EFFECT OF ESSENTIAL OILS AND ORGANIC ACIDS ON SELECTED FOOD-BORNE PATHOGENS AND PHYSICOCHEMICAL PROPERTIES OF SHRIMPS

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UNIVERSITI SAINS MALAYSIA

2016

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by

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Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

April 2016

ACKNOWLEDGEMENT

Praise to God for the success of this thesis. I would like to thank my field supervisor and my role model, Dr. Wan Norhana Bt. Md. Noordin, for giving me the opportunity to work on this project and allowing me to conduct my experiments in her lab at Product and Food Safety Division of Fisheries Research Institute, Batu Maung, Penang.

I would like to thank my supervisor, Associate Professor Dr. Nurul Huda for his supervision, valuable guidance and lessons throughout the course of study. I would also like to thank my co-supervisor, Dr. Maizura for her guidance on sensory analysis. I am also thankful to Universiti Sains Malaysia (USM) for research and library facilities. I would also like to thank, Professor Gulam Rusul for his time, critical reviews and advices on making of this thesis.

I would like to share this moment of happiness with my family and friends. I feel a deep sense of gratitude for my parents, Mr. and Mrs. Shunmugam Kamaladevi for their financial support, encouragement and motivation throughout this journey. I am also grateful to my sisters, Gahyathiri, Neetiaasree and Vaisnavi and my caring grandmother for their endless support, love, prayers, motivation and cheering me up during my hardest times. Without them, this thesis would not be successful. Sincere thanks to all the souls who directly and indirectly contributed to the success of this thesis.

Nannthini, S.
Penang, Malaysia
April 2016

TABLE OF CONTENTS

CHAPTER ONE INTRODUCTION

CHAPTER TWO LITERATURE REVIEW

CHAPTER THREE THE EFFECT OF ESSENTIAL OILS AND ORGANIC ACIDS ON MICROBIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF WHOLE SHRIMPS STORED AT 4°

CHAPTER FOUR THE EFFECTS OF GARLIC OIL AND TARTARIC ACID ON THE MICROBIAL SAFETY, PHYSICOCHEMICAL AND SENSORY QUALITIES OF SHRIMPS STORED AT 4°C

CHAPTER FIVE CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CAR	Cardamom oil
CIN	Cinnamon oil
cfu	Colony forming units
EO	Essential oil
GAR	Garlic oil
GRAS	Generally recognized as safe
LA	Lactic acid
LIME	Lime oil
Log	Logarithms
MBC	Minimum bactericidal concentration
MBS	Sodium metabisulfite
MIC	Minimum inhibitory concentration
SD	Standard deviation
SDA	Sodium diacetate
TAR	Tartaric acid
TBA	Thiobarbituric acid
TBARS	Thiobarbituric reactive substances
TCBS	Thiosulfate – citrate bile salts
TPC	Total plate count
TSB	Tryptic Soy Broth
XLD	Xylose Lysine Deoxycholate

UNITS OF MEASUREMENT

°C	degrees Celsius
h	Hour
min	Minute
sec	Second
kg	Kilogram
g	Gram
mg	Milligram
ml	Millilitre
µl	Microliter
cm	Centimetre
mm	Millimetre
v/v	Volume per volume
w/v	Weight per volume

KESAN MINYAK PATI DAN ASID ORGANIK TERHADAP PATOGEN TERPILIH DAN CIRI-CIRI FIZIKOKIMIA UDANG

ABSTRAK

Udang (*Penaeus monodon*), dikategorikan sebagai makanan yang mudah rosak dan jangka hayat bawah penyimpanan sejuk pada 4°C adalah terhad. Bahan pengawet yang sedia ada (natrium metabisulfida) berpotensi membawa risiko ke atas kesihatan manusia. Oleh itu, pengawet alternatif dari sumber semula jadi diperlukan. Tujuan kajian ini adalah untuk mengkaji keberkesanan minyak pati (kayu manis, bawang putih, limau) dan asid organik (asid laktik, asid tartarik dan natrium diasetat) untuk merencat patogen bawaan makanan, meningkatkan kualiti mikrob dan fisikokimia. Udang harimau segar dicelup dalam rawatan yang mengandungi minyak pati atau/dan asid organik selama 30 minit, dikeluarkan dan disimpan pada 4°C selama 10 hari. Gabungan minyak pati dan asid organik yang efektif dipilih melalui keputusan kualiti mikrobiologi dan fizikokimia (pH, tekstur, warna) sepanjang tempoh penyimpanan. Kombinasi tartarik asid dan minyak bawang putih dapati paling berkesan dalam merencatkan pertumbuhan bakteria aerobik, nilai *L*, a** dan kekerasan tetapi rawatan dengan natrium metabisulfida tidak memberi kesan yang signifikan (P>005). Tartarik asid dan kombinasi tartaric asid dan minyak bawang putih telah merencat pertumbuhan *L. monocytogenes, V. parahaemolyticus,* and *S.* Typhimurium dengan berkesan (P>0.05) dan juga telah memanjangkan jangka hayat penyimpanan udang selama 3 hari. Kajian ini menunjukan bahawa pati minyak boleh merencatkan pertimbuhan pathogen makanan, menurukkan bilangan mikrob (TPC) dan juga memanjangkan hayat penyimpanan.

EFFECT OF ESSENTIAL OILS AND ORGANIC ACIDS ON SELECTED FOOD BORNE PATHOGENS AND PHYSICOCHEMICAL PROPERTIES OF SHRIMPS

ABSTRACT

Shrimps (*Penaeus monodon*) are and have a limited s shelf-life under refrigerated storage. Existing preservatives (sodium metabisulfite) have potential risk on human health. There is a need for a safer alternative with reduced health effects to extend the shelf life and maintain the quality of shrimp. The aim of this study was to assess effects of selected essential oils and organic acids (singly and mixture) on microbial safety, quality and shelf-life of fresh shrimps. Initially, shrimps (*Penaeus monodon*) were dipped in essential oil (cinnamon, garlic and lime) or organic acid (tartaric acid lactic acid and sodium diacetate) (singly and mixture) for 30 mins and stored at 4 ºC to determine the most effective essential oil or mixture of essential oil and organic acid that can reduce total plate counts (TPC) and improve shelf-life of the shrimps stored at 4 ºC. Mixture of tartaric acid and garlic oil was selected as it was the most effective combination in reducing in TPC ($P<0.05$) and L^* value compared to distilled water. The effects of garlic oil and tartaric acid (singly or mixture) on microbial safety, chemical and sensorial qualities of shrimp were then evaluated. Tartaric acid and tartaric acid and garlic oil mixture were the most effective in inhibiting *L. monocytogenes, V. parahaemolyticus* and *S.* Typhimurium growth and extending shelf-life of refrigerated shrimps. Through achieving the aforementioned aims, this work was able to enhance the knowledge and literature available concerning the use of natural and safe antimicrobials in reducing the pathogens and extending the shelf-life of shrimps stored at 4°C.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Shrimps rank as one of the favourite seafood globally and are consumed in all parts of the world, with the largest market being United States, Japan and the European countries (FAO, 2012). Global shrimp production exceeded 7.87 million metric tons in 2013, a 1.35% increase (National Marine Fisheries Service, 2015). Among the various species of shrimps, *Penaeus merguensis* (white shrimp) and *Penaeus monodon* (black tiger prawn) are the most important ones.

In 2013, the world production of black tiger shrimp was about 1.02 million metric tons while white shrimp was 3.31 million metric tons. The increasing consumption over the years contributed to the increase in trade. According to the United States National Marine Fisheries Services (NMFS, 2015), annual per capita consumption of shrimp in United States increased from 3.2 kg in 2000 to 4.0 kg in 2014. In 2013 and 2014, the consumption_of shrimp was the highest among seafood consumed in The US followed by salmon and tuna (Fisheries of United States, 2014).

Despite the increasing market demand for shrimp, the production of shrimps has been decreasing over the years. The major problem faced by the shrimp industry are post-harvest losses due to poor handling and preservation. Similar to other seafood, shrimp is highly perishable as it provides a very favourable conditions for the growth of various pathogenic and spoilage microorganisms (Venugopal et al., 2001). Deterioration caused by microbial growth and enzymatic reactions leads to spoilage, loss of quality and shorter shelf life of shrimp thus resulting in economic losses (Mine and Boopathy, 2011; Wan Norhana et al., 2010). In addition, the stringent microbiological standards imposed by importers also places a heavy burden shrimp processors and exporters (Dalsgaard et al., 1995). The main bacterial species that are associated with shrimp are *Vibrio parahaemolyticus, Salmonella* Typhimurium and *Listeria monocytogenes* (Wan Norhana et al., 2010; Zarei et al., 2012). There is a need for an efficient, safe and environmental friendly preservation techniques to increase the shelf-life and quality of shrimps so as to reduce post-harvest losses rejection of shrimp consignment by the importing countries and to obtain fetch premium prices.

Icing, chilling or freezing continues to play a major role in slowing down bacterial growth and enzymatic activities in shrimp. However, these preservation methods have some effects to the quality attributes of the shrimps. On the other hand, chemicals such as sodium metabisulfite, sodium hypochlorite have also been used to extend shelf–life of shrimp and shrimp product by inhibiting microbial growth (Smid and Gorris, 2007). Sodium metabisulfite, a traditional preservative in shrimp processing plants has been widely used because of its effectiveness in controlling melanosis and microbial activity (Lu, 2009). However, sodium metabisulfite causes adverse health consequences such as severe respiratory and anaphylaxis reactions in asthmatic consumers (Otwell, 1992).

The increasingly negative consumer perception on the use traditional industrial preservatives has provoked an interest in finding natural alternatives to the use of synthetic chemicals (Nirmal and Benjakul, 2009; Zink, 1997). At present, consumers are more aware of the health effect of these chemicals and have been demanding for the use of safe, non-toxic, natural antimicrobials having less or no side effects, having high organoleptic qualities, extending the shelf life and improving the safety of seafood products and therefore, reducing health problems for consumers of seafood (Shirazinejad et al., 2010). In recent years, many researchers have reported on the effectiveness of natural antimicrobials including essential oil, spices, organic acids and salts of organic acids to control spoilage process by preventing the growth of microorganisms (Al- Dagal and Bazaraa, 1999; Burt, 2004; Ceylan and Fung, 2004; Shirazinejad, 2010, Tajkarimi et al., 2010).

Antimicrobials from plant-origin especially essential oils and their active components derived from herbs and spices have shown inhibitory effects towards the growth of microorganisms and pathogens associated with food safety and spoilage. The antimicrobial activity of these antimicrobials against food–borne pathogens in many different kinds of foods such as meat and poultry product, fish, dairy product, fruits and vegetables and rice have been extensively studied (Burt, 2004; Guttierrez et. al., 2009). However, studies on the effect of natural antimicrobials on seafood products especially shrimp and shrimp products are still lacking. Furthermore, numerous essential oils such as oregano, bay, basil, thyme, marjoram, sage and rosemary are known to have antimicrobial activities are familiar to consumers in Mediterranean, European and Western countries (Burt, 2004; Guttierrez et. al., 2009; Prabuseenivasan, et. al., 2006). There is still dearth of information on the effect of essential oils extracted cinnamon, cardamom, lime, lemongrass, lemon, garlic, turmeric, coriander and cumin on shrimps quality and safety that are commonly used in Asian cooking.

Organic acids on the other hand, have been long known for their antimicrobial attributes and are utilised as food additives and preservatives for preventing food deterioration and extending shelf life of perishable foods (Ricke, 2003). They are easily available, cheap and categorized as Generally Regarded as Safe Substances (GRAS) In addition to acid sprays used for meat processing, some organic acids and their salts are used for are used as dipping solutions for of shrimp to prevent discolouration in British trade practice (Handling and Processing Shrimp, 2001; Ricke, 2003).

Numerous studies have also being carried to determine the effect of combination of organic acids and two or more antimicrobials or preservation methods based on principles of hurdle technology. Research on combination of antimicrobials to achieve mild preservation of foods have been gaining interest due to the possible additive or synergistic effect as low concentrations of antimicrobials are used inhibit to microbial spoilage and enhance quality of shrimps. Such studies include effect on combinations of antimicrobials and preservation methods such as combination of 4-hexylresorcinol and modified atmosphere packaging in Chinese shrimp (Lu, 2009) and combination of garlic oil and modified atmosphere packaging in chilled shrimps (Maneesin et al., 2013). Combination of antimicrobials have also been studied in the past, including combination of nisin, EDTA and sodium diacetate in vacuum-packaged shrimps (Wan Norhana et al., 2012) and combination lactic acid and nisin in chilled shrimps (Shiranizejad et al., 2010).

Combination of essential oils of herbs and spices commonly used in Southeast Asian cuisine and organic acids and the possible synergistic interaction against pathogenic bacteria and in maintaining the qualities with minimum alteration on sensory properties of shrimps have yet to be studied.

1.2 Research Objectives

. The objectives of this study are:

1. To determine the effects of selected organic acids (lactic acid, tartaric acid and sodium diacetate) and selected essential oils (cinnamon oil, garlic oil and lime oil) either alone or in combination on the microbiological (total plate count) and physicochemical quality (pH, colour and texture) of whole shrimps.
2. To determine the effects of tartaric acid and garlic oil on total plate count, psychotropic count, *L. monocytogenes*, *S.* Typhimurium and *V. parahaemolyticus* pH, TBARS and sensory attributes of shrimps.

CHAPTER 2

LITERATURE REVIEW

2.1 Tiger Shrimp (*Penaeus monodon*)

Penaeus monodon is one of the most important crustaceans' species among the related species available worldwide. The species of shrimp was originally described by Fabricus in 1978 which constitute a large group of crustaceans varying in size with maximum total length of 27 cm for males and 35 cm for female shrimps and weighs from 60 to 130 g (Holthius, 1980). *P. monodon* is a marine shrimp species usually found from the coastline to about 150 m depth in deeper waters and the juveniles are commonly found mangrove swamps, estuaries and backwaters (Solie, 1988). The native range of *P. monodon* is Indo-West Pacific oceans including East Africa, South Asia, Southeast Asia, The Philippines and Australia and in Malaysia and Thailand, *P. monodon* is fished in offshore waters also obtained by both pond fishing and inshore fishing in Malaysia, Singapore and Indonesia (FAO, 2011).

Its local name in Malaysia is *udang harimau*; *udang windu* in Indonesia; *ushi-ebi* in Japan; giant tiger prawn, black tiger prawn, panda prawn and blue tiger prawn in Australia and ghost prawn in Hong Kong (Holthius, 1980). The Fisheries and Agricultural Organisation (FAO) English name for this shrimp is giant tiger prawn. There is a common confusion between the terms shrimp and prawn. The terms are indeed common English names used synonymously due to the absence of systematic basis to mark a distinction (Holthuis 1980; Wickins, 1976). The taxonomic definition of the shrimp is as follow:

Phylum	Athropoda
Class	Crustacea
Subclass	Malacostaca
Order	Decapoda
Suborder	Natantia
Family	Penaeidae Rafinesque, 1815
Genus	*Penaeus* Fabricus, 1798
Subgenus	*Penaeus*
Species	*monodon*

(Source: Solis, 1988)

Similar to other penaeid shrimp, this species of shrimp has rostrums extended beyond the tip of the antennular peduncle, sigmoidal in shape and possess 6 – 8 dorsal and 2 – 4 teeth on ventral margin and smooth, polished and glabrous shell (Motoh, 1985). The colour shades of the shrimp ranges from green, brown, red, grey, blue and the carapace and abdomen are transversely banded alternatively with blue or black to brown and yellow and the antenna are uniform pink brown (Montgomery, 2010; Solis, 1988). Figure 2.1 shows black tiger shrimp and various parts and the technical terms with taxonomic importance are shown in Appendix A.

P. monodon mature and breed in tropical marine habitats and spend their larval, juvenile, adolescent and sub-adult stages in coastal estuaries, lagoons or mangrove areas (Primavera and Lebata, 1995). Mating for this shrimps occur at night, shortly after molting while the cuticle is still soft. There the sperm are kept in spermatophore (sac) inserted inside the closed thelycum of the female and then followed by spawning which takes place at night and fertilization is a external process with females suddenly extruding sperm from the thelycum as eggs are laid in offshore waters. 12 to 15 hours after fertilization, hatching occurs where the larvae (nauplii), resembling tiny aquatic spider is released and the next larval stages including protozoea, mysis and early post larvae remain platonic for some time and are carried towards the shore by tidal currents. The post larval subsequently change their habit to feed on benthic detritus, polychaete worms and small crustaceans after six moult and possess similar characteristic to adult shrimp (Miquel, 1984).

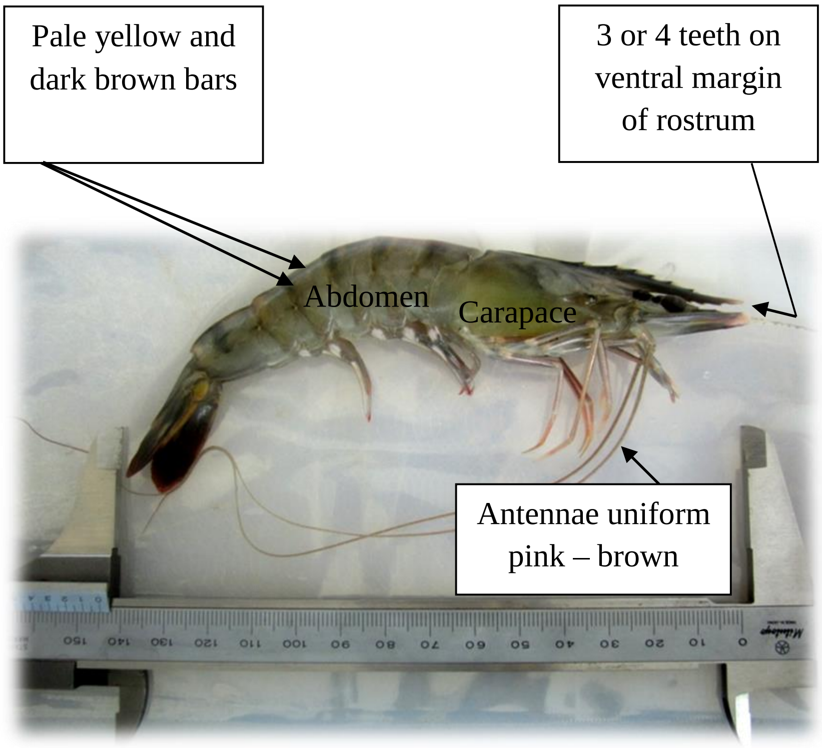

Plate 2.1 Black tiger shrimp (*Penaeus monodon)*

In general, *P. monodon* is the most prominent farmed crustacean product in international trade and has driven a significant expansion in aquaculture in many developing countries in Asia mainly Southeast Asian countries (Motoh, 1985; Ye et al, 2009). Over the period of 5 years (2007 to 2012), capture production of *P. monodon* showed a 14% decrease and therefore, the increasing demand for this shrimp is being compensated through the increase in aquaculture production. The aquaculture production of *P. monodon* reached 855, 000 metric tons per year on 2012 from 593, 000 metric tons per year on 2007 accounting to about USD 4 billion in 2012 from USD 2.9 billion in 2007 showing a 44% increase in 5 years (FAO, 2014) (Table 2.1).

Shrimp is regarded as a favourite delicacy for its uniqueness of flavour and texture (Erickson et al., 2007; Wan Norhana et al., 2010). Shrimp is a good source of protein and consists of highly polyunsaturated fatty acids such as eicosapentanoic and docosahexanoic acid also is a principal source of omega-3-fatty acids after salmon making it a healthy choice of food (Mahaffey et al., 2008; Sriket et al., 2007). The proximate composition of *P. monodon* is listed in Table 2.2.

Table 2.1 World production in metric tons of *P. monodon*

Production	2007	2008	2009	2010	2011	2012
Aquaculture	593 649	720 365	769 139	684999	796 925	855 055
Capture	248 501	254 919	223 485	189 941	221 818	212 504

(Source: FAO, 2014)

Table 2.2 Proximate composition of *P. monodon*

Proximate content	Amount (%)
Moisture	80.47
Ash	0.95
Protein	17.1
Fat	1.23

(Source: Sriket et al., 2007)

2.2 Quality Deterioration of Shrimps

Generally, consumers tend to relate freshness of seafood to the inherent quality of freshly harvested as it is a common perception that if the time lapse after harvest is short, the seafood retains its original characteristic and of high quality (Venugopal, 2006). The quality of shrimp is influenced by both intrinsic and extrinsic factors. Species, sex, size, composition, spawning, presences of parasites and toxins, contamination with pollutants and cultivation conditions are the intrinsic factors and the location of catch, season, methods of catch, on-board handling, hygienic conditions on the fishing vessel, processing and storage conditions are the extrinsic factors (Venugopal, 2006).

Theoretically, freshly caught shrimps are at their highest quality level; however, their quality begins to deteriorate soon after the harvest due to high amount of non-protein nitrogenous compounds that can be easily metabolised by microorganisms and a variety of chemical reaction involving mainly microbial enzymes that causes breakdown of proteins (Broekaert et al., 2013; Venugopal, 2006). Post-harvest decomposition begins and involves microorganisms which originate from the marine environment or from contamination during handling or washing and storage temperature plays a very important role in influencing growth rate and spoilage microorganisms in highly perishable food products such as shrimp.

Therefore, in order to slow down the deterioration, low temperature storage using ice is mostly used in the seafood industry as well as in the retail market. Refrigeration temperature (0 – 10 ºC) is limited for the local production and retail market and frozen temperature (below than 0 ºC) is employed for shrimps intended for long storage and exportation (Attala, 2012). At temperature below 10 ºC, growth of mesophiles, which constitute a major proportion of the initial population of microflora are restricted, however, the growth of psychotropic bacteria continues and eventually dominate the microflora (Ashie et al., 1996). In addition, the loss of quality in shrimp continues to occur mainly as a result of the action of enzymes produced by the microorganisms present on the skin and in the intestinal and respiratory system (Gorga and Ronsivalli, 1988).

The use of chill temperature alone can only slightly delay microbial spoilage. In order to further extend product shelf-life, other strategies such as method of packaging, type of preservatives or their combinations, involving the application of hurdle technology would have to be adopted.

2.2.1 Quality deterioration due to microbial growth

Spoilage in shrimp as in other seafood is caused by microbial growth and metabolism through which amines such as putrescine, histamine and cadaverine, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable odours and off-flavours are produced (Ghaly et al., 2010; Gram and Dalgaard, 2002). In chilled shrimp, most predominant bacteria associated with spoilage are *Pseudomonas* spp., *Shewanella putrefaciens* and *Shewanella putrefaciens*-like bacteria because during chilled storage, there is a shift in bacterial type from a mixture of aerobic, psychotropic, Gram positive and facultative anaerobic Gram negative, rod-shaped bacteria to psychotropic (Chinivasagam et al., 1998; Venugopal, 2006).

The shelf-life of shrimp is the storage time the shrimps reaches the point of unacceptability through spoilage. The endpoint of spoilage may be defined by a certain maximum acceptable bacterial load in the range of 7.0 or 8.0 log cfu/g in untreated

Penaeus shrimp (Outtara et al., 2001). Other microbiological (aerobic plate count) standards available are The International Commission on Microbiological Specification for Foods (ICMSF) of 7.0 log cfu/g in frozen shrimp and Malaysian Food Regulations 1985, log 6.0 cfu/g in ready-to-eat fish and fish products (Cadun et al., 2005; Food Regulations, 1985). The European Economic Community (EEC) Council Directive 91/493/EEC, stipulated that the maximum aerobic plate count for ready-to-eat shrimp as log 5.0 cfu/g (EEC, 1991; Huss, 1993). The predominant factors determining the shelf-life of the product are the numbers and types of bacteria initially present and their subsequent growth (Lalitha and Surendran, 2006). Total viable count or total plate count is usually used as an index for acceptability to establish standards, guidelines and specification (Olafsdottir et al., 1997).

2.2.2 Quality deterioration due to physicochemical changes

2.2.2(a) pH changes

The increase in lactic acid generated in anoxic condition from glycogen during the first few days of storage causes decrease in pH and then after few days of storage, lactic acid content ceases due to rapid oxidation resulting increase in pH of shrimp (Venugopal, 2006). The changes in pH also depend on the liberation of inorganic phosphate and ammonia due to enzymatic degradation of adenosine triphosphate (ATP) and on the inherent buffering capacity of the muscles (Venugopal, 2006). During the latter part, the decomposition of nitrogenous compounds and accumulation of basic compounds generated from both autolytic reactions by endogenous enzymes and microbial enzymatic actions will lead to increase of pH in shrimp (Seabra et al., 2011; Sikorski et al., 1990). pH value can be used as a measure of spoilage and quality, pH range of 7.7 and below indicates a good quality shrimp (Mehmet et al., 2009).

2.2.2(b) Lipid oxidation

Lipid oxidation is one of the main causes of quality deterioration in various types of fresh foods including shrimps. It is a complex process in which lipids especially polyunsaturated fatty acids are degraded via free radical formation causing the deterioration of flavour, texture, colour, aroma, taste, consistency, nutritional benefits. After harvest, the muscle tissues are liable to react with oxygen when exposed to air. Oxygen may react with many of the biochemical components tissues including lipids. The oxidation of polyunsaturated fatty acids causes the development of off-flavours and aromas referred to as 'rancidity' (Ashton, 2002; Nirmal and Benjakul, 2009).

Thiobarbituric acid reactive substances (TBARS) test has been widely used to measure the degree of lipid peroxidation and where it measures the auto-oxidation products of protein bound lipids and phospholipids to aldehydes and ketones (Li et al., 2012). The test procedure involves heating the food with strong acid in order to release malondehyde from some precursor as well as for the condensation of malondehyde with of 2 – thiobarbituric acid (TBA). Then, unstable hydroperoxides form and decompose into shorter chain hydrocarbons and is detected as TBA (Benjakul et al., 2005). In short, the test is based on the formation of pink or red complex with strong absorbance at 532 to 535 nm when thiobarbituric acid (TBA) and oxidation products react. The spectrophotometric determination of this red or pink pigments have been used to measure rancidity in a wide range of food products and are expressed as mg malonaldehye/ kg sample. Nishimoto (1985) proposed the value of 3.0 mg malonaldehyde/ kg sample to indicate a good quality fish. However, in a more recent study, the consumption limit of 7.0 or 8.0 mg malonaldehyde/ kg was proposed (Cadun et al., 2008).

2.2.2(c) Textural changes

Texture is an important quality parameter influencing purchasing decision. Generally shrimp has a softer texture than red meat because of its low content of connective tissues and cross-linking. Expectedly, during storage, texture deterioration facilitated by endogenous protease including cathepsin, calpains, trypsin, chymotrypsin, alkaline protease and collagenases takes place causing gaping between the muscles and hence causing loss of hardness and firmness of shrimp (Venugopal, 2006). In a study, it was revealed that the degradation of connective tissues in iced freshwater prawns was caused by enzymatic degradation of collagen which contributed to softening of the prawn (Mizuta et al., 1997; Yi et al., 2013). From the various shrimp texture attributes (hardness, springiness, cohesiveness, gumminess and chewiness), only hardness is correlated with deterioration compared to the other attributes (Imran et al., 2013).

2.2.2(d) Colour changes

The colour of shrimp is an important quality attribute that influences consumers' acceptance. The pigments in shrimp that causes impact in the quality are those of natural origin as well as those resulting from post-mortem changes. Shrimp colour is dependent upon the amount of astaxanthin present in the external tissues especially in the exoskeleton and in the epidermal layer between the abdominal muscle and the exoskeleton (Tume et al., 2009). Astaxanthin is commonly present in free and esterified forms with fatty acids. Besides astaxanthin, small amounts of luteins and zeaxanthin also contribute to the colour in shrimp (Sachindra et al., 2005). These carotenoids may present as carotenoproteins especially in the exoskeleton. During storage, fading of carotenoid may take place due to autooxidation of conjugated double bonds, enzyme activity and formation of lipid hydroperoxide from carotenoid and products of lipid oxidation resulting in fading of the blue-green pigments in black tiger prawn (Tume et al., 2009; Venugopal, 2006).

Other than fading of carotenoids during storage, surface discoloration and formation of black-spots from the carapace to exoskeleton forming blackbands outlining the sections of exoskeleton caused by polyphenoloxidase enzyme reduce the marketability and value of shrimp (Balfour et al., 2014). The blackspots or melanins production are the result of enzymatic browning termed as melanosis. Melanosis in crustaceans is one of the most studied problems as the occurrence of black spots gives the product an unacceptable appearance and low market value (Haard, 2002).

2.2.3 Changes in sensory quality

The change in sensory properties during storage is measured through sensory evaluation. It is the rapid method for food quality assessment and is defined as the scientific-discipline used to evoke, measure, analyse and interpret characteristics of food perceived with the five senses of sight, smell, touch, hearing and taste (Venugopal, 2006). Sensory evaluation can be done through discriminative, descriptive and affective tests. Discriminative test is used to measure the perception of difference between two or more samples of a certain attribute, descriptive test measures quantitative descriptions (nature and intensity) of samples by a group of qualified and trained subjects and affective test is used to measure personal response (acceptance or preference) by a group of current or potential consumers (Botta, 1995).

Fresh and high quality shrimp has a translucent appearance, greyish-green colour, fresh ocean air odour, firm texture, sweet taste and firmly attached cephalothorax and abdomen. Changes in surface coloration of shrimp as well as altered colour of the flesh result mainly from enzymatic and non-enzymatic oxidation. Shrimp become darker due to the formation of melanin especially at cephalothoraxes; traumatism and molting may affect this deficiency more than the storage condition. The loss of texture of shrimp from firm involves increase in softness or mushiness and loss of springiness during storage. Fresh shrimp exude a fresh seaweedy odour which on storage become less intense, neutral and flat-sweet until off-odour appears, indicating intensive malodorous spoilage. Putrid odour is caused by the presence of indole, putrescine, cadaverine and other diamines from bacterial degradation of amino acids (Pardio et al., 2011; Sikorski et al., 1990).

2.3 Pathogens associated with Shrimp

Pathogenic bacteria are naturally present on live fish and crustaceans including shrimp whether from the marine or estuarine environment (Nilsson and Gram, 2002). Shrimps are commonly contaminated and associated with several microorganisms including *Listeria monocytogenes, Salmonella* Typhimurium and *Vibrio parahaemolyticus* as summarised in Table 2.3 (Nillson and Gram, 2002; Salem and Amin, 2012; Wan Norhana et al., 2010; Wang et al., 2013). Given that shrimps are usually consumed whole including their intestinal tract they possess a health risk to the consumer. The microbiological criteria for cooked crustaceans stipulated a maximum count of 10 cfu/g for *E.coli* and absence of *Salmonella* in 25g of sample (EC, 2005).

2.3.1 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, rod-shaped and non-spore forming bacterium that is frequently found in both raw and ready–to–eat foods. *Listeria* species are ubiquitous in nature and are found in soil, water, sewage, silage, plants and in intestinal tract of domestic animals. According to Marshall (2004), *L. monocytogenes* has resistance to harsh environmental conditions such as heat, cold and chemicals and therefore, ingestion of the bacterium is a common occurrence. In addition, the bacterium survives in extreme environments, including broad pH ranges (4.1 to 9.6), high salts concentration (up to 10%) and in presence of antimicrobial agents (Bhunia, 2008). The bacterium grows rapidly during temperature abuse and grows slowly in a long shelf-life food. Food is the main vehicle of infection and meat, vegetables, fish and dairy products are potential source for transmission. Post-processing contamination of products is a major concern in ready-to-eat (RTE) or minimally processed foods such as sausages, smoked fish, deli meats, fruits, vegetables and cooked RTE crustaceans (Bhunia, 2008).

In Malaysia, so far, listeriosis outbreak has not been reported due to poor surveillance but previous study by Arumugaswamy et al. (1994) and Kuan et al. (2013) showed prevalence of *L. monocytogenes* in seafood including raw and RTE prawns, squids and clams. The prevalence of *L. monocytogenes* has been reported in raw shrimp in Iceland (Gudmundsdottir et al., 2006), Russia (Beleneva, 2011) and India

(Moharem et al., 2007). However, in the other parts of the world, the occurrence of this pathogen in fresh and frozen raw shrimp at retail, wholesale and import level is quite common and prevalence varies from very low to 50 %. These products pose risk to susceptible populations when consumed raw or lightly cooked.

2.3.2 *Salmonella* Typhimurium

Salmonella, the member of the *Enterobacteriaceae* family, is a Gram-negative, non-spore forming bacillus (Bhunia, 2008). *Salmonellae* are facultative anaerobes, mostly motile and present in various environmental conditions including the intestinal tract of birds, reptiles, turtles, insects, farm animals and human and outside living in a desiccated state (Bhunia, 2008; Wan Norhana et al., 2010). They are mesophilic, with optimum growth temperature between 35 and 37 ºC and a growth range of 5 to 46 ºC (Amagliani et al., 2012). However, they are unable to grow under desiccated conditions and are killed by pasteurization temperature and time, sensitive to low pH and unable to multiply at water activity of 0.94 (Amagliani et al., 2012, Wan Norhana et al., 2010).

The most common factors contributing to salmonellosis outbreaks are improper cooking, inadequate storage, cross-contamination and use of contaminated raw ingredients in the preparation of food (Amagliani et al., 2012). *Salmonella* has the ability to colonize the gastrointestinal of warm blooded animals and cold blooded animals and capable of causing illness in a wide range of animals. However, the bacteria usually exist as harmless and short-lived component of the intestinal microflora. Therefore, *Salmonella* contamination in food is predominantly due to faecal contamination of food or produce during processing, manure application to fields, water used for irrigation or human waste (Maurer and Lee, 2005). Studies revealed that *Salmonella* prevalence in shrimp culture environment especially in the tropics through animal manure, contaminated feeds and water (Amagliani et al., 2012; Bhaskar et al., 1995).

Other than that, several findings reported presence of *Salmonella* in fresh and frozen shrimp collected from landing centres, retailers, wholesalers, importers and processors (Arumugaswamy et al., 1995; Wan Norhana et al., 2010). Post-processing cross-contamination of *Salmonella* is very much of a concern as RTE shrimp has been demonstrated to support the survival and growth of this pathogen (Wan Norhana et al., 2010). *Salmonella* prevalence in shrimps has been reported in Asian countries including India, Indonesia, Philippines and Thailand but not Malaysia (Koonse et al., 2005; Kumar et al., 2008).

2.3.3 *Vibrio parahaemolyticus*

V. parahaemolyticus is a major cause of food-borne illness caused by eating contaminated seafoods (Wong et al., 2000; Sujeewa et al., 2009). *V parahaemolyticus* is a curved, rod shaped Gram-negative bacteria, 1.4-2.6 µm long and 0.5- 0.8 µm width, *Vibrios* are widespread in the marine and estuarine environment and grow well in neutral to alkaline pH 9.0, acid sensitive, the optimum pH is 8.0 to 8.8 and the optimum growth temperature is 20 to 37 ºC (Zarei et al., 2012, Bhunia, 2008). *V. parahaemolyticus* has been frequently isolated from seafood including shrimp and oyster and is infectious and a dose of 5.0 to 7.0 log cfu (Bhunia, 2008; Sani et al., 2013). Infection with this pathogen causes diarrhoea, vomiting, abdominal cramps, and in rare case, fever, but however, the illness is mild and usually occurs at four to 96 h after ingestion and lasts for 2.5 days (Lin et al., 2005).

Due to the ubiquitous nature of this species, it is impossible to obtain shrimp and shrimp products free from this bacterium (Su and Liu, 2007). Studies revealed that most of *V. parahaemolyticus* isolated from the environment or seafood are not pathogenic except for strains that were able to produce thermostable direct haemolysin (TDH) enzyme that was originally detected on Wagatsuma agar (Bhunia, 2008). The TDH and TDH- related haemolysin (TRH) have been recognized as the major virulence factor in *V. parahaemolyticus*, owing to the gastroenteritis (Su and Liu, 2007).

The pathogen is a major cause of food-borne illness in the world and in Asia, approximately half of the food poisoning outbreaks in Japan, Taiwan and several Southeast Asian countries and has been recognised as one of the major food-borne pathogens infecting shrimp in Chinese market (Sujeewa et al., 2009; Xu et al., 2014; Zarei et al., 2012). In Malaysia, food-borne illness associated with consumption of *Penaeus monodon* (black tiger prawn) contaminated with *Vibrio parahaemolyticus* was reported to be between 49 and 197 cases per year (Sani et al., 2013).

2.4 Control of Spoilage and Pathogenic Bacteria on Shrimp

Due to high perishability potency of shrimp, several preservation techniques have been applied to prolong their shelf-life and to reduce the risk of health hazards. Such methods and techniques include gamma irradiation (Hocaoglu et al., 2012; Perng and Yang, 1990), frozen storage from -5 to -15 °C (Ali, 2011; Tsironi et al., 2009), chilled storage (Imran et al., 2013; Rogerio et al., 2001), cryogenic freezing (Guo et al., 2013), edible coating (Arancibia et al., 2014; Guo et al., 2013; Wang et al., 2013), vacuum frying (Pan et al., 2015), modified atmosphere packaging (Arvanitoyannis et al., 2011; Lu, 2009; Nirmal and Benjakul, 2011; Noseda et al., 2012; Young et al., 2014), ozone treatment (Okpala, 2014), high pressure processing (Bindu et al., 2013; Jantakoson et al., 2012; Yi et al., 2012), treatment with ozonated water (Guo et al., 2013; Lu, 2009), vacuum packaging (Young et al., 2014), brine marination (Mejlholm et al., 2012) and the use of chemical preservatives such as chlorine dioxide (Andrews et al., 2002) and sodium metabisulfite (Januario and Dykes, 2005).

Although some of these methods such as chilled storage may be effective to some extent in controlling bacterial growth and spoilage activity in shrimp and shrimp products, refrigerated temperatures between 4 ºC and 8 ºC are not sufficient to ensure complete cessation of growth of psychrotrophs and pathogenic bacteria. Therefore, additional treatments are needed to enhance the existing technique in order to maintain quality and to assure the safety of chilled shrimps. The preferred preservation techniques are those based on a combination of different factors to delay, prevent or inhibit the growth of undesirable microorganisms in order to enhance the safety, stability and sensory quality of the products (Gould, 2000). The potential hurdles used for food preservation are temperature, pH, water activity, modified atmosphere, pressure, ultrasound, radiation and preservatives (Leistner, 1994; Shalini and Singh, 2014).

For a long time, preservatives of chemical origin have been used as reliable preservative factors to achieve sufficiently long shelf-life for foods and high degree of safety with respect to food-borne pathogenic microorganisms (Smid and Gorris, 2007). However, in recent years, consumers' demands have strongly increased for the need of safe, high-quality foods preserved with natural preservatives. Consequently, researches on the usage of natural antimicrobials to replace the existing preservative are receiving greater attention. Such researches in extending shelf- life and microbial safety of shrimp using include herbs and spices: galangal (Weerakkody et al., 2011), essential oils: garlic oil (Maneesin et al., 2013), rosemary (Seabra et al., 2011), thymol oil (Mastromatteo et al., 2010), plant extracts: tea extract (Nirmal and Benjakul, 2011), grape seed extract (Gokoglu and Yerlikaya, 2008), organic acids: citric acid and acetic acid (Attala, 2012), lactic acid (Benner et al., 1994; Wang et al., 2013) and bacteriocins: nisin (Shirazinejad et al., 2010).

2.5 Natural Antimicrobials as Preservatives

Natural antimicrobials are derived from plant, animal and microbial sources which provide potential for utilization in food preservation and safety and a broad range of natural antimicrobials from microorganisms, plants, and animals. The main advantages of using these antimicrobials are the minimal damage to product flavour and texture (Gould, 1996). The application of natural antimicrobials on shrimp that have been reported is summarized in Table 2.3. The application of some of the plant based antimicrobials including spices, herbs and essential oils studied in the past are galangal (Weerakkody et al., 2010) and rosemary (Cadun et al., 2008; Seabra et al., 2011; Weerakkody et al., 2010), grape seed extract (Gokoglu and Yerlikaya, 2008), green tea (Nirmal and Benjakul, 2011; Sundararajan et al., 2011), cinnamon oil (Arancibia et al., 2014), cinnamaldehyde (Mu et al., 2011; Outtara et al., 2001), garlic oil (Maneesin et al., 2013) and thyme oil (Outtara et al., 2001).

Table 2.3 Effect of different antimicrobial compounds on different microorganisms present in shrimps

Antimicrobials	Type of shrimp	Bacterial species	Inhibition	Reference
Potassium sorbate	Fresh, raw	Psychotropic bacteria	Moderate	Harrison and Heinsz, 1989
Potassium sorbate/ sodium benzoate/ Sodium diacetate	Fresh, vacuum packed	Native microflora	Slight	Wan Norhana et al., 2012
Lactic acid	Fresh, raw	Aerobic bacteria	Very slight	Benner et al., 1994
Lactic acid	Fresh, raw	Native microflora	Slight	Shiranizejad et al., 2010
Citric acid	Fresh, raw	Aerobic bacteria, coliforms, *Staphylococcus aereus*	Moderate	Attala, 2012
Citric acid, acetic acid	Fresh, raw	*Vibrio parahaemolyticus*	High	Amami and Amin, 2012
Olive oil	Fresh, raw	Aerobic bacteria, coliforms	High	Ahmed et al., 2014
Thymol oil	Ready-to-eat, peeled	Aerobic bacteria	Slight	Mastromatteo et al., 2010
Thyme oil, cinnamaldehye	Cooked	*Pseudomonas putida*	Very slight	Outtara et al., 2001
Cinnamaldehyde	Fresh, raw	Aerobic bacteria	High	Mu et al., 2011
Bilimbi extract	Fresh, raw	Aerobic bacteria, *Listeria monocytogenes, Salmonella* Typhimurium	Moderate	Wan Norhana et al., 2009

Natural antimicrobials can be applied in food products as spraying agent, dipping solution, marinating ingredient or incorporated in packaging or known as active packaging. The benefits of using natural antimicrobials include reducing the need for antibiotics, controlling microbial contamination in food, improving shelf-life by delaying microbial spoilage and eliminate undesirable pathogen and decreasing the development of antibiotic resistance by pathogenic microorganisms (Tajkarimi et al., 2010). In addition, the application of natural antimicrobials in different combinations with each other and with other preservative factors will create multiple hurdle approaches that will have result in more effective and safer preservation of the products (Sivarooban, 2007).

2.5.1 Essential Oils

Essential oils (EOs) are aromatic oily liquids extracted from different parts of plants for instance, leaves, barks, seeds, flowers and peels (Tongnuanchan and Benjakul, 2014). They can be obtained by expression, fermentation, effleurage or extraction but among all the methods, steam distillation and hydro distillation are widely used for commercial production of EOs (Burt, 2004; Tajkarimi et al., 2010). EOs possesses antibacterial and antiviral properties and has been screened as a potential source of novel antimicrobial compound, alternatives to hazardous chemical preservatives and agents promoting food preservation (Solorzano-Santos and Miranda-Novales, 2011).

Cinnamon, known as “kayu manis” in Malaysia brought by Arab traders from the Far East to Egypt and the West, where the spice was highly treasured (Raghavan, 2007). The spice since then been an important spice used in cooking as a flavouring agent in India, Southeast Asia, USA and in European countries. Essential oil of