6.53 ± 1.6^{bBC}	6.15 ± 1.0^{bC}	7.44 ± 2.0^{aA}	7.21 ± 1.7^{aAB}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) ($n=20$).

Combination treatments of HHP with herb extracts and crude bacteriocins did not make any significant differences ($p>0.05$) in weight loss (%) except at the pressure at 300 MPa which differed in last day of storage (Table 5.12). High pressure treatment of sliced pork loins at these medium pressure levels of 200 and 300 MPas and with combination of crude bacteriocins and both herb extracts did not make any significant differences ($p>0.05$) in Aw and pH (Table 5.13 and Table 5.14).

Table 5.11 Hardness of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean±SD)

Pressure	Day of storage	Hardness(N)			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	53.29±3.1^{aA}	50.12±3.6^{aA}	39.89±2.8^{B}	50.91±2.0^{aA}
	3	52.68±5.6^{aA}	42.67±4.2^{aB}	37.01±3.7^{C}	41.93±4.2^{bBC}
	6	40.22±5.2^{bA}	35.25±3.4^{bB}	39.03±5.5^{A}	32.26±4.6^{cB}
	9	42.27±4.5^{bA}	37.59±3.8^{bB}	36.97±3.4^{B}	31.86±4.3^{cC}
300 MPa	0	36.88±1.2^{bBC}	34.08±2.6^{bcC}	51.70±4.2^{aA}	38.99±4.6^{bB}
	3	48.81±5.5^{a}	46.21±3.7^{a}	45.06±2.2^{b}	43.17±3.0^{a}
	6	40.43±2.3^{bA}	31.48±5.2^{cB}	40.25±3.1^{cA}	33.39±2.9^{cB}
	9	41.19±6.5^{bA}	36.25±2.7^{bB}	37.57±4.2^{cAB}	35.35±3.4^{bcB}

Upper case letters are significantly different in the same row ($p<0.05$).
Lower case letters are significantly different in the same column ($p<0.05$) (n=20).

Table 5.12 Weight Loss (%) of vacuum packed pork chops treated by HHP (200 and 300 MPa) in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean ±SD) (n=4)

Pressure	Day of storage	Weight loss (%)			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	27.1±1.8	32.1±3.1	29.3±2.8	31.1±0.2
	3	28.7±2.5	27.3±4.8	25.1±4.3	30.8±0.5
	6	24.9±4.2	28.2±0.9	25.4±8.0	26.2±1.1
	9	25.9±2.0	25.2±0.4	22.7±3.8	27.7±1.3
300 MPa	0	30.02±1.4^{a}	31.09±1.7	28.35±4.1	30.65±3.2
	3	27.92±0.4^{a}	30.05±2.1	29.65±1.6	29.77±2.9
	6	29.70±0.2^{a}	29.36±3.9	25.29±2.3	25.67±3.3
	9	22.94±1.8^{b}	25.25±0.5	26.84±4.4	24.18±4.0

Lower case letters are significant difference in the same column ($p<0.05$).

Table 5.13 Water activity of vacuum packed pork chops treated by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3), and crude bacteriocins (B) (Mean±SD) (n=4)

Pressure	Day of storage	Aw			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	97.0±0.3	96.6±0.1	97.1±0.6	96.6±0.1
	3	96.7±0.6	96.4±0.4	96.6±0.1	97.1±0.1
	6	97.0±0.4	97.4±0.5	96.7±1.0	96.7±0.1
	9	97.5±0.1	97.3±0.1	96.9±0.4	96.8±0.1
300 MPa	0	95.9±0.1	97.2±0.8	96.9±0.4	96.8±0.4
	3	96.9±0.0	97.1±0.4	97.2±0.0	97.0±0.6
	6	96.3±0.7	97.0±0.1	97.1±0.9	97.0±0.9
	9	97.0±0.4	96.9±0.0	97.0±0.1	97.3±0.9

Table 5.14 pH of vacuum packed pork chops by HHP (200 and 300 MPa) and in combination with herb extracts (E1, E3) and , crude bacteriocins (B) (Mean±SD) (n=6)

Pressure	Day of storage	pH			
		HHP	HHP+B	HHP+E1	HHP+E3
200 MPa	0	5.9±0.4	5.6±0.0	5.7±0.0	5.6±0.0
	3	5.7±0.0	5.6±0.1	5.7±0.1	5.8±0.2
	6	5.7±0.1	5.7±0.1	5.8±0.0	5.8±0.1
	9	5.8±0.1	5.7±0.1	5.7±0.1	5.7±0.1
300 MPa	0	5.8±0.2	5.7±0.1	5.6±0.1	5.7±0.0
	3	5.7±0.1	5.7±0.2	5.7±0.1	5.8±0.1
	6	5.8±0.1	5.8±0.1	5.8±0.0	5.9±0.2
	9	6.0±0.0	5.8±0.0	5.8±0.0	5.8±0.0

5.5 Conclusions

There was no significant difference ($p>0.05$) in total bacteria counts of pork samples treated by HHP at 200 MPa when packed in both package conditions. When pork samples pressurized at 300 MPa and aerobically packed, total bacterial counts differed among samples from the treatment of HHP+E3 which had lower counts($p<0.05$) than those from HHP and HHP in combined with crude bacteriocins treatment in the last day of storage. HHP in combination with crude bacteriocins and herb extracts did not affect the weight loss, Aw and pH of pork samples packed in aerobic condition while higher pressure caused higher meat tenderness. The color of aerobically packed pork samples was lighter when higher pressure was applied except when crude bacteriocins was combined.

For vacuum packaging condition application of high pressure resulted in lighter, more redness and slight increase in yellowness. The moderate pressure of 200 and 300 MPa did not ensure complete inactivation of bacteria growth. However, it was obvious that it could allows improvement of microbiological quality and extended shelf life of sliced pork in the stage dependent on the level of initial contamination of raw material. HHP could affect physicochemical qualities of fresh pork meat, particularly depending on the pressure level applied, and thus typical characteristic associated with fresh meat particularly texture and color could be remarkably modified. HHP treatment caused lighter color of pork meat products. In addition, tenderness of fresh pork was improved.

5.6 References

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BIOGRAPHY

Thananun Yuwang was born in July 3rd, 1962 in Pichit, Thailand. He studied for high school at Taphanhin School (1974-1979). In 1984, he received the degree of Bachelor of Science in from Chieng Mai University, Chieng Mai. In 2005, after graduation, he has been employed under the position of lecturer at Vocational and Technology College Surin campus. He continued and received the degree of Master of Food Science and Technology from Chieng Mai University, Chieng Mai. In 2007, he received government foumdation from Rajamangala University of Technology Isan studied Doctor of Philosophy in Suranaree University of technology. Now he served in position of lecturer in Agro-Industry department at Faculty of agriculture and technology, Rajamangala University of Technology Isan Surin campus.