THE HONG KONG POLYTECHNIC UNIVERSITY

DEPARTMENT OF APPLIED BIOLOGY & CHEMICAL TECHNOLOGY

SHRIMP FRESHNESS PRESERVATION:
Effects of Chemical Preservatives and Modified Atmosphere Packaging on Shrimp (*Penaeus monodon*) Stored at Chilling Temperature

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by

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TABLE OF CONTENT

SECTION 3 MATERIALS AND METHODS

SECTION 4 FINDINGS AND DISCUSSION

Abstract of thesis entitled 'Shrimp Freshness Preservation : Effects of Chemical Preservatives and Modified Atmosphere Packaging on Shrimp (*Penaeus monodon*) Stored at Chilling Temperature'

Submitted by HO YIU FAI

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ABSTRACT

Shrimps (*Penaeus monodon)* were caught in the local coastal waters and were kept alive in water tanks on board. Immediately after arrival to pier, the shrimps were sorted by size (12 to 15 cm) and kept alive in aerated tanks during the transportation to the laboratory within half an hour. The shrimps were then chilled to death by crushed ice in the laboratory. For whole shrimps, they were soaked in preservatives for 5 minutes and either stored in plastic boxes (packed with ice cubes) or packed in Nylon/PE bags. For peeled shrimps, they were de-headed, de-gutted and peeled before they were soaked in preservatives. The bags were then filled with gases of different composition by using vacuum packing machine. Samples packed with ice cubes were stored at 0-2°C while those packed in bags were stored at 2-4°C.

Objective tests in the research included Aerobic Plate Count (APC), lightness (L) measurement, Trimethylamine (TMA) determination, and Total Volatile Basic Nitrogen (TVBN) determination. Subjective tests conducted were sensory evaluations in which each panelist of the experienced 10-member panel evaluated 12 raw and 2 cooked shrimps. Cooked samples were made by blanching raw shrimps for 3.5

minutes. Panelists were asked to evaluate samples for odor, texture, integrity (shell-on shrimps only), color, appearance and overall acceptability (OA), using a 1 to 9 hedonic scale; and to evaluate melanosis using a 6 interval (0,2,4,6,8,10)scale.

The shell-on shrimps treated with the combination of 1.25% sodium metabisulphite and 100ppm lysozyme could be kept in ice for 8 days which doubled that of the control samples. The shelf life of peeled shrimps treated with the same combination of preservatives stored in 40% CO_2 and 60% N_2 was at least 46 days while the shell-on control samples can be kept for only about 2 days. APC showed that the bacterial load in treated samples were still acceptable after such storage.

TMA values increased generally, but there was no significant difference between different treated samples.

The trend for the TVB-N study showed that TVB-N contents in shell-on shrimps were much higher than that in peeled shrimps. The TVB-N value may be used as a freshness indicator for the species because the OA scores correlated well with TVBN values. The TVBN level of samples were determined to be ranged from 44.9 to 58.3 mg/100g with average of 51.6 mg/100g at time of odor rejection.

The TVBN levels in two batches of fresh shrimps were 27.3 mg/100g (standard deviation = 0.84) and 13.3 mg/100g (standard deviation = 0.73) respectively.

Moreover, experimental data showed that lightness may also be used as a fresh indicator for raw shell-on shrimps because the lightness decreases while the OA score increases. The surface lightness of samples were determined to be ranged from 14.8 to 18.1 with average of 16.5 at time of melanosis rejection. The averaged surface

lightness in two batches of fresh shrimps were 17.49 (standard deviation = 1.60) and 21.5 (standard deviation = 1.29) respectively.

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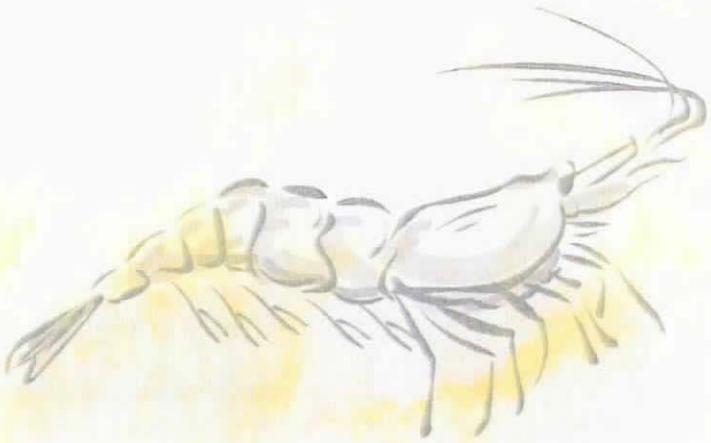
LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

Section 1

Introduction



SECTION 1

Introduction

1.1 Introduction

Shrimp is one of the popular seafood in Hong Kong. Shrimps are characterized by their nutritional values, ease of digestion and flavors. The deterioration and destruction of local marine habitats due to pollution and over-fishing resulted in drastic decrease in fish supply. Consequently, fishermen were driven to go further away from the coast to catch shrimps and therefore shrimps should be stored for a rather long period of time before they are transported to market. Since shrimps are very perishable, good preservation techniques must be applied in order to supply high quality shrimps to the local market.

1.2 Aims & Objectives

The aims of the present research are:

1. To study the effects of preservatives and modified atmosphere packaging on a selected shrimp specie stored under chilling temperature, in the hope to extend their shelf life.
2. To monitor the spoilage of the selected shrimp specie during storage by subjective sensory evaluation and some objective analyses, so as to develop a Shrimp Freshness Index.

Section 2

Literature Review

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Literature Review

2.1 Significance of shrimp preservation

In Hong Kong, the land reclamation projects carried out recently destroyed the natural marine habitat for shrimps (Yang & Wong, 2000). Moreover, the deterioration and destruction of local marine habitats due to pollution and over-fishing resulted in drastic decrease in shrimp supply in the near coast. Fishermen need to move far away from the coast in order to capture shrimps.

However, shrimps were perishable because of their high water content and nutritional value. The typical raw, fresh shrimp has a relatively high protein content of 18.1% w/w. Generally, shrimp flesh contains only about 0.5 to 0.8% w/w fat while the carbohydrate content is about 1.5 % w/w (Stansby, 1963). The amino acids in shrimps were higher than that in fish. This made shrimps spoil more rapidly (Mukundan *et al*, 1981).

The demand for shrimps was generally increasing during the past decades. The worldwide production of shrimps had grown tremendously from about 25 Mt in 1988 to 29 Mt in 1992 (Fatima, 1994). Unfortunately, the shelf lives of shrimps were limited to several days and this seriously affected their distribution. Moreover, the marketability of shrimps was affected by the deterioration in their freshness and this can causes heavy economic losses (Hanpongkittikun *et al.*, 1995). Therefore,

freshness preservation of these perishable shrimps, which were one of our major high quality food resources, was continuously addressed by researchers.

2.2 Freshness Deterioration of Shrimps

Deterioration of shrimps were mainly caused by autolysis (Hobbs, 1982), microbial spoilage (Cobb III *et al.*, 1976) and physical handling. Loss of quality during early stage was mainly caused by autolysis, whereas long-term deterioration was the result of bacterial action (Fatima *et al.*, 1988).

2.2.1 Autolysis

Important endogenous reactions occurred above the freezing point included glycolysis, proteolysis, ATP catabolism, lipid hydrolysis and lipid oxidation (Haard, 1992). These reactions were brought about by the autolyzing enzymes present mainly in the gut of shrimps. Together with the proteolytic enzymes present in shrimps flesh, they broke down organs and surrounding tissues and facilitated the microbial enzymatic activities.

Proteolytic enzymes present in shrimp muscle play an important role in spoilage by degrading muscle proteins and polypeptides and then forming amino acids. These amino acids enrich the natural substrate and speed up the growth of spoilage microorganisms (Pedraja, 1970). Since these enzymes are naturally present in the shrimp, autolysis is unavoidable. However, we can lower the storage temperature so that the enzymatic activities are slowed down.

2.2.2 Melanosis

Melanosis or blackening occurred rapidly after the death of shrimps. The process was caused by endogenous enzyme activities of tyrosinase (Bailey *et al.*, 1960). This enzyme acted on aromatic acids to form malanins which was possibly polymerized indole quinines. Melanosis occurred most frequently on the head first and then on the body. It was in no doubt that this phenomenon would make shrimps less marketable. The chemical mechanism of the process was described in figure 2.2.1.

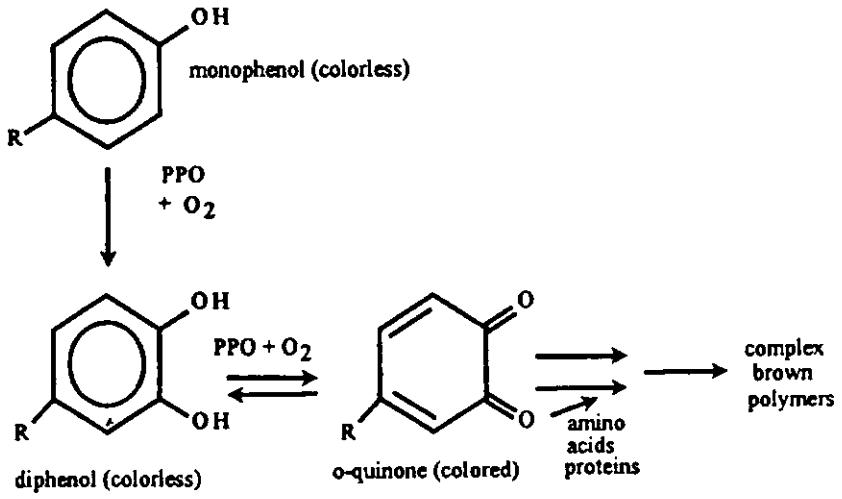

Figure 2.2.1 The mechanism of melanosis occurred in shrimps (Bailey *et al.*, 1960).

From figure 2.2.1, we can see that the first two steps of the series are enzymatic reactions catalyzed by polyphenoloxidase (PPO). Melanosis can be prevented by removing the head, gut and blood containing the enzyme and tyrosine. Moreover, other methods like inactivation of the enzyme by boiling, immediate freezing, pH adjustment, vacuum packing, and treatment with antioxidants may be used to control melanosis (Inoue & Kimura, 1999).

2.2.3 Microbial Spoilage

Because of the chemical and metabolic effects of autolysis and physical damage of shrimps, microorganisms can make use of these abundant nutrients on shrimps to grow and cause spoilage. Seafood harvested from temperate waters spoil relatively more quickly than do their tropical or warm-water counterpart because they are 'reinoculated' with psychrotropic Gram-negative spoilage bacteria (Smith *et al.*, 1992; Listen, 1980), such as *Micrococcus*, *Coryneforms* and *Bacillus* (Ashie *et al.*, 1996; Shamshad *et al.*, 1990). Psychrotrophic bacteria, particularly *Achromobacter* are capable of reducing TMAO to TMA (Dalgaard *et al.*, 1993). Other compounds produced as a result of microbial activity are acids, various amines and ammonia from amino acids, lower fatty acids from sugars such as glucose and ribose, carbonyl compounds from lipids, and indole from proteins. Such derived compounds cause the change in shrimp flavor during storage.

Besides spoilage, microorganisms may cause food poisoning. As found by Dalsgaard (1994), *Salmonella* and *Vibrio cholerae* were common food poisoning species found in the South-East Asia.

2.3 Shrimp Freshness Determination Methods

There is wide range of methods for the determination of seafood freshness. They include subjective sensory evaluation, objective physical, chemical and microbiological examinations.

2.3.1 Sensory Evaluation

Spoilage of shrimps could lead to the formation of malodorous substances, flavor deterioration, toughness, mushiness, juiciness, dryness and discoloration (Pedraja, 1970). All these were perceived as defects which could be detected by the human senses including odor, touch, sight, and flavor (Botta, 1995). Such usage of our senses for the examination of the food was sensory evaluation.

The definition of sensory evaluation prepared by the Sensory Evaluation Division of the Institute of Food Technologists, USA (Stone & Sidel, 1985) was quoted below:

> Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the sense of sight, smell, taste, touch and hearing

This definition makes it clear that sensory evaluation encompasses all the senses and not solely a taste testing. Sensory Evaluation was important in shrimp quality assessment. All physical, chemical and microbiological analyses need to be correlated well with sensory evaluation before they can be used as an indicator of freshness. Therefore, the shrimp freshness determination would be inadequate without sensory evaluation. If sensory analytical tests are properly conducted, with appropriate control of operational variables, they can yield reliable and reproducible data (Sawyer, 1987).

2.3.2 Physical Measurement

In order to determine the freshness of fish, many physical and chemical methods have been proposed other than sensory methods. Physical methods included electric resistance, hardness of meat, turbidity of crystalline lens, color and water holding

capacity (Ohashi, 1991). However, only limited researches studied their effectiveness for shrimps.

2.3.2.1 Surface Lightness Measurement

Surface lightness measurement was a measurement of the lightness on the surface of shrimps by a tri-stimulus colorimeter. Some researchers proposed the use of colorimeter for measuring fishes qualities (Botta, 1995; Bonnell, 1994) by measuring the color (lightness to darkness, yellowness to blueness, and redness to greenness) in fishes and compare them to the standard. They found this was a quick and convenient process. However, the researches studied only the use of colorimeter on fishes and some of the measurements were destructive. No such attempt was make on using portable colorimeter for measuring lightness on shrimps. Melanosis in shrimps led to the formation of dark spots/patches on the body surface within a few hours after their death. Therefore, surface lightness measurement might be a method for measuring melanosis, which in turn reflected the freshness, of shrimps if a good correlation could be established between them.

2.3.3 Chemical Analysis

Chemical tests were used more generally than physical methods for seafood freshness determination. They included pH, total volatile basic nitrogen (TVBN), volatile acid, trimethylamine (TMA), betains, polyamines, free basic amino acids and K value etc (Ohashi, 1991). The most widely used analyses for seafood freshness determination included TMA and TVBN which were detailed in the following sub-sections.

2.3.3.1 Trimethylamine Determination

Recently, researchers developed many different TMA determination methods which included gas sensor (Ohashi, 1991), diagnostic test strip (Wong, 1988), headspace analysis (Krzymien & Elias, 1990) and enzymatic determination (Wong & Gill, 1987).

TMA may be derived possibly partly by intrinsic enzymes (Partmann, 1965) but certainly by bacterial enzyme action from TMAO (Shewan, 1977). Trimethylamine oxide (TMAO) was an odorless substance that naturally present in seafood and was used for osmoregulation (Kelly & Yancey, 1999). When the fish or shellfish died, upon bacterial action by such as *Pseudomonas putrefaciens* (responsible for 80% TMA production) and *Achromobacter* (Laycock & Regier, 1971), TMAO was degraded to TMA which generated fishy smell when combined with fatty substances. Numerous studies on iced or refrigerated fish species have used TMA concentration as an index of freshness because TMA in muscles of various spoiling seafood has correlated well with sensory scores, primarily due to its volatility and low odor threshold at 600 ppb (compare to ammonia, 110,000 ppb)(Ikeda, 1979, Connell, 1990, Colby *et al*, 1993).

The chemical structures of TMAO and TMA were shown below:

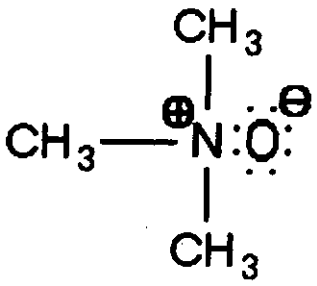

Figure 2.3.1 Trimethylamine Oxide

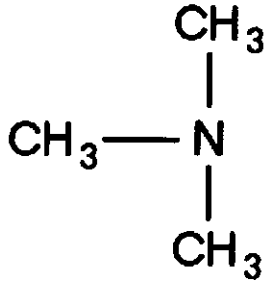

Figure 2.3.2 Trimethylamine

The enzyme involved will also use compounds such as trimethylamine oxide, $(CH_3CH_2)_3NO$, and tripropylamine oxide, $(CH_3CH_2CH_2)_3NO$, as substrates, and thus has been called a triamineoxidase. The apparent function of the enzyme is to activate the substrate so that a dehydrogenase in the bacterial wall can then act on the substrate. A number of compounds can serve as a hydrogen source. Two compounds that probably are physiologically important are lactic acid and pyruvic acid:

$$CH_3CHOHCO_2H + TMAO \rightarrow CH_3COCO_2H + TMA + H_2O$$

Lactic Acid — Pyruvic Acid

$$CH_3COCO_2H + TMAO + H_2O \rightarrow CH_3CO_2H + CO_2 + TMA + H_2O$$

Pyruvic Acid — Acetic Acid

2.3.3.2 *Total Volatile Basic Nitrogen Determination*

Since some species contained very low TMAO level, the detection of TMA is very difficult. Therefore, any increase in TMA seemed inconspicuous. On the contrary, TVBN could provide a better picture for any change in spoilage because the measurement of TVBN actually included TMA, DMA which was formed by autolytic enzymes during frozen storage, and ammonia which was produced via protein degradation and enzymatic breakdown of nucleotides (Pivarnik *et al.*, 1998).

2.3.4 Microbiological Examination

As we know, spoilage of seafood was mainly caused by microbial activities, and some pathogenic bacteria could cause food poisoning. It was important for us to examine microbial load in shrimps so as to monitor the spoilage and to monitor the hygiene condition. Basavakumar *et al.* (1998) reported that the aerobic plate count was negatively correlated to the mean organoleptic scores. However, the long detection time of traditional microbiological methods made it inconvenient for applying in the determination of seafood quality. Alternative microbiological techniques such as direct epifluorescent filter technique and biosensors were recently developed that could provide much shorter microbial detection time (Hanna, 1992).

In the hygienic point of view, the maximum microbial count of 10^7 cfu/g had been suggested by Farooqui *et al.* (1978) as the acceptable condition in shrimps.

2.4 Principles of Shrimp Preservation

It was sure that spoilage could be delayed under freezing and chilling temperatures because the rate of biochemical reactions in the spoilage microflora was reduced. Some researchers studied the effect of chilling and freezing storage on freshness of shrimps (Basavakumar *et al.*, 1998, Yamagata & Low, 1995). Chilled shrimps were more preferred by consumers then frozen shrimps. The price for frozen shrimps could be as low as 20% of the fresh ones. However, the energy cost in providing chilling temperatures would be high, especially in the tropical region. Thus, other preservation methods should also be developed in combination with refrigeration to lower the expenditure in preservation. To inhibit the growth of bacteria in shrimps, we could change some factors in the storage environment, such as temperature, pH, presence of preservatives, availability of substrates and water activity etc. These factors were regarded as 'hurdles' for the survival of microorganisms. The critical value of a particular factor for the survival of bacteria could change if other preservative factors were present in shrimp, and these leading to the simultaneous effects of different preservative factors, if added together, could be synergistic. This hurdle concept was first introduced to the food industry in 1978 by Leistner. Although microbes could generate stress shock proteins under stressed conditions (Leistner, 1999) such as extreme heat, pH and water activity. However, simultaneous exposure to different stresses would require much energy to synthesize stress shock proteins, which in turn might cause the microbes metabolically exhausted (Leistner, 1996).

Modified/Controlled atmosphere packaging (Gopakumar, 1998), freezing, irradiation (Thorne, 1991), edible films/coatings packaging (McHugh & Krochta, 1994), lactic

acid culture (Kim & Hearnsberger, 1994) innoculation, utilization of glucose oxidase (Pivarnik, 1986), acid bath and addition of preservatives (Russell & Gould, 1992) were currently used preservation methods. Besides, there were traditional ones such as salting, smoking, drying etc. (Frazier & Westhoff, 1988). Some more advanced preservation techniques employ lysozyme, light and electricity (Rice, 1994) as well as copper (II) ascorbate (Graf, 1994) for food preservation.

The combined usage of various inhibiting factors could avoid extreme use of any single treatment (Gould & Jones, 1989) which would generate potential hazards. The following sections revealed some principles of these preservation methods that were employed in this project.

2.4.1 Physical methods

Physical methods included cooling, cooking, coating, electricity application etc. Chilling was the most widely used method. Advanced fishing boat in the South-East Asia might use built-in freezer to blast freeze while small boats might use ice blocks and heat-insulated storage cabins for keeping their seafood.

2.4.1.1 Chilling

Ratkowsky *et al.* (1982) showed that the growth rate of a wide range of spoilage bacteria fitted a square law. Solving their equation for spoilage rates of shrimp stored at temperatures between 0 and 15°C, Storey (1986) obtained the relative rate function $R=(0.1T +)^2$, where T was the temperature in °C, and confirmed its relevance to data from sensory analyses. On the basis of the above research, one could see that shrimp

spoiled more than twice as fast at 5°C than at 0°C, and 4 times as fast at 10°C (Davis, 1993).

However, if we used subzero temperature for storage and make the shrimps frozen, the flesh after thawing would become poor in quality. It was because the cells in shrimps were broken in freezing and the fluid inside flowed out of them during thawing. Moreover, freezing required more energy when compared with chilling. Chilling caused minimal changes to the sensory properties and appealed more to consumers (Keizer, 1995). Therefore, chilling would be a better choice if it could be supported by other preservatives such as those mentioned in the hurdle concept.

2.4.1.2 Controlled/Modified Atmosphere Packaging

The use of barriers to separate air from the food was successful in preserving many foodstuffs. Two types of preservation methods made use of the concept of barriers namely, controlled/modified atmosphere packaging (MAP) and surface coating were commonly used. Actually the term Controlled Atmosphere Packaging (CAP) refer to the packaging that the atmosphere inside has a known, intended composition at all times while in Modified Atmosphere Packaging (MAP), the atmosphere might change during subsequent storage. However, the two terms are often used synonymously. Strictly speaking, MAP was a mixture of physical and chemical methods as different gases were used in filling the packaging bags. The technology in MAP had been developed rapidly in the recent years. Numerous types of packaging films and gas combinations were developed and their effectiveness for the fishery industry were

studied (Gopakumar, 1993). Table 2.4.1 and 2.4.2 showed the physical properties and gaseous permeability of various packaging films.

Table 2.4.1 Physical properties of various packaging films (Dulin 1978).

Packaging	Specific Gravity	Tensile Strength kg/cm^2	Elongation %	Tear Strength g/25 mic	Bursting Strength kg/cm^2 25 mic	Yield m^2/kg, 25 mic
LDPE	0.91-0.93	70.4-240	200-800	100-400	0.70	42.6
LLDPE	0.91-0.92	246-492	640-680	80-800	-	42.0
HDPE	0.95-0.96	173-357	10-500	15-300	1.40	4.12
HMHDPE	0.95	438-493	350-500	-	-	41.0
IONOMER	0.94	173-337	300-400	48-264	-	42.0
CPP	0.91	306-398	50-600	40-330	2.10	44.0
PVC	1.23-1.35	459-561	120-375	-	1.7-2.8	28.4-32.7
PVDC	1.65-1.69	844	40-100	10-20	1.4-2.8	22.7-32.7
PET	1.35-1.39	1785	70-100	13-80	3.2-3.5	28.4
NYLON-6	1.12	704-1264	250-500	20-50	7.0-12.6	34.1
PC	1.20	704-755	95-115	20-25	19.3	32.3
PS	1.05	357-847	1-3	5	3.5	37.1
EVOH	1.13-1.21	398-1601	235-325	400-500	16.2-23.2	32.6
EAA	0.94	430-454	520-545	374-467	-	41.8
MST CELLO	1.44	632-1264	15-23	2-10	2.1	29.4
MXXT CELLO	1.44	632-1264	25-50	7-15	2.0	29.4

Remarks:
1 MPa = 10.2 kg/ cm2 = 145 psi
1 N – 0.1020 kgf

Table 2.4.2 Gaseous permeability of various packaging films (Dulin, 1978).

Film 25 micron	WVTR g/m^2, 24hr. 38°C/90%RH	Gas Transmission Rate ml. 25 mic/m^2, 24hr. atm at 25°C		
	Water Vapor	**Oxygen**	**Carbon dioxide**	**Nitrogen**
LDPE	18.6	7750	41850	2790
HDPE	4.6-10.0	2868	8990	651
IONOMER	19-27	4820-6850	9360-15500	-
CPP	7.8-10.0	2325-3720	7750-12400	620-744
PVC	3-40	124-465	310-465	16-155
PVDC	1.5-4.6	12-107	59-682	2-23
PET	20.1	47-62	233-387	11-16
NYLON-6	388	40	155-186	14
PC	150	4650	16682	775
PS	108-155	3875-5425	13950	1500
EVOH	22-59	0.5-20	0.7-24.6	0.02-40
EAA	22.3	4557	-	-
MST CELLO	7.8	8	6-93	8-25
MXXT CELLO	7.0	3	40-50	-

Many combinations of gas mixtures had been examined experimentally for their effects on seafood preservation. Some researchers reported successful preservation with lower level (11.5 to 25%) of CO_2 while others recommended 100% of it. Despite of this, an initial CO_2 concentrations between 30 and 60% were most widely recommended. On the other hand, the concentrations of O_2 recommended by researchers were more divergent. Some recommended no O_2 while others up to 40% (Davis, 1993). N_2 was used in MAP as it had a low solubility in water and lipid. Moreover, it was inert to microbial activities. N_2 could be added to avoid the collapse of packs while high concentration of CO_2 was used, and could enable the full effects of CO_2 (Gill & Penny, 1988) in preserving foodstuffs.

2.4.2 Preservatives

They covered chemical preservatives and lysozyme as follows. Preservatives either targeted at destroying the biochemical functions or physical structure of microbes. There were numerous types of chemical preservatives ranging from most simple one like table salt to more complicated ones like 4-Hexylresorcinol. Preservatives used in this research were illustrated below.

2.4.2.1 Lysozyme

Lysozymes were important components in preventing bacterial growth in foods of animal origin such as hen eggs (Mayes & Takeballi, 1983; Ng & Garibaldi, 1975) and milk (Brunner, 1981, Vakil, 1970). Lysozyme from egg white has outstanding performance as a food preservative because it was able to destroy bacterial cell walls (peptidoglycan) but harmless to human beings. Moreover, industrial methods have been developed for its economical recovery from egg whites.

Figure 2.4.1 presented diagrammatically the three-dimensional structure of egg-white lysozyme.

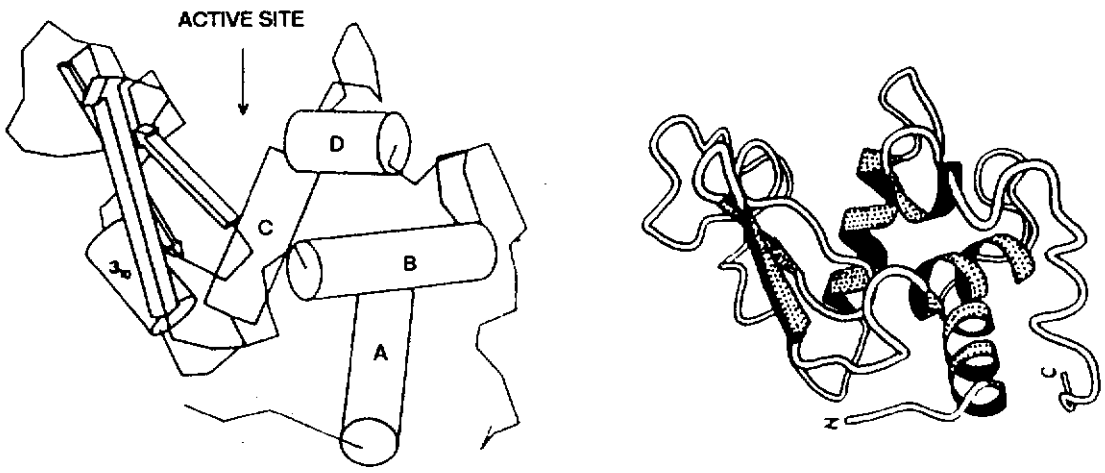

Figure 2.4.1 The three-dimensional structure of egg-white lysozyme (Adapted from Law & Goodenough, 1991).

Chander and Lewis (1980) studied the effect of lysozyme and sodium ethylenediaminetetraacetate (EDTA) on shrimp microflora. The results showed that lysozyme had the potential in extending the shelf life of shrimp. Hughey and Johnson (1987) found that lysozyme might have selected applications in food preservation, especially when thermophilic sporeformers created the problem. They also found that lysis of several spoilage bacteria was enhanced when lysozyme was used in combination with EDTA particularly with certain strains of *C. botulinum*, many of which were completely refractory to lysozyme alone but were inhibited and lysed by lysozyme plus EDTA. EDTA could allow partial removal of certain cell wall components and promote penetration of lysozyme to the peptidoglycan. Since cell wall destruction occurred most effectively in lowered temperatures, lysozyme was suitable for preserving chilled seafood. Other potential applications included its usage in heat-sterilized products to reduce thermal requirements, its inclusion in immobilized enzyme columns to prevent contamination (Fox & Morrissey, 1980), and its usage as

a supplement to foods such as poultry, sausage, and sake as a preservative (Fox & Morrissey, 1980; Hayashi, 1981; Palumbo, 1986; Samuelson *et al*, 1985).

Biochemical reaction performed by lysozyme was hydrolysis of 1,4-β-linkages between N-acetylmuramic acid and 2-acetamido-2-deoxy-D-glucose residues in a mucopolysaccharide or muropeptide. Therefore, the enzyme specifically lysed bacterial cell wall and avoided spoilage when used in foods but was harmless to humans.

Because lysozymes occurred naturally in humans, and since egg white lysozyme was destroyed in the human stomach and during cooking, it was likely that lysozyme can serve as a safe and widely used food preservative.

However, it was also possible that lysozyme might increase the risk of food poisoning by promoting the release of intracellular toxin because it might lyse certain cells that contained the toxin (Hughey and Johnson, 1987).

2.4.2.2 Sodium Propionate

Propionic acid and its salts had been used as antimicrobial agents for a long time since 1938 (Hoffman *et al.*). Virtually all countries with industrialized bread production permitted the use of sodium and calcium propionate, and some countries even allowed propionic acid itself, for the preservation of bread and in certain instances also other baked goods. As a food preservative, propionic acid accumulated in the bacteria and blocked metabolism by inhibiting enzymes and lowering intracellular pH value (Salmond *et al.*, 1984). Owing to its low dissociation constant, propionic acid could be used for preserving foods with a high pH value. The antimicrobial action of

propionic acid and its salts was weak in comparison with other preservatives. In practical food preservation, they has to be employed in relatively high dosages.

Mold, yeast and Gram-negative bacteria (major microflora of shrimps) were inhibited by sodium propionate while some yeast e.g. Torula spp. were capable of utilizing propionic acid in their metabolism.

2.4.2.3 Sodium Erythorbate

Sodium erythorbate was a stereoisomer of sodium ascorbate. Its antimicrobial effects were relatively weak (only 5 to 7 % of ascorbate). It was because sodium erythorbate was a reducing agent that was capable of reducing reactive orthoquinones back to diphenols (Lambrecht, 1995). Thus, it could be used for controlling enzymatic browning or melanosis in shrimps.

Therefore sodium erythorbate was used mainly for preserving colors in foodstuffs. For example, the browning of mushrooms could be controlled by 4 to 5 % of sodium erythorbate. It was hoped that sodium erythorbate could be useful in controlling blackening of our shrimp samples as well.

2.4.2.4 Sodium EDTA

Sodium EDTA reacted with divalent metal ions and might act synergistically with other antimicrobial agents. EDTA could be regarded as a supportive agent because it increased the permeability of bacterial cell membranes to other preservatives. For examples, Gram-negative bacteria were normally resistant to the action of lysozyme, but they could be lysed in the presence of EDTA (Conner, 1993).

EDTA could also complex with the cations in the microbial cell wall and caused leakage of cell solutes (Leuck & Jager, 1997).

2.4.2.5 Sorbic Acid

Sorbic acid had been used for a long period of time for inhibiting the growth of mold and yeast in foodstuffs. It was effective in inhibiting both Gram-positive and Gram-negative bacteria. The permitted usage level of sorbic acid was varied in different countries but the effective antimicrobial concentrations of which was in the range of 0.05 to 0.3% (Sofos, 1989). The FDA/USA permitted maximum sorbic acid level was 0.2%.

Some researchers found that the combination of sodium acetate and *Bifidobacterium breve* culture could prolong shelf life of shrimps by three days (Al-Dagal *et al.*, 1999). The drawback of using sorbic acid was that it generated acid odor and taste in foodstuffs.

2.4.2.6 4-Hexylresorcinol

4-Hexylresorcinol aroused the interest of food scientist recently for using it as an alternative of sodium metabisulphite. It was because some researchers reported that sodium metabisulphite could be allergic and caused asthma in some individuals after consumption of sulfite-treated shrimps (Taylor *et al.*, 1986).

4-Hexylresorcinol could be used for inhibiting melanosis at concentration as low as 0.005%. Moreover, it was evaluated by FDA as 'generally recognized as safe' (GRAS) substances. McEvily (1991) demonstrated that 1 minute dip of shrimps in 0.005% 4-Hexylresorcinol could preserve their shell life up to 12 days.

Unlike the action of sodium erythorbate or other reducing agents, 4-Hexylresorcinol reacted irreversibly with the polyphenoloxidase involved in the browning reaction. The enzyme was then inactivated and could not transform monophenol to diphenol and then o-quinone any more.

2.4.2.7 Sodium Metabisulphite

Similar to all other reducing agents, sodium metabisulphite was capable of reducing the o-quinone (the intermediate product of the browning reaction) back to diphenol which was colourless. However, the dosage of sodium metabisulphite was much higher than 4-Hexylresorcinol at about 1 to 2% (McEvily *et al.*, 1991).

Beside the function of melanosis inhibition, sodium metabisulphite reacted with water to form sulfurous acids in foods. The undissociated acids were capable of penetrating the microbial cell walls and reacting with acetaldehyde, reducing disulfide linkages in enzymes, and finally interfering the respiratory reactions (Ashie *et al.*, 1996).

Section 3

Materials and Methods

SECTION 3

Materials and Methods

3.1 Shrimp Samples

The shrimp specie selected for the research was *Penaeus monodon* with common name of Black Tiger Shrimp or Grass Shrimp. Grass Shrimp was the major shrimp source in the South East Asia region. Plate 3.1.1 showed the appearance of the shrimp specie.

Plate 3.1.1 A fresh Grass Shrimp (*Penaeus monodon*), the target shrimp specie of this research.

3.1.1 Sample Source

The shrimps were caught in the coastal waters of Hong Kong by shrimp boats during the summer season from May to October. Shrimps caught on board the shrimp boats were kept alive in seawater, aerated without feeding and were shipped to the loading pier (Aberdeen, Hong Kong) within 6 to 8 hours.

Shrimps were then loaded into aerated tanks and transported to the laboratory within half an hour.

3.1.2 Sample Preparation

The preservation systems studied in this research were summarized in table 3.1.1.

Table 3.1.1 Summary of preservation systems.

Phase	*Preservatives*	*Shell-On/Off*	*Storage Condition*	*Analyses*
I	Control	On	In tap water ice-cubes, 0-2°C, air	1.Sensory Evaluation, 2.Surface Lightness Measurement
	100mg/L Lysozyme	On	In *modified ice-cubes, 0-2°C, air	
	1% Na Propionate	On		
	4.5% Na Erythorbate	On		
	1mM Na_2EDTA	On		
	0.2% Sorbic Acid	On		
	0.0025% 4-HR	On		
	0.005% 4-HR	On		
	0.01% 4-HR	On		
	1.25% $Na_2S_2O_5$	On		
II	Control	On	In tap water ice-cubes, 0-2°C, air	1.Sensory Evaluation, 2.Trimethylamine Determination
	0.01% 4-HR + 100mg/L Lysozyme	On	In *modified ice-cubes, 0-2°C, air	
	0.01% 4-HR + 1% Sodium Propionate	On		
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	On		
	1.25% Na $Na_2S_2O_5$+ 1% Sodium Propionate	On		

Table 3.1.1 (con't) Summary of preservation systems.

Phase	*Preservatives*	*Shell-On/Off*	*Storage Condition*	*Analyses*
III	Control	On	In tap water ice-cubes, 0-2°C, air	1.Sensory Evaluation, 2.Aerobic Plate Count, 3.Surface Lightness Measurement
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	On	In *modified ice-cubes, 0-2°C, air	
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme + 1mM Na2EDTA	On	In *modified ice-cubes, 0-2°C, air	
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme + 0.01% 4-HR	On		
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme + 1mM Na2EDTA + 0.01% 4-HR	On		
IV	Control	On	0-4 °C, MAP (Air)	1.Sensory Evaluation, 2.Aerobic Plate Count
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	On		
	Nil.	Off		
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off		
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off		

Table 3.1.1 (con't) Summary of preservation systems.

Phase	*Preservatives*	*Shell-On/Off*	*Storage Condition*	*Analyses*
V	Control	On		1.Sensory Evaluation, 2.Total Volatile Basic Nitrogen Analysis, 3.Aerobic Plate Count, 4.Anaerobic Plate Count
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off	0-4 °C, MAP (Air)	
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off	0-4 °C, MAP (CO_2:N_2:O_2 = 4:3:3)	
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off	0-4 °C, MAP (CO_2:N_2 = 4:6)	
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off	0-4 °C, MAP (CO_2)	
VI	Control	On	0-4 °C, MAP (Air)	1.Sensory Evaluation, 2.Total Volatile Basic Nitrogen Analysis, 3.Aerobic Plate Count
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off	0-4 °C, MAP (Air)	
	Nil.	Off	0-4 °C, MAP (CO_2:N_2 = 4:6)	
	1.25% Na $Na_2S_2O_5$+ 100mg/L Lysozyme	Off	0-4 °C, MAP (CO_2:N_2 = 4:6)	

*Remarks: *modified ice was prepared by freezing the corresponding preservative solutions in plastic moulds (2cm x 2cm x 2cm) in freezer (-3 to -8°C).*

The following sections described how the shrimps were preserved by the above systems.

3.1.2.1 Shell-On Samples

Living shrimps arrived the laboratory were chilled to death with crushed ice (Connell, 1990) to reduce deterioration before experiment. Only healthy shrimps with firm texture and with body length ranged from 12 to 15 cm (approximately 20 to 23g) were sorted for experiment. Sorted shrimps were then soaked in freshly made

preservative solutions contained in clean plastic trays for 5 minutes. Handling of samples were conducted as hygienic as possible e.g. disposal gloves and clean plastic trays for containing samples were used. The ratio of kilogram shrimps to liter preservative solution was set at 1 to 2. Then the shrimps were drained, packed in a plastic container (with air holds on the lids) and stored in chiller.

For shrimps that were packed with modified ice, they were put into plastic boxes with holes at the bottom of them. The holes allowed draining of water come from melted ice. The ratio of kilogram sample to kilogram ice was set at 1 to 2. Maximally two layers of shrimps were allowed so as to avoid damage of bottom shrimps. Ice cubes were distributed evenly around each shrimp and were replenished from time to time during storage.

For MAP samples which were stored in bags (see plate 3.1.2), the sharp nostrums in them were cut away to prevent it from stabbing through the packaging bags. Every bag contained 3 shrimps. The ration of gram sample to ml bag volume was about 1 to 3. The volumes inside the bags were checked by its displacement of water inside a beaker.

Plate 3.1.2 Shell-on MAP shrimp samples.

The following parameters were set for the vacuum machine used for MAP (see plate 3.1.3):

Vac = 999

Gas = 500

Seal = 2.0

Cut = 1.8

Prevent = 0

Plate 3.1.3 Vacuum machine for packing MAP shrimp samples.

3.1.2.2 Peeled Samples

Treatment for peeled shrimps were similar to that of shell-on shrimps. However, peeled shrimp samples were first decapitated, de-gutted, and then rinsed under water before putting into the preservative solutions. Please note that all peeled shrimps were for MAP experiments only and therefore they were all kept in bags.

Samples were randomly drawn from the groups during sampling. The drawn MAP sample bags were not allowed to be resealed again and all samples contained inside of them were used up in a single sampling time, otherwise, the atmosphere inside the bags would be diluted by air.

3.1.3 List of Materials and Equipment

Special chemicals such as the preservatives and major reagents, and special equipment (except general glassware) were listed here.

3.1.3.1 Special Chemicals

- Lysozyme : from chicken egg white, Sigma Chemical., 47,000 units/mg powder
- Sodium Propionate : C3H5O2Na, FW 96.06, assay =99%.
- Na_2EDTA : BDH Chemicals Ltd., England.

  $[CH_2.N(CH_2.COOH).CH_2.COOK]_2 2H_2O$, FW 404.47, assay =98%.
- 4-Hexylresorcinol : (4-Hexyl-1,3-dihydroxybenzene), $C_{12}H_{18}O_2$, FW 194.3, AR.
- Sodium Metabisulphite : (Sodium disulphate (IV)), Philip Harris Ltd., England, $Na_2S_2O_5$, FW 190.10, =90%.
- Trimethylamine : (Trimethylamine hydrochloride), Sigma Chemical, $C_3H_9N.HCl$, FW 95.57, AR
- Trichloroacetic acid : RDH, RG.
- Sodium Hydroxide : Peking Chemical, AR.
- Peptone : Difco, USA.
- Tryptone Soya Agar : Oxoid, England.

3.1.3.2 Special Equipment

- Tri-stimulus reflection colorimeter : X-Rite Incorp. USA, Model X-Rite 918.
- Spectrophotometer : Perkin Elmer UV/visible, Model Lambda 3B.
- Blender : New Hartford, CT, Waring Products Division, Model 1120.

- Compressed Air : air tap located in laboratory CF 703, ABCT, The Hong Kong Polytechnic University.
- Gas Cylinder : mixture of 40% CO_2 and 60% N_2, Chun Wang Industrial Gases, Hong Kong.
- Gas Cylinder : mixture of 40% CO_2, 30% O_2 and 60% N_2, Chun Wang Industrial Gases, Hong Kong.
- Gas Cylinder : pure CO_2, Hong Kong Oxygen, Hong Kong.
- Vacuum Packing Machine : Type VC999® 06i, Inauen Maschinen AG, Switzerland.
- MAP bags : Nylon/D/LLDPE 15 70, gases permeabilites at 20°C, 90% RH, 24 hrs, atm are CO_2 : 1458cc/m^2, O_2 : 2573cc/m^2, N_2 : 148cc/m^2 Hong Kong Packaging Co. Ltd., Hong Kong.

3.2 Shrimp Freshness Determination Methods

The various methods deployed for monitoring the changes in shrimps during storage included sensory evaluation, physical measurement of surface lightness, chemical determinations of TMA level and TVBN level, and microbiological determination of aerobic and anaerobic plate count.

3.2.1 Sensory Evaluation

Sensory tests were usually classified into Hedonic (affective) Quality Testing and Sensory Quality Testing. The former provided information on such as pleasantness or unpleasantness, acceptability, like or dislike while the latter gave the similarities or

differences of samples on sensory attributes such as texture, odor, and taste (Sawyer, 1987).

Hedonic quality testing was conducted in the research. For a particular sensory attribute, say odor, even though the difference between that of the control and that of the preserved samples might be great but it did not necessarily mean the preserved samples were unacceptable. For example, there were two different preserved samples, one of which had acidic smell and the other had pungent smell, both of their odors were greatly different from that of the control, yet the pungent one would obviously be rejected by consumers while the other one might not.

Sensory quality attributes evaluated were odor, texture, color, integrity, general appearance, melanosis and overall acceptability. These properties are widely used for sensory evaluations (Poole, 1994; Wong, 1988; Scott 1986; Stone & Sidel, 1985). The 9-point hedonic scale used in this research was limited to nine intervals, except melanosis evaluation which had 6 intervals (0,2,4,6,8,10). The scale was rather easy to operate and was used frequently by researchers. Details can be seen in Appendices.

3.2.1.1 Materials and Equipment

- Sensory evaluation forms (see Appendices)
- Pens
- Labels for coding samples
- Disposable gloves for hygienic shrimp handling
- 1 liter beaker, plastic sieve and hot plate for cooking samples

3.2.1.2 Procedures

1. A shrimp was put on a container with an evaluation form and a pen placed next to it.
2. Panelists were asked to evaluate all the samples in turn.
3. The attributes evaluated was in the order of odor > appearance/colour > integrity > texture > overall acceptability. Odor evaluation was done first to avoid loss of odor to the hands of panelists during the evaluation process. This also prevented the samples from cross contaminating from each other.
   3.1 The evaluation of odor was done by wafting 3-4 sniffs of the samples.
   3.2 The evaluation of appearance, color and melanosis was done by observing the surfaces on the shrimps.
   3.3 The evaluation of integrity was done by holding the shrimps and check the junction between the head and the thorax, the legs and the tails.
   3.4 The evaluation of texture was done by pressing the shrimp bodies and observe the suppleness.
4. The 'pass mark' for the evaluation was 5 except melanosis in which the pass mark was 4 (Otwell & Marshall, 1986).
5. When all panelists finished, all the samples were rinsed by tap water for 1 minute and blanched for 3.5 mins for the subsequent cooked sample sensory evaluation.
6. Cooking of shrimps was done by heating 750 ml water in a 1 liter beaker put in a hot plate (see Plate 3.2.1).

Plate 3.2.1 Cooking of shrimp samples.

7. Cooked samples were then be evaluated in the similar way as raw shrimps except that cooked samples were chewed when the assessors evaluate the texture of them.

Actions had been taken to minimize the biases or confusions of assessors and to collect most reliable and representative information (Larmond, 1977):

- A panel consisted of 6 to 10 well-experienced assessors was responsible for the sensory evaluations. They were technicians, research assistants, researchers, and housewives that had exposure to the sensory assessment of shrimps.
- Panelists were screened, individuals having low sensitivities to the sensory attributes and allergies on shrimps were not qualified for the panel.

- The same bench was used for sensory assessment every time.
- Communication between panelists was prohibited to allow independent judgments.
- Panelists did not know what treatments were done on the samples because no information about the samples were provided before evaluation. It was because such information might introduce expectation error.
- Lightings and air-conditionings were fixed for every evaluation.

Other equipment e.g. the container for sample was the same for all samples in order to minimize variation in experimental conditions.

3.2.2 Physical Measurement

The physical measurement carried out in the research was surface lightness determination.

3.2.2.1 Surface Lightness Determination

Many physical analyses were developed for freshness determination of seafoods, such as torrymeter (Bonnell, 1994) and test strip (Wong, 1988). However, the idea of using portable colorimeter for assessing shrimp freshness was innovative. Shrimps would turn black during spoilage; if we could correlate their lightness with their melanosis, lightness measurement could be a useful method for measuring melanosis and therefore the freshness. Moreover, the portable Tri-stimulus Reflection Colorimeter could provide rapid, non-destructive measurement on the shrimp lightness.

3.2.2.1.1 Materials and Equipment

- Tri-stimulus Reflection Colorimeter
- Sample cartridge (Hand made)

3.2.2.1.2 Procedures

The following settings of the colorimeter were used:

Illuminants : C

Obervers : 2

1. 7 to 12 shrimps samples were randomly drawn from the storage box.
2. Samples were placed on the groove in the cartridge as shown in plate 3.2.2 and plate 3.2.3.

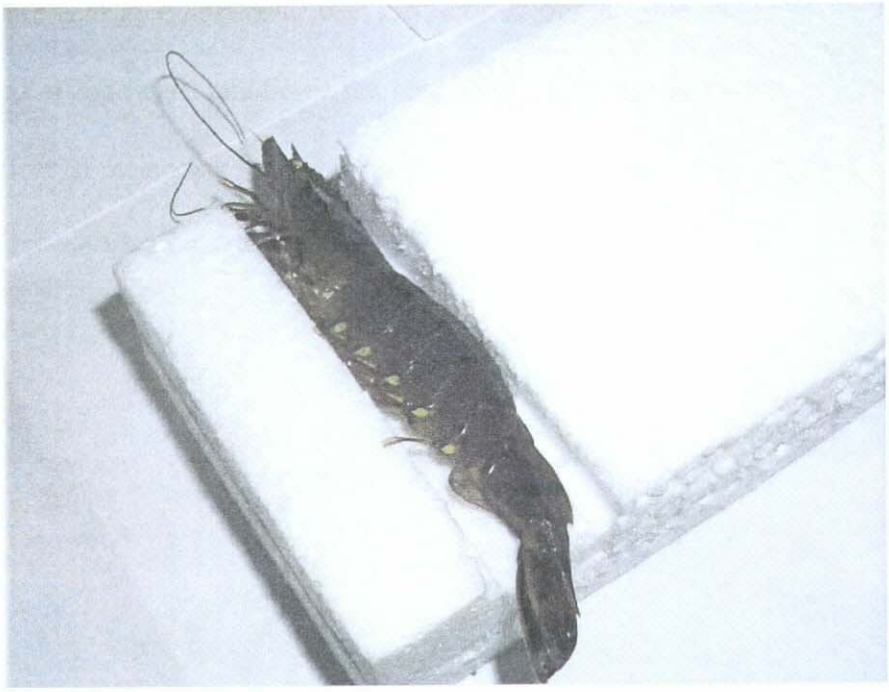

Plate 3.2.2 Shrimp placed on the foam cartridge.

Plate 3.2.3 Measurement of shrimp surface lightness by use of portable Tri-stimulus Reflection Colorimeter.

3. Measurement by colorimeter was then carried out in three defined sampling points located at the head, middle of the main body, and the tail part in order to reduce the effect of melanosis variation in different parts of the body (see plate 3.2.4).

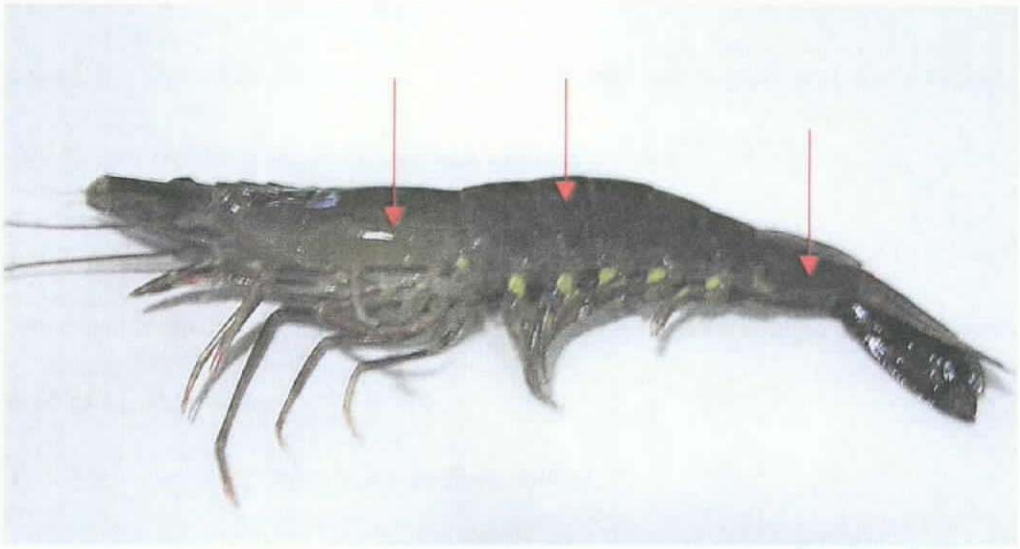

Plate 3.2.4 The three defined sampling points for the surface lightness measurement.

4. Results were automatically averaged and displayed on the LCD screen on the colorimeter.

3.2.3 Chemical Analysis

The chemical analyses carried out in the research were TMA determination and TVBN determination.

3.2.3.1 Trimethylamine Determination

In this colorimetric procedure (Dyer's Method) a sample containing trimethylamine was made alkaline with potassium carbonate in the presence of formaldehyde. The function of formaldehyde was to suppress reaction between ammonia and monomethylamine, otherwise, they would affect results. The alkaline added was for facilitating extraction of the amine to the toluene phase during extraction. The extracted amine in toluene would then react with a toluene solution of picric acid to form trimethylamine picrate. The yellow color was measured at 410 nm.

Although subjected to interferences and occasional errors, the picric acid method developed by Dyer had been most widely used and evaluated and the method was adopted by the Association of Analytical Chemists (AOCS, 1971).

3.2.3.1.1 Materials and Equipment

1. Trichloroacetic acid (7.5% aqueous solution) - by dissolving 75g trichloroacetic acid in 1L D.I. water
2. Toluene - Dry over anhydrous sodium sulfate.

3. Picric acid solutions – by dissolving 2 g dry picric acid in 100 ml of dry toluene; then diluting 1 ml of this stock solution to 100 ml with dry toluene to give working solution.
4. Potassium carbonate solution – by dissolving 100 g potassium carbonate in 100 ml D.I. water.
5. Formaldehyde solution – by shaking 1 liter formalin (40%) with 100 g magnesium carbonate, and filter. Dilute 10 ml to 100 ml with D.I. water.
6. Trimethylamine (TMA) standard solution - To 0.1 g of trimethylamine, add 1 ml concentrated hydrochloric acid (25%) and dilute to 100 ml with D.I. water to give working solution.
7. Spectrophotometer with mica cuvettes
8. Blender (National Mulinex)
9. Filter funnel x 5
10. 250 ml beaker x 5
11. 5 ml pipette x 10
12. Whatman # 2 paper
13. 25 ml test tubes with stoppers
14. 0.2 ml autopipette
15. 1 ml autopipette
16. 5 ml autopipette
17. Test tubes with stoppers

3.2.3.1.2 Procedures

1. Weight about 20g (reading recorded as W) shrimp samples (homogenized shrimp paste of 3 shrimps). Add 40 ml 7.5% trichloroacetic acid and blend (use National Mulinex Blender) for 1 minute.
2. Filter the extract by use of filter paper, No.2 (Whatman) or equivalent.
3. Pipette 1 ml of filtrate into a test tube and add 1 ml water. For standards, prepare several test tubes containing e.g. 10, 20, 35, 50 and 75 µl of the TMA working standard solution and add 2 ml water into each test tube. For blank, use 2.0 ml water.
4. Add 0.5 ml of the formaldehyde solution, 5 ml toluene, and 1.5 ml potassium carbonate solution.
5. Stopper tube and swing by hand approximately 30 times.
6. Pipette 2.5 ml of the toluene extract (upper layer) into a test tube and add 2.5 ml picric acid in toluene solution and mix by swirling 30 times.
7. Determine the Ab at 410 nm of the sample and TMA standards against a water blank subjected to the entire procedure. The color should be stable.
8. If the original sample aliquot contains out-ranged amount of TMA, dilute the filtrate in the filtration step with trichloroacetic acid and repeat the determination.
9. The sampling and analysis were repeated for 3 times for each sampling day.

Calculations:

TMA mg/100g = A x 80[a]/W

Where A = Corresponding absorbance of the sample as found from the standard curve

W = gram sample used for analysis

3.2.3.2 Total Volatile Basic Nitrogen Determination

TVBN analysis was carried out according to the method used by Pivarnik *et al* (1998). The volatile basic nitrogen content was extracted by aqueous trichloroacetic acid from the shrimp samples during homogenization. The volatile basic nitrogen was then liberated by the addition of an alkaline followed by steam distillation in the distillation apparatus. The volatile bases vaporized from the distillation were cooled down to liquid and were trapped in boric acid solution. The concentration in the boric acid was determined by titration with a standard acid at appropriate concentration with the aid of an indicator.

3.2.3.2.1 Materials and Equipment

1. 7.5% Trichloroacetic acid (TCA) solution - by dissolving 75g trichloroacetic acid in 1L D.I. water
2. 2% N Sodium hydroxide (NaOH) solution - by dissolving 4 g NaOH in 500ml D.I. water

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[a] Calculation of dilution was as follow:
(A) 1 ml of sample was drawn from 40 ml extract and dilution was 40.
(B) 2.5 ml of toluene extract mixed with 2.5 ml toluene/pricric and dilution was 2
(C) The total dilution become 40 x 2 = 80.

3. 4% Boric acid solution - by dissolving 40g boric acid (RG grade, Sigma) in 1L D.I. water
4. Mixed indicator solution - by dissolving 40mg methyl red and 1g bromocresol green with 60ml 95% ethanol
5. Standard sulphuric acid solution was prepared with reference to AOAC (1990) Sec 890.01 by diluting approximate 2.8ml 95-98% H_2SO_4 10L. The exact concentration of the acid was determine by standardization by Standard borax method, AOAC (1990) Sec 936.15E
6. Waring blender
7. Filter paper, No.1 (Whatman) or equivalent
8. Steam distillation apparatus
9. General laboratory glassware

3.2.3.2.2 Materials and Equipment

1. Three shrimp samples were randomly drawn, weighted and put into the blender.
2. 2ml of TCA solution was added to each gram of sample and blended for exactly 1 minute at high speed.
3. The homogenate was filter into a conical flask,
4. 5ml of the filtrate was transfer quantitatively to a 250ml quickfit round bottom flask with a few pieces of boiling chips. The flask was then fitted into the steam distillation set up. 10ml of 2% NaOH solution was added to the round bottom flask.

5. The content was steam distilled with distillate output rate of 5ml/min for 20 minutes. The distillate was collected with exactly 10ml 4% boric acid solution with 4 drops of indicator.
6. The boric acid trap was titrated from blue color to the end point (the original yellow color) with standard 0.01N H_2SO_4 solution. The volume (V) of acid used for titration was recorded.
7. Distillation was conducted in duplicate.
8. Blank determination was conducted using TCA solution to replace sample filtrate. The volume (B) of acid used for titration was recorded.
9. The sampling and analysis were repeated for 3 times for each sample.

Calculations:

TVB-N, mg/100g = (V-B) x N x 840 [b]

where V = average volume of H_2SO_4 used for titration

B = volume of H_2SO_4 used for blank determination

N = concentration of H_2SO_4 determined by standardization

3.2.4 Microbiological Examination

The microbiological examination covered aerobic and anaerobic plate count. Anaerobic plate count was carried out for MAP samples in order to find out the

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[b] The formula for TVB-N calculation was derived as follows,
(1) amount of sample in 5ml TCA extract = 5ml x 1g sample/(1g sample +2ml TCA) = 1.667g
(2) amount of Nitrogen (mg) in 5ml TCA extract = (V-B)xNx14 where 14 is the molecular wt of nitrogen
(3) therefore TNB-N mg N/100g sample = (V-B)xNx14/1.667 = (V-B) xN x840

difference in growth patterns of aerobes and anaerobes under modified atmospheres.

3.2.4.1 Aerobic Plate Count

The aerobic plate count method described by Shamshad *et. al.* (1990) was referenced for this research. The shrimps were homogenized with water by a blender. Homogenates were then put into test tubes for series of dilutions. Then the diluents were transferred into petri dishes and mixed with molten agar. Finally, dishes were inverted and incubated for 72 hrs. During storage, the microbial profile will change from predominantly mesophiles to psychrophiles (Stannard, 1997). Therefore, the temperature of 25°C, which flavors the growth of psychrophiles, was used for incubation.

3.2.4.1.1 Materials and Equipment

1. Molten TSA - by dissolving 40g of TSA powders in 1L D.I. water in a conical flask; cap the flask with aluminium foil and then heat it by hot plate to melt all TSA powders and autoclave the solution for 15 min at 121°C.
2. Sterilized saline - by dissolving 8.5 g of sodium chloride in 1L D.I. water and autoclave for 15 min at 121°C.
3. Blender.
4. Autopipette, 1 ml, 5 ml or dispensers.
5. Scale.
6. General microbiology laboratory equipment.

3.2.4.1.2 Procedures

1. Three shrimps were randomly drawn and weighted aseptically.
2. Put the samples into a sterilized blender and add sterilized saline water (4 times of dilution).
3. Cover the blender and blend at low speed for 30 seconds and then high speed for 30 seconds.
4. Draw one gram of the homogenates to a test tube filled with 9 ml of sterilized saline water to make 10 fold dilution.
5. Do a series of dilutions in other test tubes with sterilized saline water.
6. Vortex test tubes after addition of samples.
7. 1 ml of solution as drawn from each test tube were transferred to a petri dishe.
8. Repeat step 7 to obtain duplicated results.
9. Pour about 15 ml molten agar (cooled down to less than 40 °C) to the petri dishes and swirl 5 times clockwise and 5 times anticlockwise.
10. Allow the agar to solidify, then invert and incubate the dishes at 25 °C for 72 hrs.
11. Steps 1-9 were repeated three times for a particular sample treatment.
12. After incubation, dishes containing 30-300 colonies of bacteria were counted and recorded.

Calculations:

APC (cfu/g) = count x dilution

where count = cfu counted in point 11 above

dilution = dilution made for the sample

3.2.4.2 Anaerobic Plate Count

The materials and methods used for anaerobic plate count were similar to that for aerobic plate count. The differences of which were listed in the sections below.

3.2.4.2.1 Materials and Equipment

In addition to the materials and equipment as listed in 3.2.4.1.1, the followings were required for the anaerobic plate count.

1. Anaerobic jars with catalyst
2. Anaerobic kits (Oxoid, England)
3. Anaerobic atmosphere indicator (Oxoid, England)

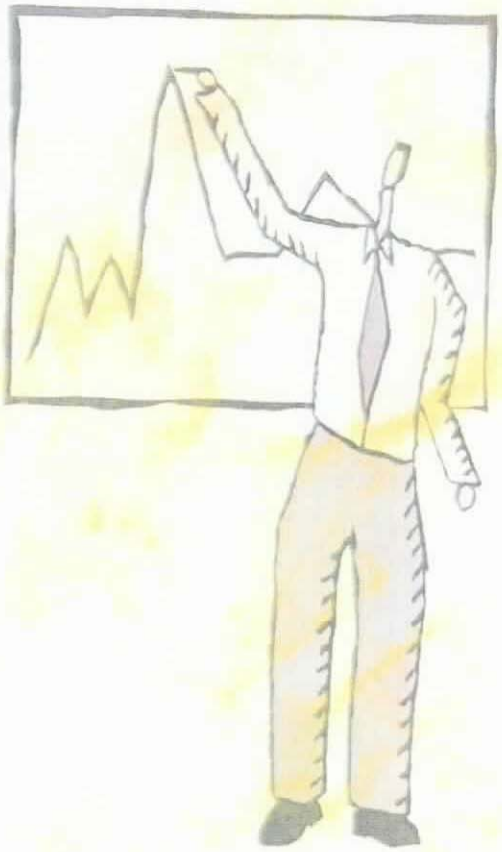
3.2.4.2.2 Procedures

In addition to the procedures as listed in 3.2.4.1.2, the followings were required for the anaerobic plate count.

1. Finished agar plates were put into the anaerobic jars
2. Inject 10ml of water to the anaerobic pouch and place it immediately to the jar.
3. Put catalyst (heated for 2 hrs at 160 °C and cooled down to room temperature) inside the anaerobic jars.
4. Put anaerobic atmosphere indicator into the jars immediately and close the jars tightly.

Section 4

Results and Discussion



SECTION 4

Findings & Discussion

4.1 Phase I Experiment

In this phase of experiment, the effectiveness of individual preservatives were studied. The best performers would be picked out for the next phase of experiment in which individual preservatives were mixed in different combinations.

4.1.1 Sensory Evaluation

The various sensory attributes studied were Odor, Texture, Integrity, Melanosis and Overall Acceptability.

The results of sensory evaluation were shown in Fig. 4.1.1 to Fig. 4.1.5.

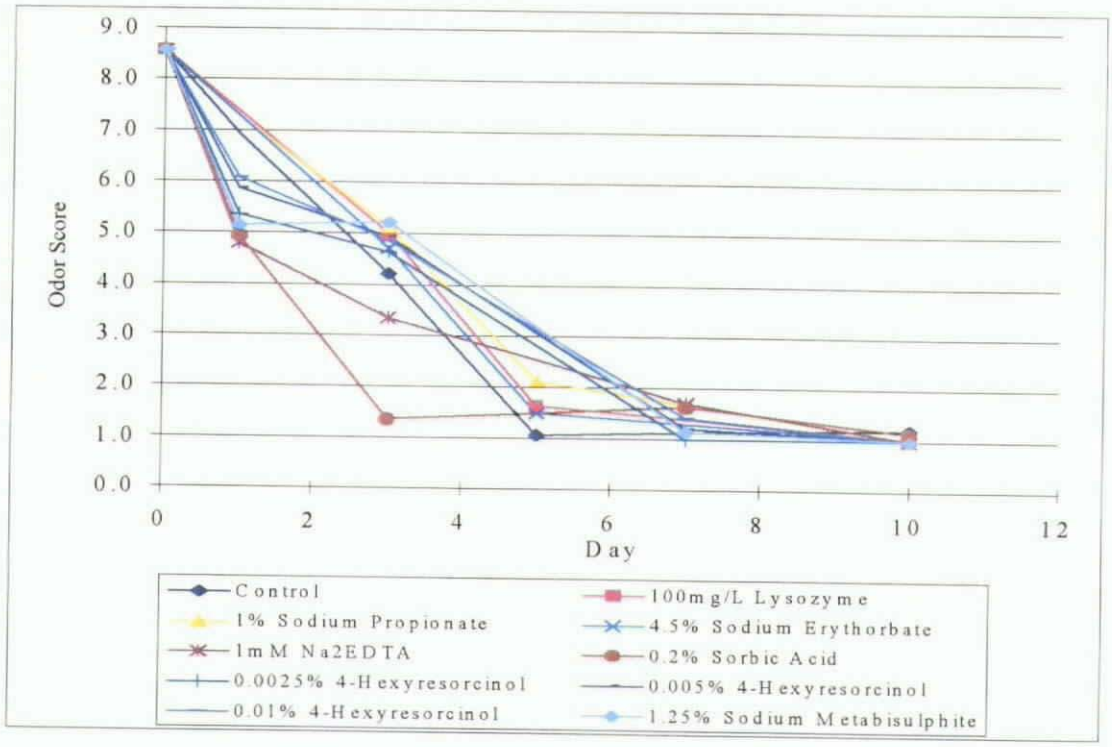


Figure 4.1.1 Change of odor scores of shrimp samples during storage in Phase I experiment.

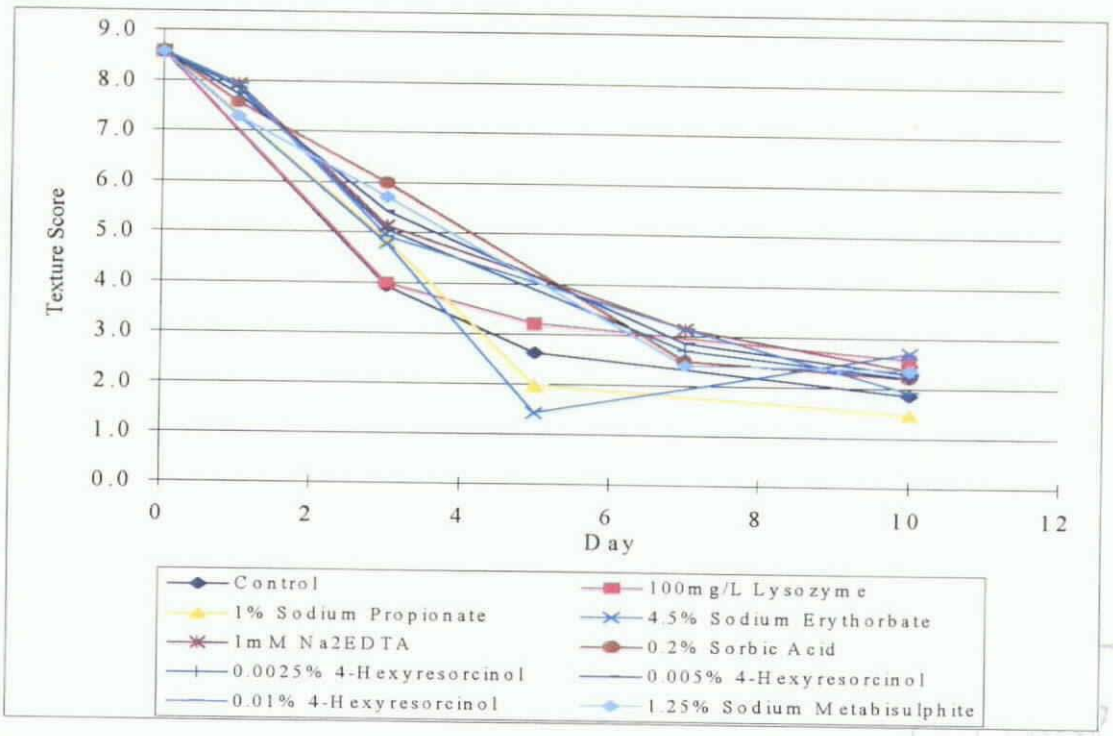


Figure 4.1.2 Change of texture scores of shrimp samples during storage in Phase I experiment.

Amongst the sensory evaluation results, we could found that odor and melanosis were the two quality attributes that deteriorated most rapidly during the spoilage. The following table showed the respective 'sensory-quality-attribute-shelf-lives' of samples as determined graphically from figure 4.1 to 4.5. Sensory-quality-attribute-shelf-life was defined as the shelf life of a sample as determined by a particular sensory quality attribute when the sensory score for that attribute dropped/rose beyond the passing mark (4 for melanosis, 5 for others).

Table 4.1.1 Sensory-quality-attribute-shelf-lives of samples in Phase I experiment.

Treatment	Sensory Quality Attributes Shelf Life (day)				
	Odor	Texture	Integrity	Melanosis	Overall Acceptability
Control	2.3	2.3	4.4	1.5	2.1
Na Propionate	3.0	3.0	4.0	3.0	3.0
Na_2EDTA	1.0	3.3	5.1	1.5	2.7
0.0025% 4-HR	2.0	3.1	4.1	3.0	3.8
0.01% 4-HR	2.7	2.9	4.6	7.0	4.0
Lysozyme	3.0	2.3	3.7	2.1	2.1
Na Erythorbate	2.7	2.8	4.1	2.4	2.5
Sorbic Acid	1.0	4.1	3.8	1.0	2.2
0.005% 4-HR	2.7	3.7	4.2	2.1	3.8
Na Metabisulphite	3.1	3.9	4.2	3.6	3.8
Average	2.35	3.14	4.22	2.72	3.00

From Table 4.1, the odor and melanosis shelf lives of samples were shorter than other shelf lives. That implied odor and melanosis were the two most critical attributes we should preserve as they lead to unacceptable quality of samples in a rather short period of time.

This observation also helped in determining the combinations of preservatives for the next phase of experiment. It was rational to use preservatives that were the best in preserving odor and melanosis. For melanosis, the most outstanding performer was 0.01% 4-hexyresorcinol, and the next one was sodium metabisulphite.

However, in terms of preserving odor, sodium metabisulphite was the best performer, and 0.01% 4-hexyresorcinol was not. Whereas, sodium propionate and lysozyme were the second best ones in odor control.

Therefore, 4-hexyresorcinol and sodium metabisulphite were selected for controlling melanosis while sodium propionate and lysozyme were for controlling odor in the next phase of experiment and combined effects of them would be studied.

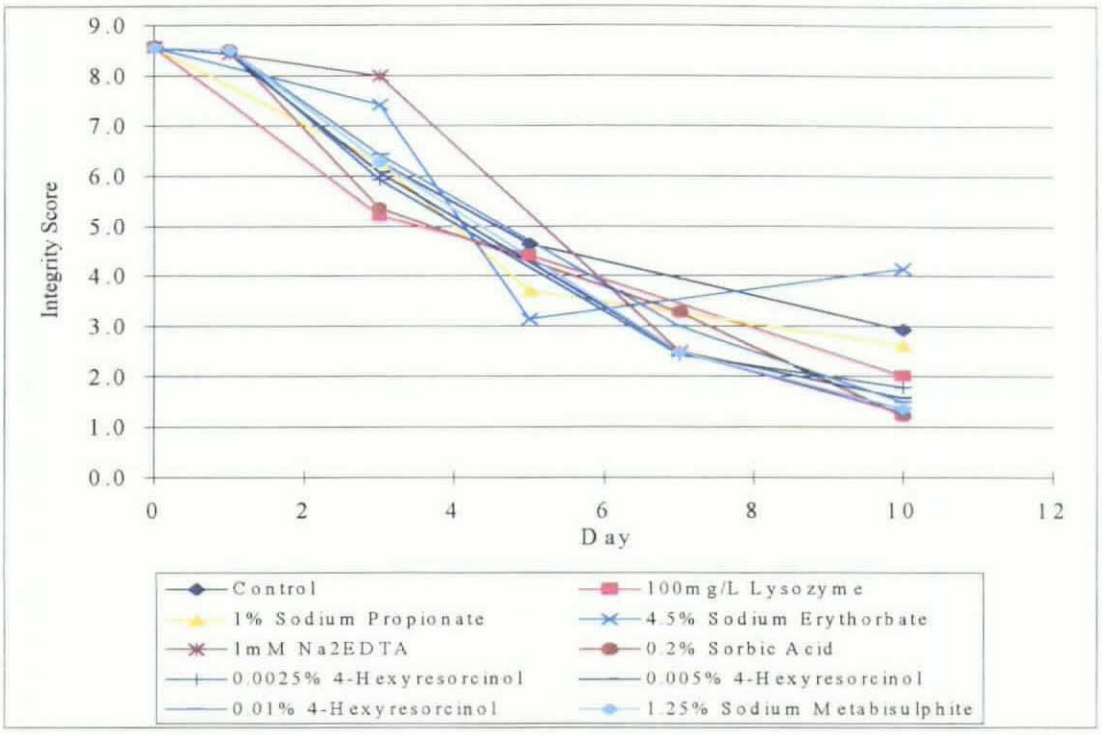


Figure 4.1.3 Change of integrity scores of shrimp samples during storage in Phase I experiment.

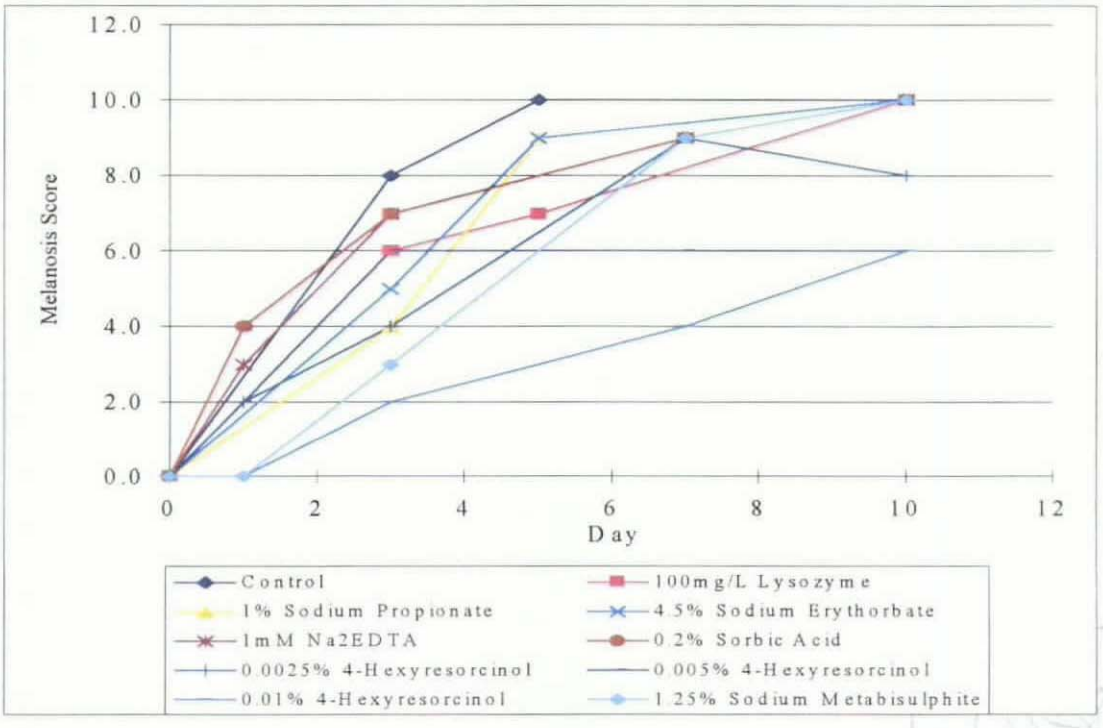


Figure 4.1.4 Change of melanosis scores of shrimp samples during storage in Phase I experiment.

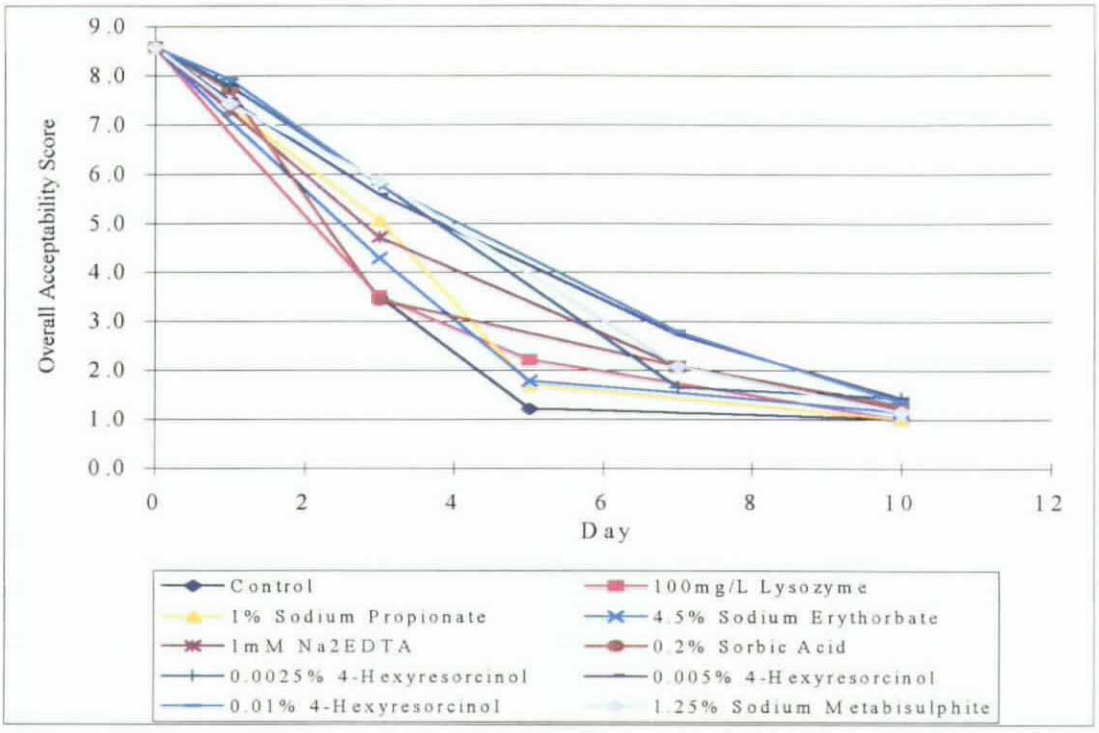


Figure 4.1.5 Change of overall acceptability scores of shrimp samples during storage in Phase I experiment.

4.1.2 Surface Lightness Measurement

Surface lightness measurement was used to monitor the change in lightness/darkness of shrimps during storage. This can be regarded as an objective melanosis measurement and we hope that the method can replace the subjective sensory evaluation of melanosis once the correlation between lightness and sensory score for melanosis could be established.

The surface lightness measurement results were shown in Figure 4.1.6.

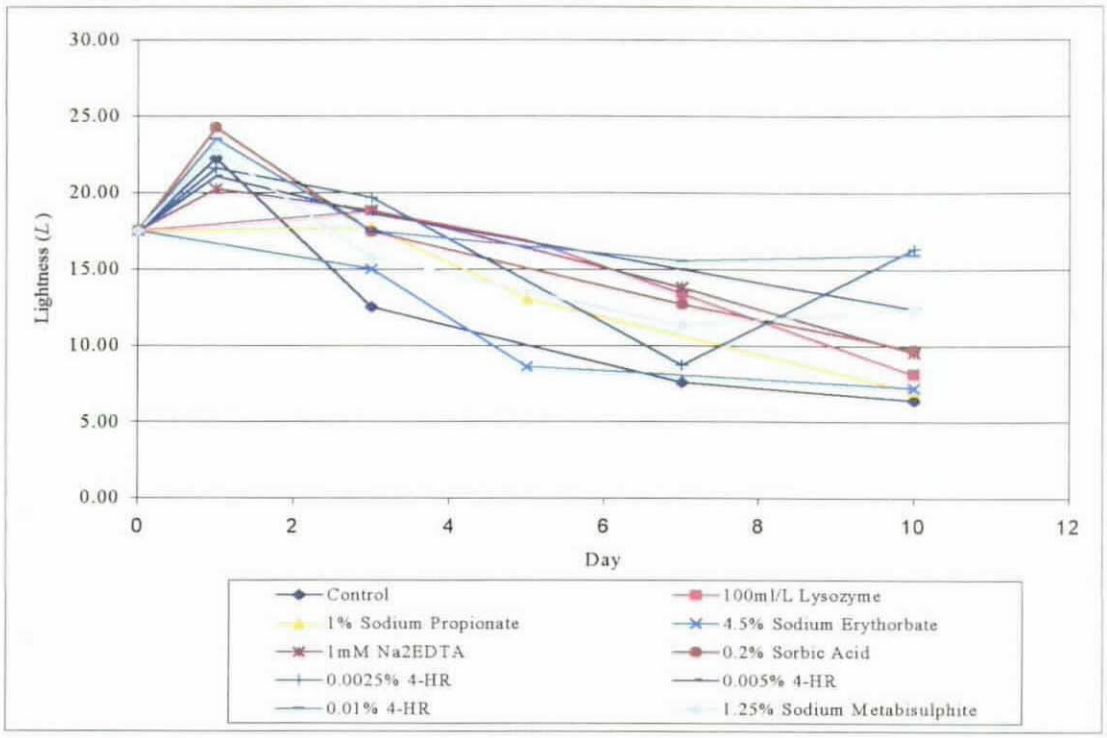


Figure 4.1.6 Change of surface lightness of shrimp samples during storage in Phase I experiment.

The surface lightness (L) measurement results were shown in figure 1.6. We can see that the surface lightness of samples were generally decreasing during the storage. The control samples had the lowest values of lightness and therefore they were the darkest among the samples. After 10 days of storage, the samples treated by 4-hexyresorcinol and sodium metabisulphite had highest lightness values. This finding matched with the sensory evaluation results in which 4-hexyresorcinol and sodium metabisulphite had longest melanosis shelf life. Figure 4.1.7 visualized the correlation between melanosis evaluation and lightness measurement of control samples in Phase I experiment.

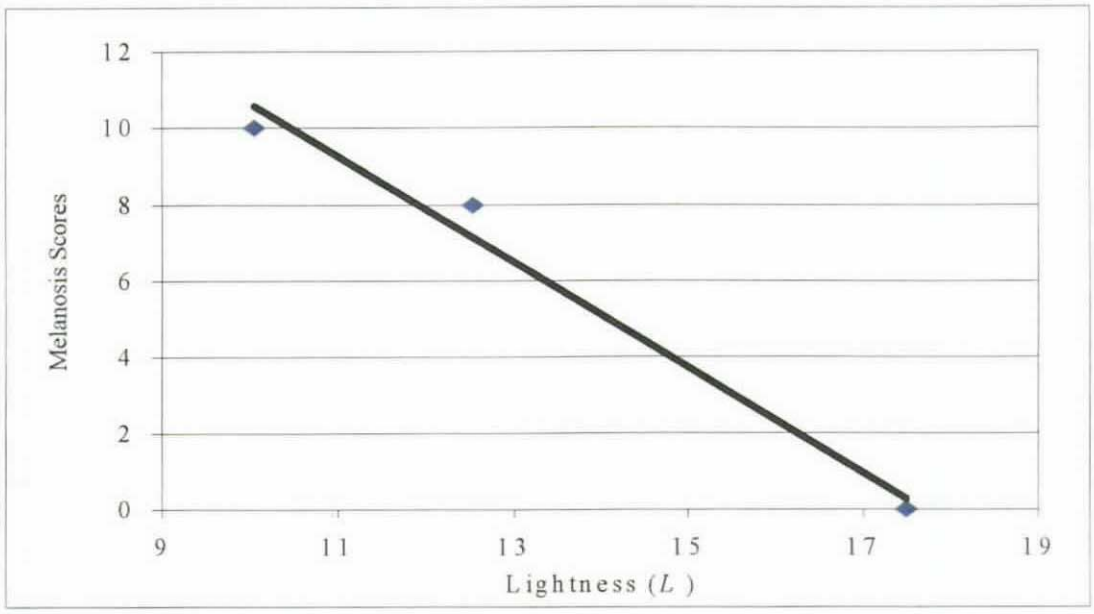


Figure 4.1.7 Correlation between melanosis evaluation and lightness measurement of control samples in Phase I experiment.

The correlation coefficient was –0.99 at 95% confidence level. From figure 4.1.7, the lightness of samples, at the time of melanosis rejection (score = 4), was determined to be 14.8.

The averaged surface lightness of fresh shrimps was determined to be 17.49 (standard deviation = 1.60).

4.2 Phase II Experiment

Combined preservatives were studied in this phase of experiment. It was expected that some of the individual preservatives could act additively or synergistically when they were used together.

4.2.1 Sensory Evaluation

The sensory evaluation results of Phase II experiment were shown in Figure 4.2.1 to 4.2.6.

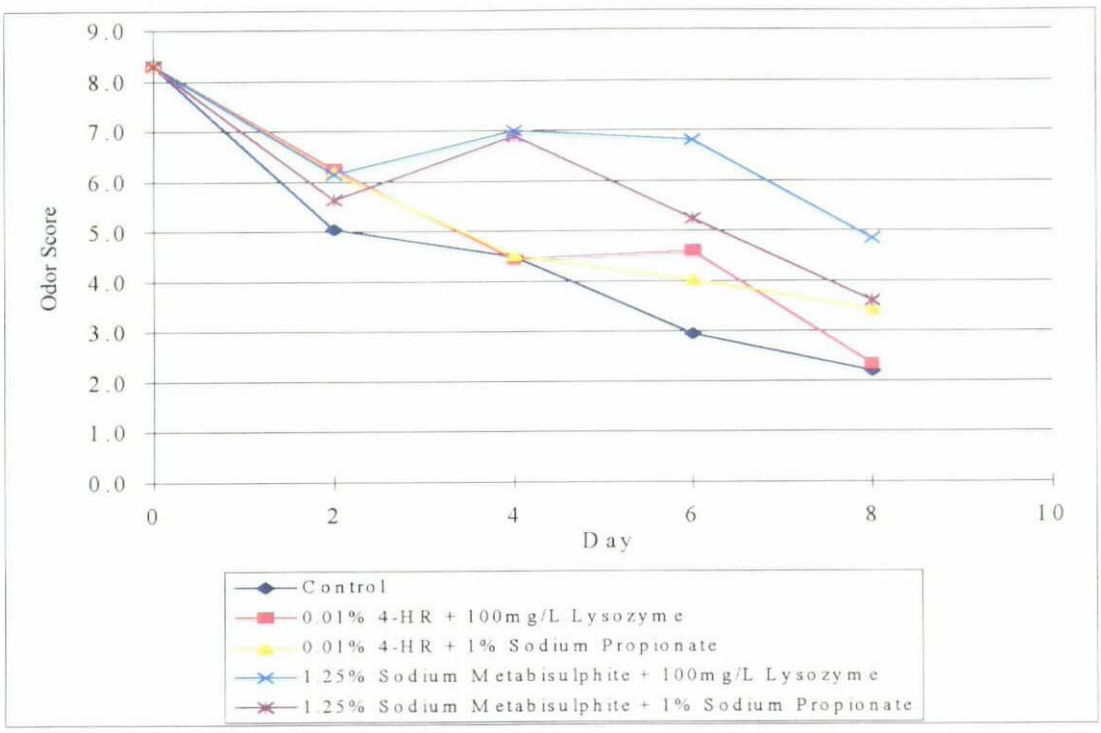


Figure 4.2.1 Change of odor scores of shrimp samples treated with combined preservatives during storage in Phase II experiment.

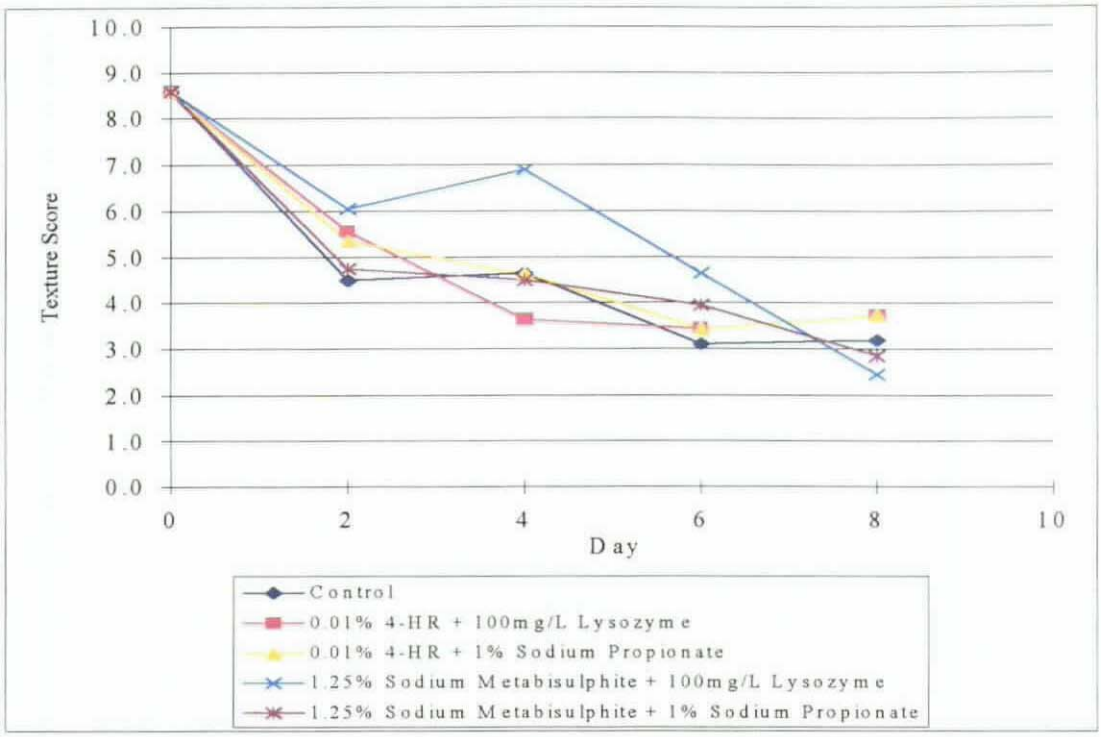


Figure 4.2.2. Change of texture scores of shrimp samples treated with combined preservatives during storage in Phase II experiment.

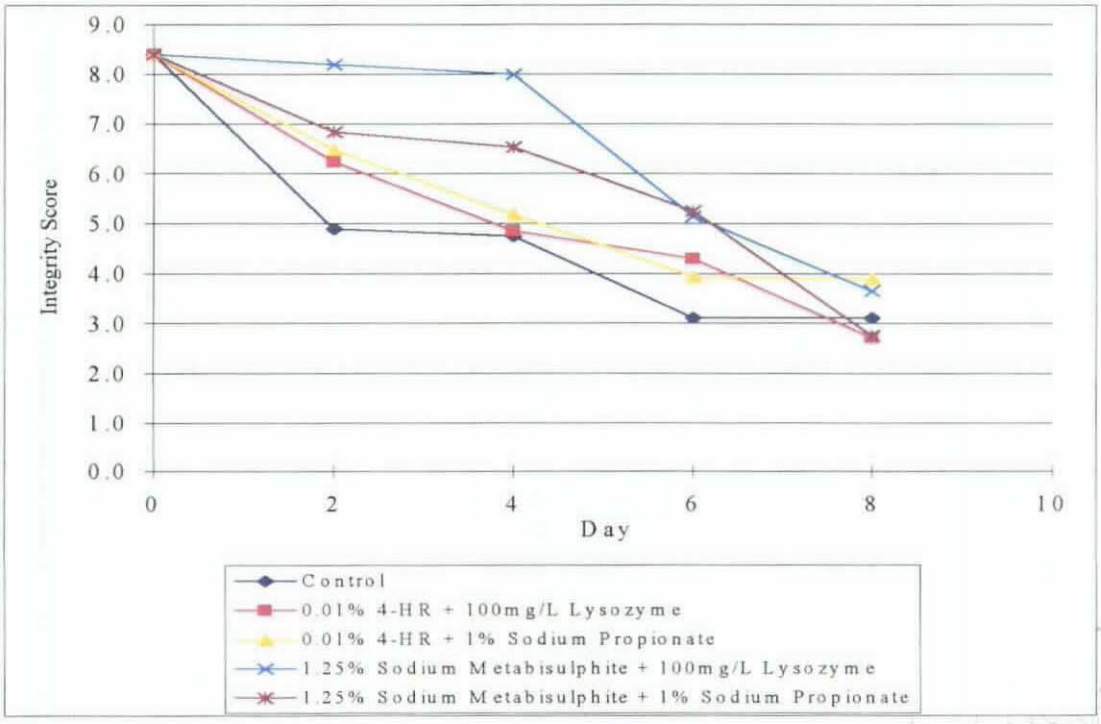


Figure 4.2.3 Change of integrity scores of shrimp samples treated with combined preservatives during storage in Phase II experiment.

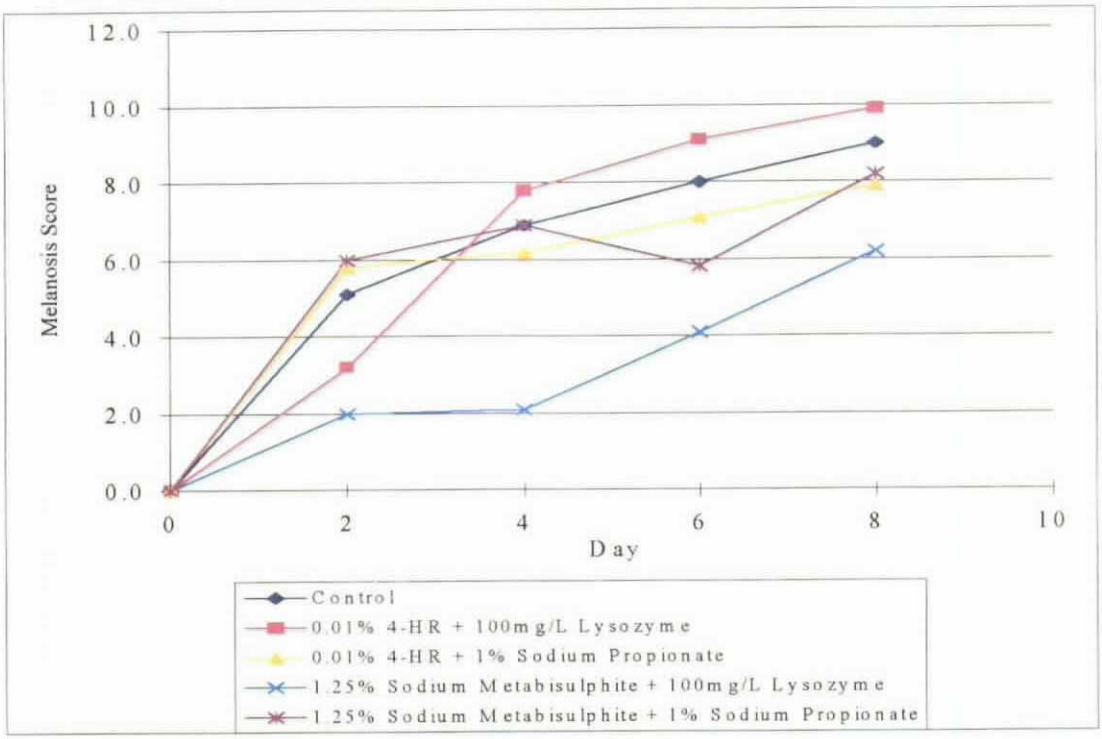


Figure 4.2.4. Change of melanosis scores of shrimp samples treated with combined preservatives during storage in Phase II experiment.

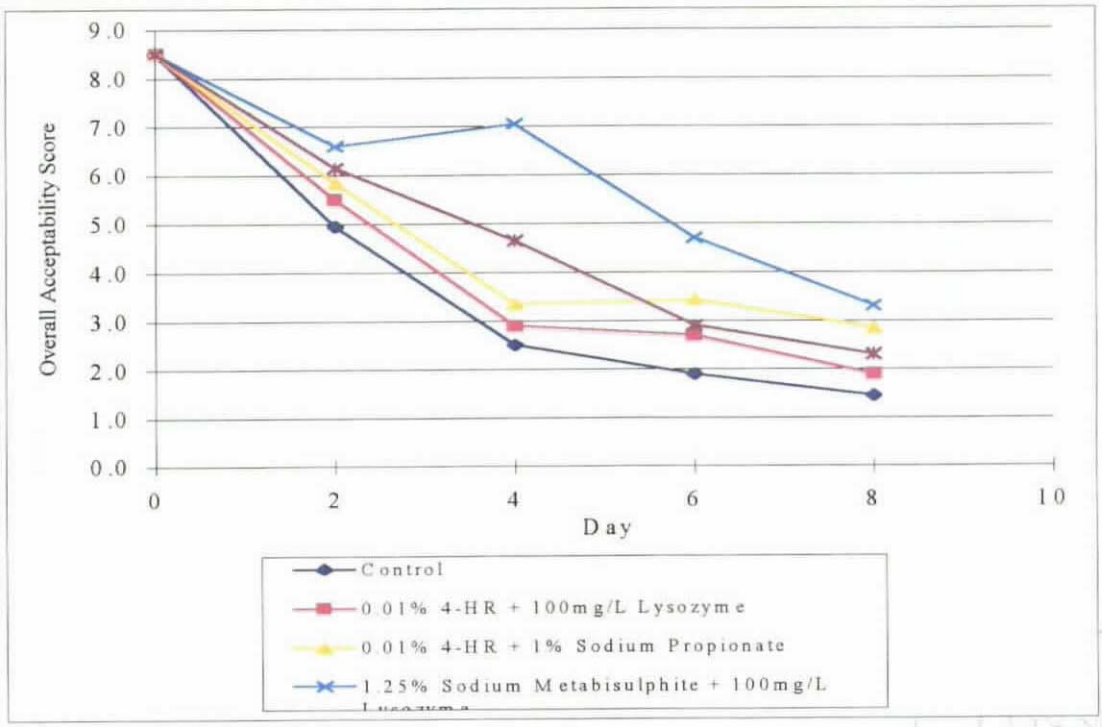


Figure 4.2.5 Change of overall acceptability scores of shrimp samples treated with combined preservatives during storage in Phase II experiment.

Table 4.2.1 Sensory-quality-attribute-shelf-lives of samples treated with different combined preservatives in Phase II experiment.

Treatment	Sensory Quality Attributes Shelf Life (day)				
	Odor	Texture	Integrity	Melanosis	Overall Acceptability
Control	2.1	1.8	1.9	1.7	1.9
0.01% 4-HR + 100mg/L Lysozyme	3.4	2.5	3.8	2.3	2.3
0.01% 4-HR + 1% Sodium Propionate	3.5	3.0	4.3	1.3	2.6
1.25% Na Metabisulphite + 100mg/L Lysozyme	7.9	5.7	6.2	5.9	5.7
1.25% Na Metabisulphite + 1% Sodium Propionate	6.3	1.9	6.2	1.2	3.6

Table 4.2.1 showed that the combination of 1.25% Na Metabisulphite + 100mg/L Lysozyme was superior in extending sample shelf life. Samples treated by this combination of preservatives had the longest sensory-quality-attribute-shelf-lives. In contrary to the Phase I experiment, the odor shelf life of samples treated by this combination of preservatives became the longest. Moreover, the overall acceptability shelf life of such samples was increased by 200% (3.8 days) when it was compared with that of the controls (1.9 days).

It was because 1.25% Na Metabisulphite + 100mg/L Lysozyme was a very successful combination in preserving our shrimp samples. The combination was then used as a base for the further experiments.

4.2.2 Trimethylamine Determination

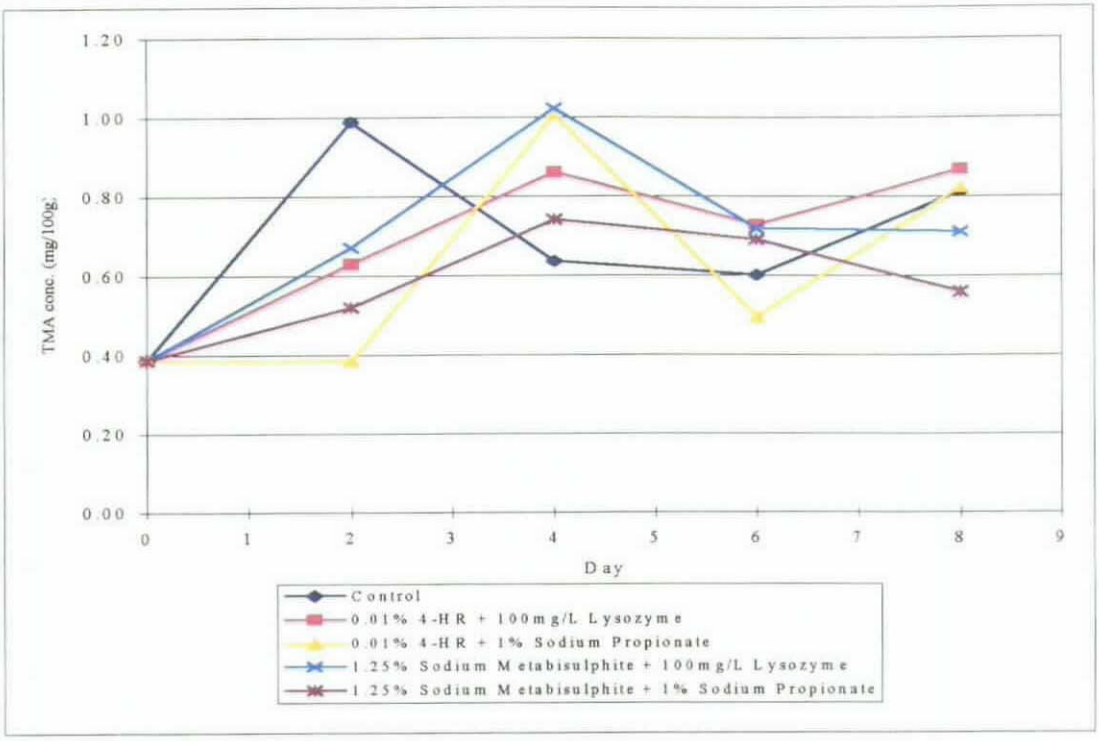


Figure 4.2.6 Change of trimethylamine concentration of shrimp samples treated with combined preservatives during storage in Phase II experiment.

Figure 4.2.6 showed that the concentration of TMA in shrimp samples did not increase drastically during the storage. After 8 days of storage, the TMA levels did not exceed 1 mg/100g. Moreover, the TMA concentration went up and down in the curves without clear pattern. However, the shrimp samples were evaluated as unacceptable in sensory evaluation after 8 days of storage (figure 4.2.1-4.2.5). Due to this founding, it could be concluded that the change in TMA concentration could not reflect the change in sensory scores and it could not be used as a freshness indicator for our shrimp samples, at least in the initial stage of storage. The results confirmed the discovery of Dalgaard *et al.* (1993) who stated that significant amounts of TMA were not produced in shrimps until after the bacterial lag phase.

4.3 Phase III Experiment

It was revealed that EDTA can enhance the function of lysozyme in killing bacteria (Conner, 1993). EDTA was added to the preservative combination in the previous experiment in order to examine its enhancing effect in extending shelf life of shrimps. 4-HR was also added to the combination in order to further suppress the melanosis of shrimps.

4.3.1 Sensory Evaluation

The sensory evaluation results of Phase III experiment included that of raw and cooked shrimp samples. The raw sample results were shown in Figure 4.3.1 to 4.3.5 and the cooked sample results in Figure 4.3.6 to 4.3.10.

4.3.1.1 Raw Shrimps

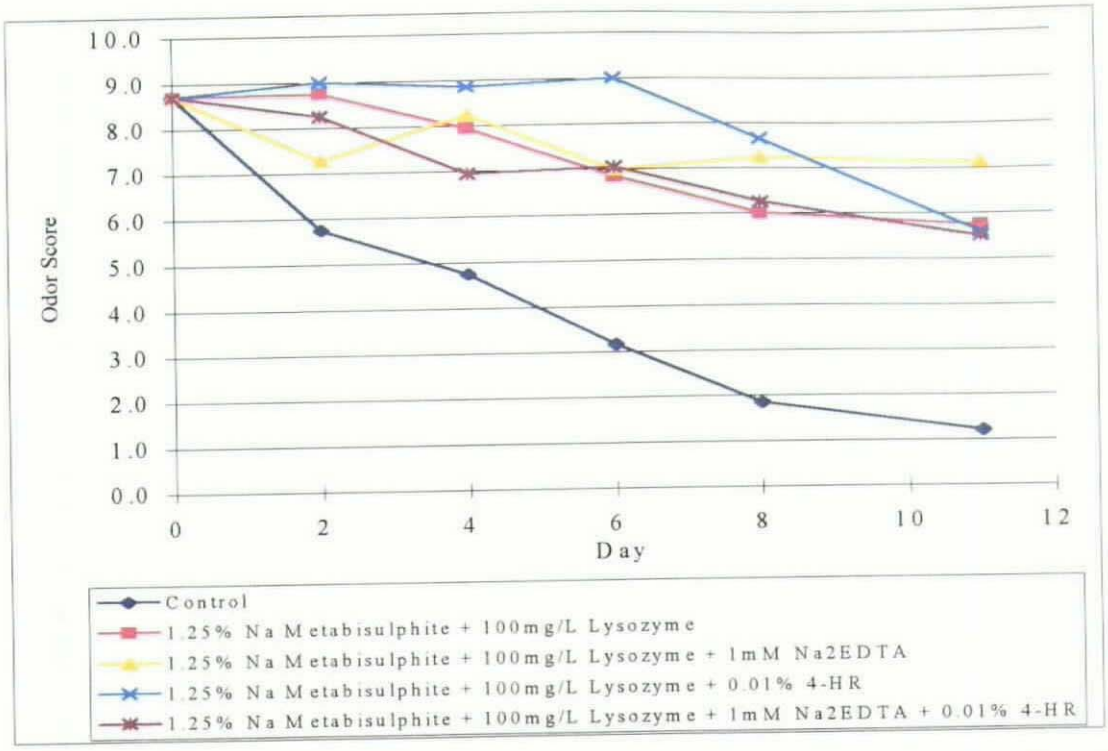


Figure 4.3.1 Change of odor scores of raw shrimp samples treated with combined preservatives during storage in Phase III experiment.

From figure 4.3.1, the results showed that the additional preservatives to the basic combination of sodium metabisulphite + lysozyme did not significantly extend the odor shelf life of samples. After 11 days of storage, the odor of samples, except the control, was still acceptable.

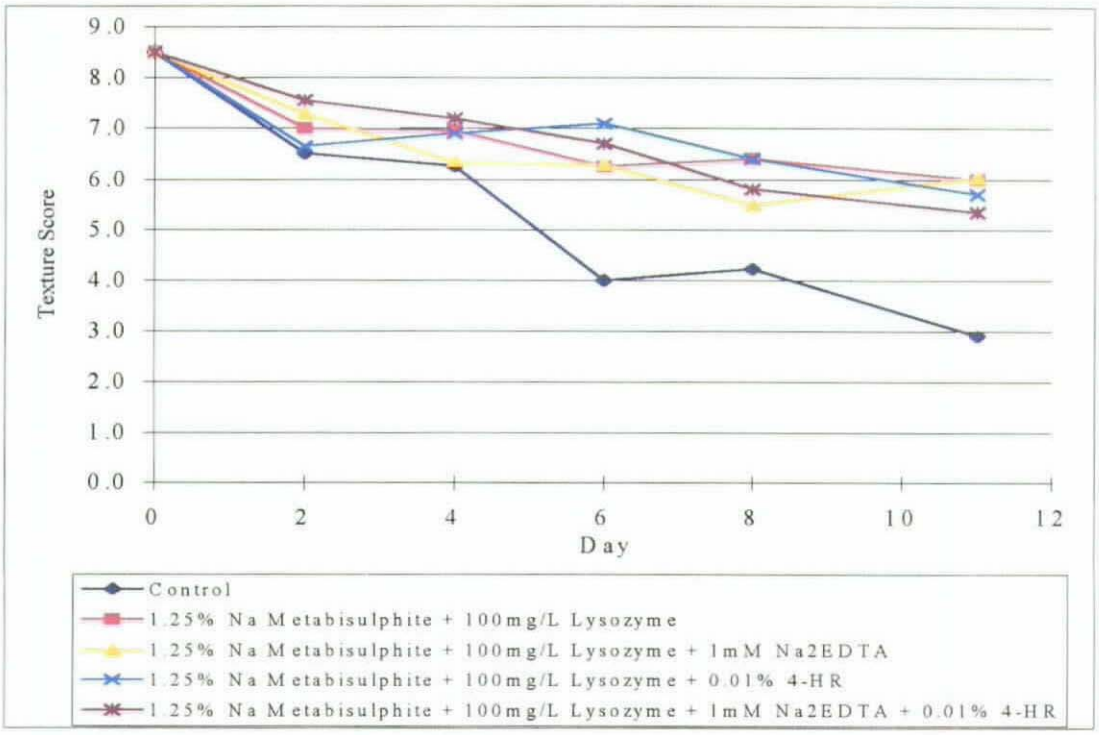


Figure 4.3.2 Change of texture scores of raw shrimp samples treated with combined preservatives during storage in Phase III experiment.

The similar situation was observed in change of texture scores as shown in figure 4.3.2. All samples, except the control, were acceptable in odor.

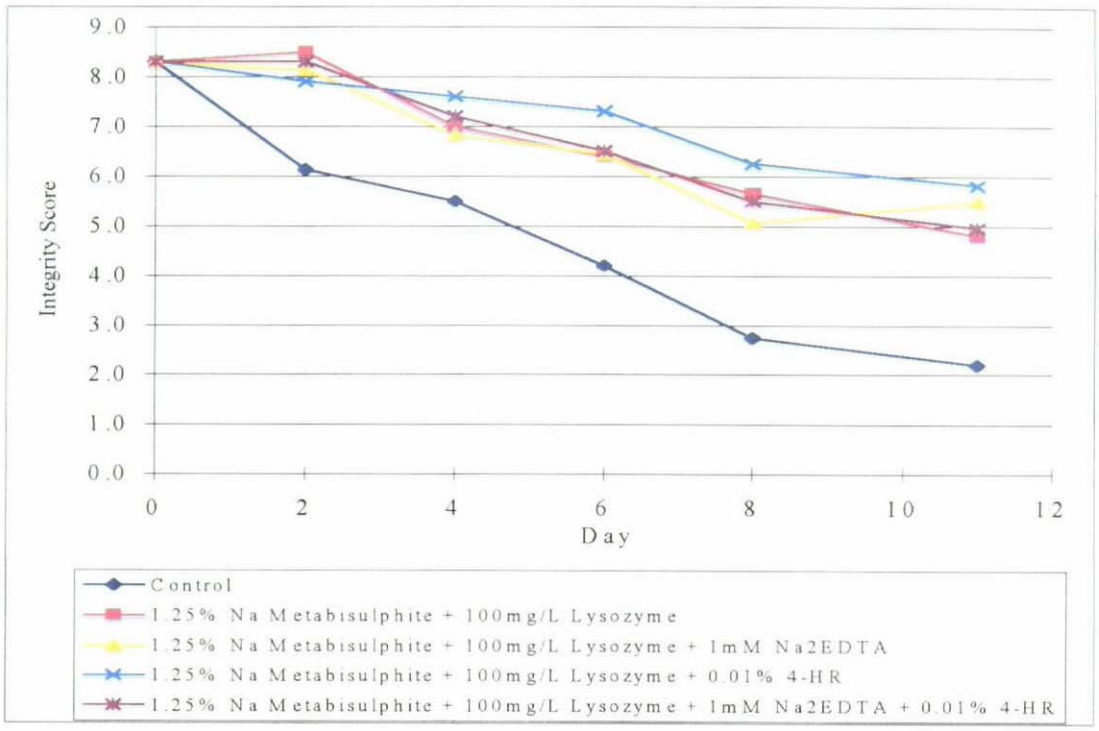


Figure 4.3.3 Change of integrity scores of raw shrimp samples treated with combined preservatives during storage in Phase III experiment.

From figure 4.3.3, since not all integrity scores of samples dropped below 5, the effects of additional preservatives can not be determined.

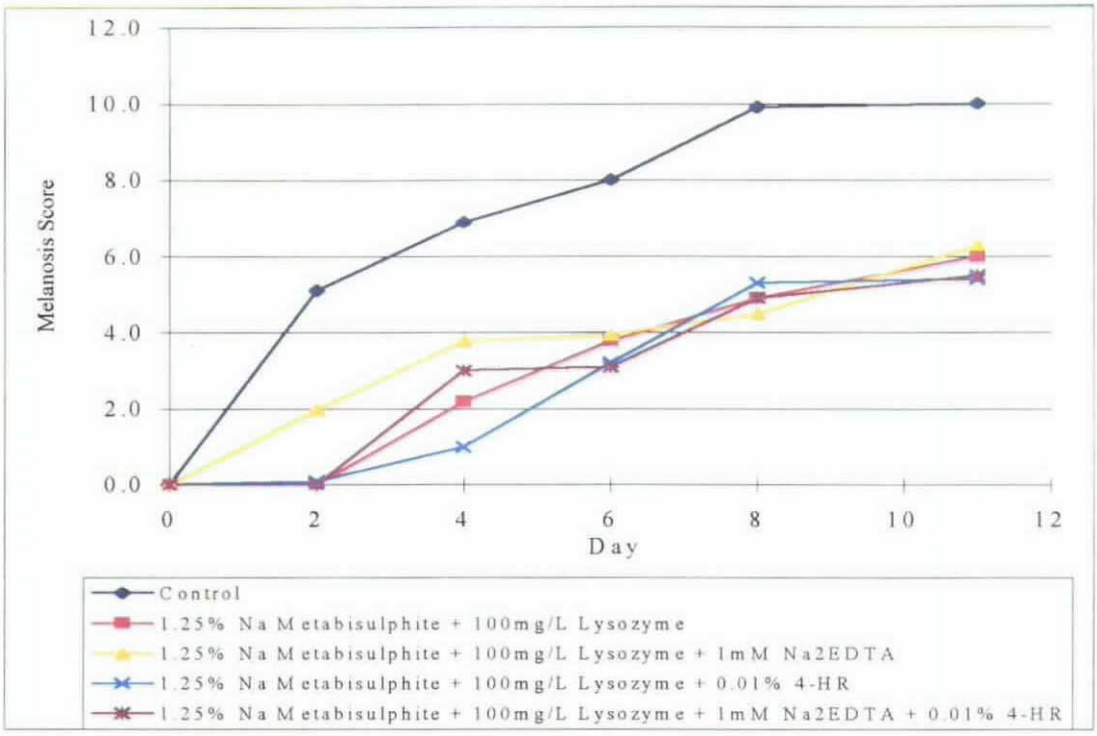


Figure 4.3.4 Change of melanosis scores of raw shrimp samples treated with combined preservatives during storage in Phase III experiment.

In comparing the results in figure 4.3.1 to 4.3.4, we found that melanosis was the only quality attribute deteriorated rapidly in the storage. After 8 days of storage, melanosis scores of all preserved shrimps increased to above 4 which means all shrimp samples were rejected by the sensory panel.

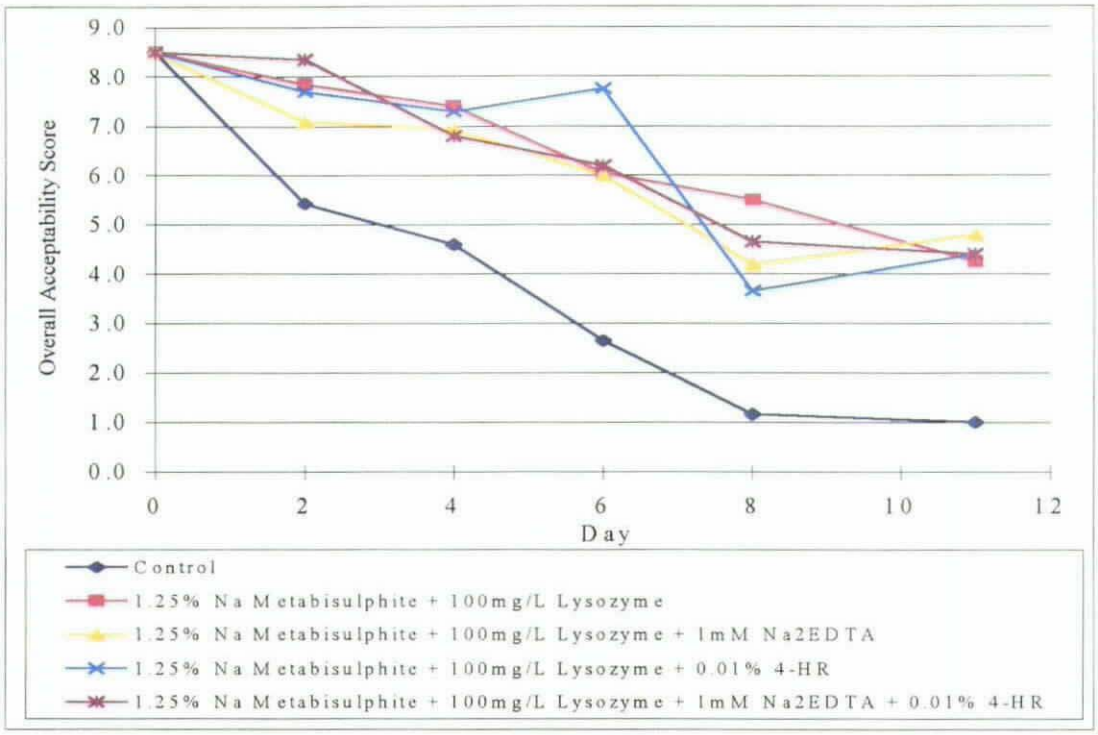


Figure 4.3.5 Change of overall acceptability scores of raw shrimp samples treated with combined preservatives during storage in Phase III experiment.

The sensory-quality-attribute-shelf-lives of raw shrimp samples in Phase III experiment were determined and showed in table 4.3.1.

Table 4.3.1 Sensory-quality-attribute-shelf-lives of raw shrimp samples treated with different combined preservatives in Phase III experiment.

Treatment	Sensory-Quality-Attributes-Shelf-Life (day)	
	Melanosis	Overall Acceptability
Control	1.5	3.0
1.25% Na Metabisulphite + 100mg/L Lysozyme	6.3	9.2
1.25% Na Metabisulphite + 100mg/L Lysozyme + 1mM Na2EDTA	6.3	7.0
1.25% Na Metabisulphite + 100mg/L Lysozyme + 0.01% 4-HR	6.7	7.3
1.25% Na Metabisulphite + 100mg/L Lysozyme + 1mM Na2EDTA + 0.01% 4-HR	6.9	7.5

From table 4.3.1, we noted that all the samples, except control, had similar melanosis shelf lives. Moreover, the overall acceptability shelf lives of samples treated with additional preservatives were even lower than that of samples treated with the basic combination of sodium metabisulphite + lysozyme.

4.3.1.1 Cooked Shrimps

In order to further investigate the effectiveness of preservatives on cooked shrimps, the raw shrimp samples were cooked and then evaluated by the sensory penal. The results of which were visualized in figure 4.3.6 to 4.3.10.

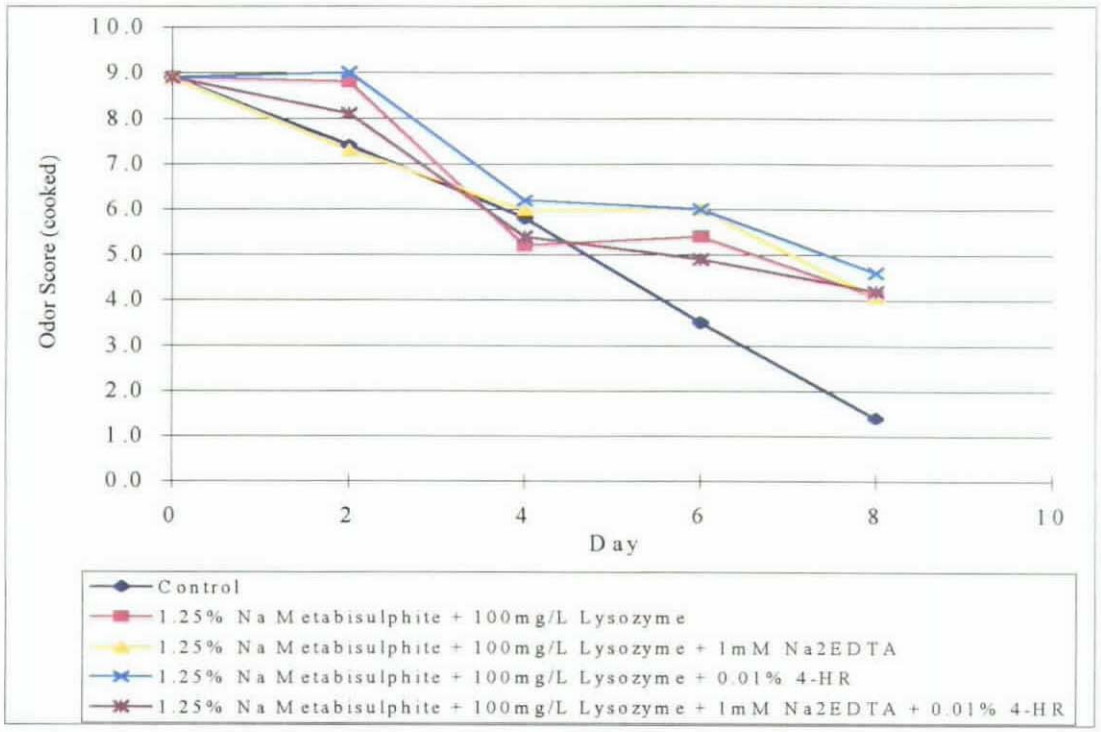


Figure 4.3.6 Change of odor scores of cooked shrimp samples treated with combined preservatives during storage in Phase III experiment.

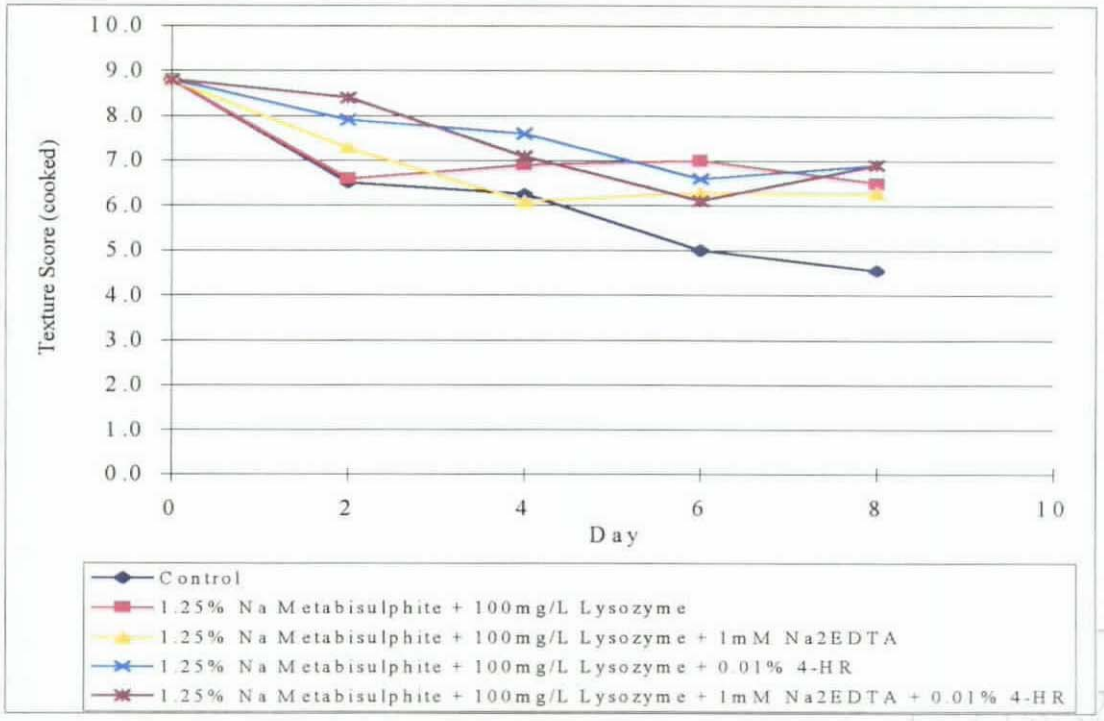


Figure 4.3.7 Change of texture scores of cooked shrimp samples treated with combined preservatives during storage in Phase III experiment.

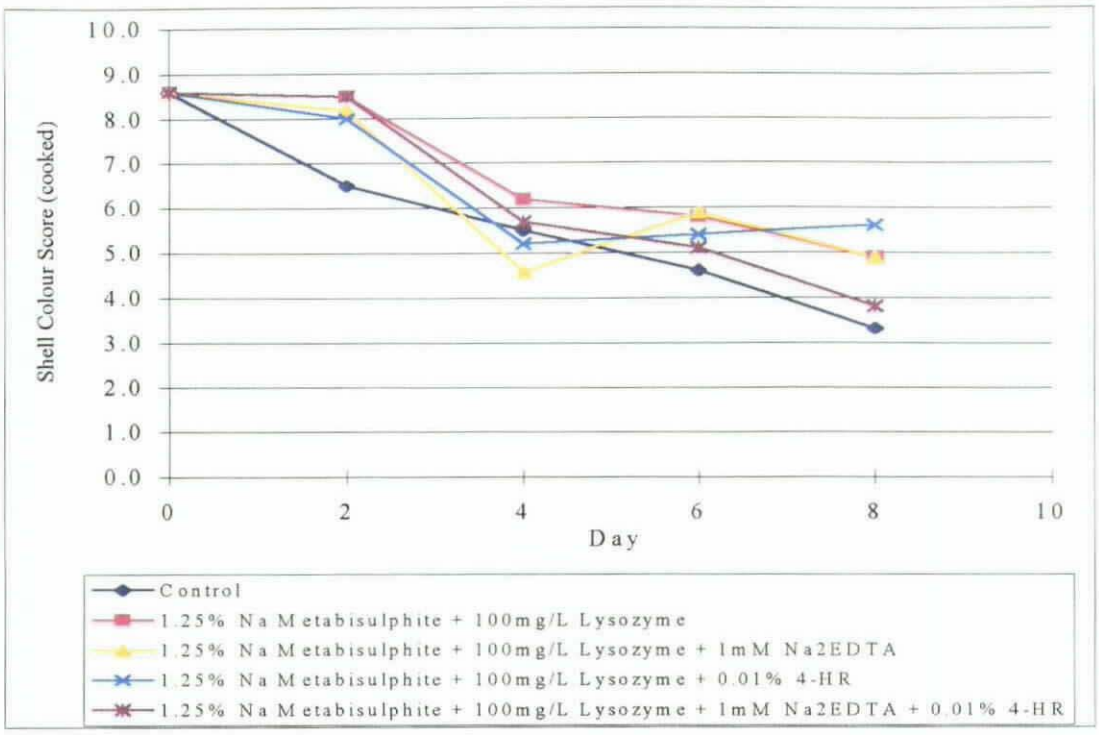


Figure 4.3.8 Change of shell colour scores of cooked shrimp samples treated with combined preservatives during storage in Phase III experiment.

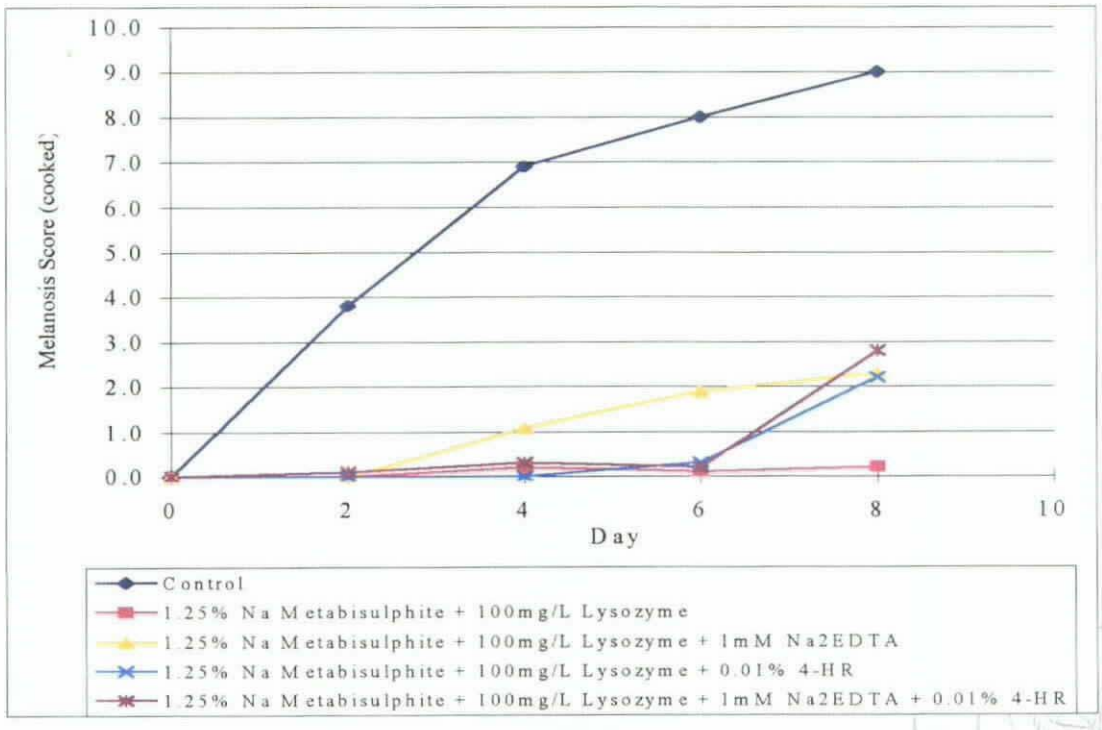


Figure 4.3.9 Change of melanosis scores of cooked shrimp samples treated with combined preservatives during storage in Phase III experiment.

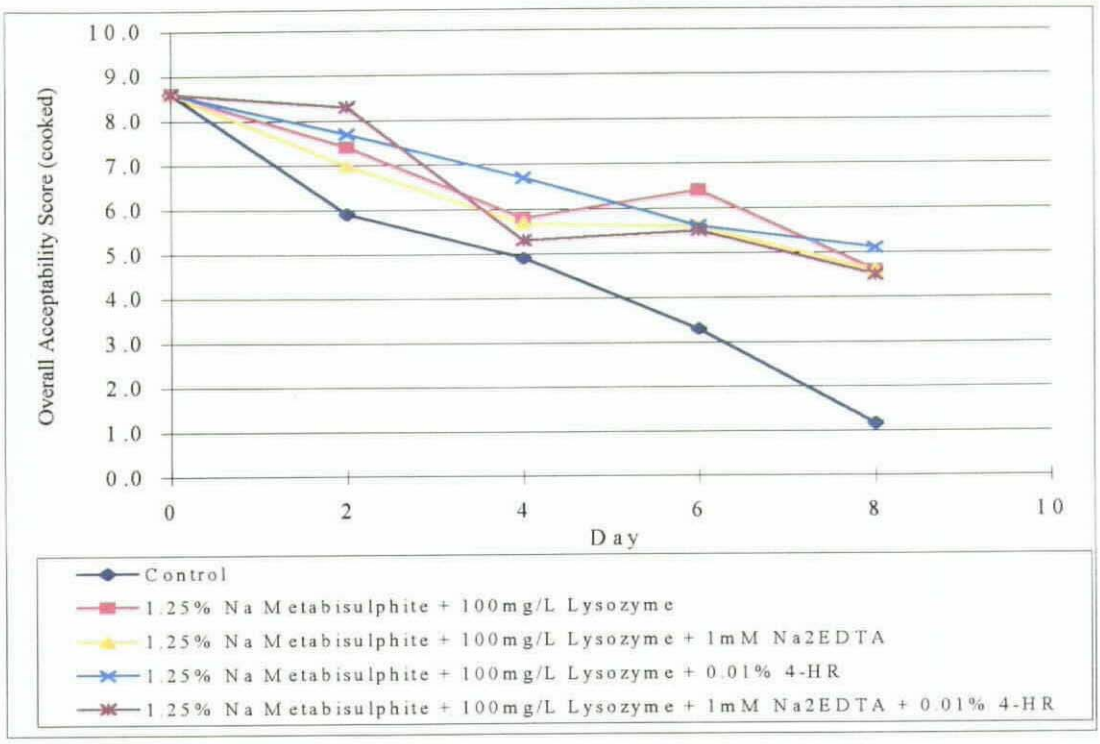


Figure 4.3.10 Change of overall acceptability scores of cooked shrimp samples treated with combined preservatives during storage in Phase III experiment.

Differ from the raw sample results, cooked samples did not fail in melanosis after 8 days of storage. It was probably because the dark pigments were dissolved into the boiling water in the cooking process. On the other hand, the odor of samples failed after about 6 to 8 days of storage. When we compare this with the raw samples as shown in figure 4.3.1, you may be surprised that even though raw shrimps were acceptable in odor, they could be fail in it after they were cooked! In contrary to our prediction that the cooking process might have dissolved or evaporated away large amount of odor causing chemicals, such chemicals might have actually been trapped inside the shrimp's shell. I gave the about suggestions based on the discovery that the bad odor was significantly sensed by penalists only after the shrimps were peeled. Moreover, it was reported that the tail part of shrimps generated the strongest odor

after cooking. If the 'odor trapping' hypothesis could be established, the strong odor in the tail could be explained as follows: During the cooking process, bad odor in the cephalo-thorax region of the shrimps might be evaporated out through the joint between the head and the thorax. After such long period of storage, the membranes between the head and the thorax of shrimp samples were usually broken. Thus, comparatively large amount of bad odor could be trapped in the tail region.

The following table showed the odor and overall acceptability shell lives of cooked shrimp samples in Phase III experiment.

Table 4.3.2 Sensory-quality-attribute-shelf-lives of cooked shrimp samples treated with different combined preservatives in Phase III experiment.

Treatment	Sensory-Quality-Attributes-Shelf-Life (day)	
	Odor	Overall Acceptability
Control	4.6	3.7
1.25% Na Metabisulphite + 100mg/L Lysozyme	6.6	7.5
1.25% Na Metabisulphite + 100mg/L Lysozyme + 1mM Na_2EDTA	7.0	7.1
1.25% Na Metabisulphite + 100mg/L Lysozyme + 0.01% 4-HR	7.4	8.0-8.5*
1.25% Na Metabisulphite + 100mg/L Lysozyme + 1mM Na_2EDTA + 0.01% 4-HR	5.7	6.9

Remarks: * estimated value.

Overall speaking, the additional preservatives could not help in extending the sample shelf life, therefore, the basic combination of sodium metabisulphite + lysozyme ($Na_2S_2O_5$/Lys) was used for subsequent experiments.

The photos for shrimp samples were taken during the experiment and were shown below:

	Control	1.25% Na Metabisulphite + 100mg/L Lysozyme
Day 0		
Day 2		

Day 5		
Day 7		
Day 10	**End**	

Plate 4.3.1 Photos of raw shrimp samples taken during Phase III experiment.

	Control	1.25% Na Metabisulphite + 100mg/L Lysozyme
Day 0		
Day 2		
Day 5		

Day 7		
Day 10	**End**	

Plate 4.3.2 Photos of cooked shrimp samples taken during Phase III experiment.

4.3.2 Surface Lightness Measurement

The surface lightness measurement was performed again in this experiment. The results of which were shown in figure 4.3.11.

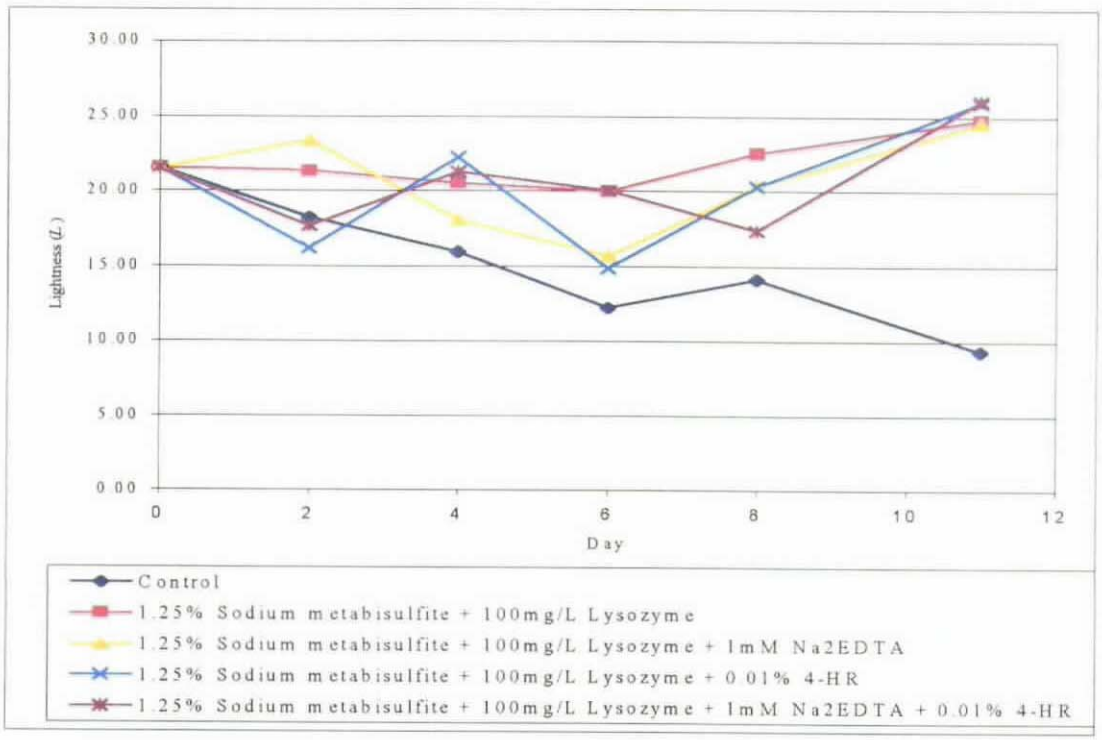


Figure 4.3.11 Change of surface lightness of shrimp samples during storage in Phase III experiment.

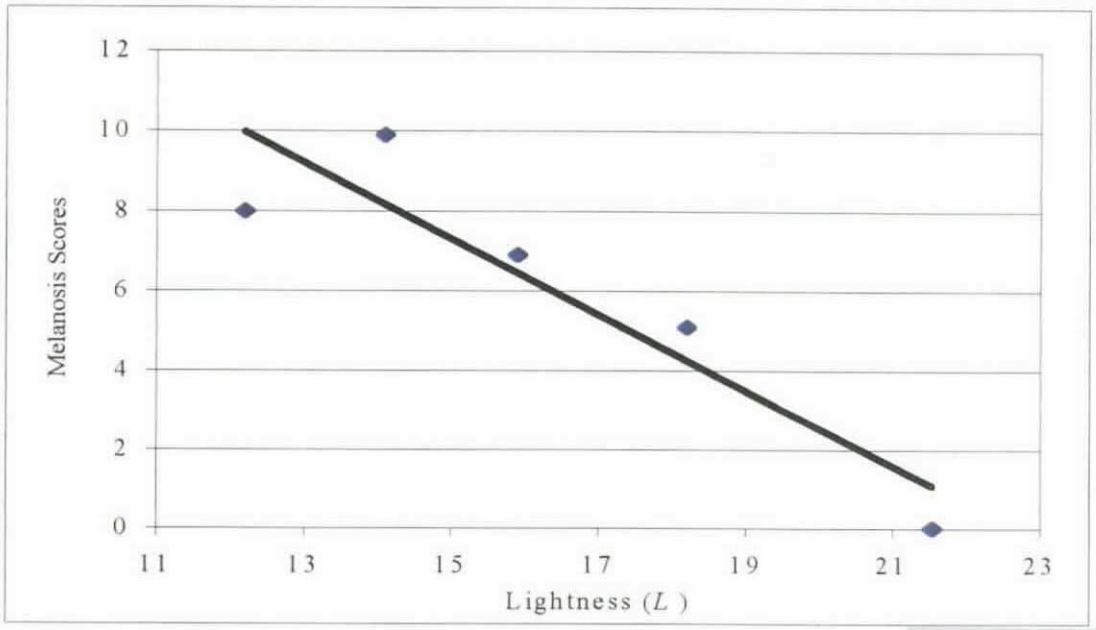


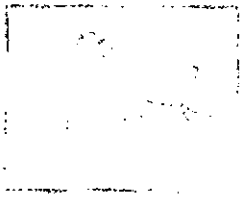
Figure 4.3.12 Correlation between melanosis evaluation and lightness measurement of control samples in Phase III experiment.

The correlation coefficient was –0.92 at 95% confidence level. From figure 4.3.12, the lightness of samples, at the time of melanosis rejection (score = 4), was determined to be 18.1. Together with the results obtained in Phase I experiment, the lightness averaged out at time of melanosis rejection was 16.5. The average surface lightness of fresh shrimps were determined to be 21.5 (standard deviation = 1.29).

Since the correlation coefficients were high, surface lightness measurement could be used as an objective melanosis measurement to replace subject sensory evaluation of melanosis. The measurement take only about 1 minute for assessing one shrimp. Moreover, no special training is required for the operation of the colorimeter, a layman can learn how to use it in no time and the readings can be obtained directly without any complicated calculations. This objective method could replace the traditional melanosis sensory evaluation method from which only subjective decisions could be made.

4.3.3 Microbiological Examination

Microbiology examination was performed in this phase in order to determine whether the organoleptically acceptable samples were hygienic or not. Figure 4.3.13 showed the aerobic plate counts of samples during storage.



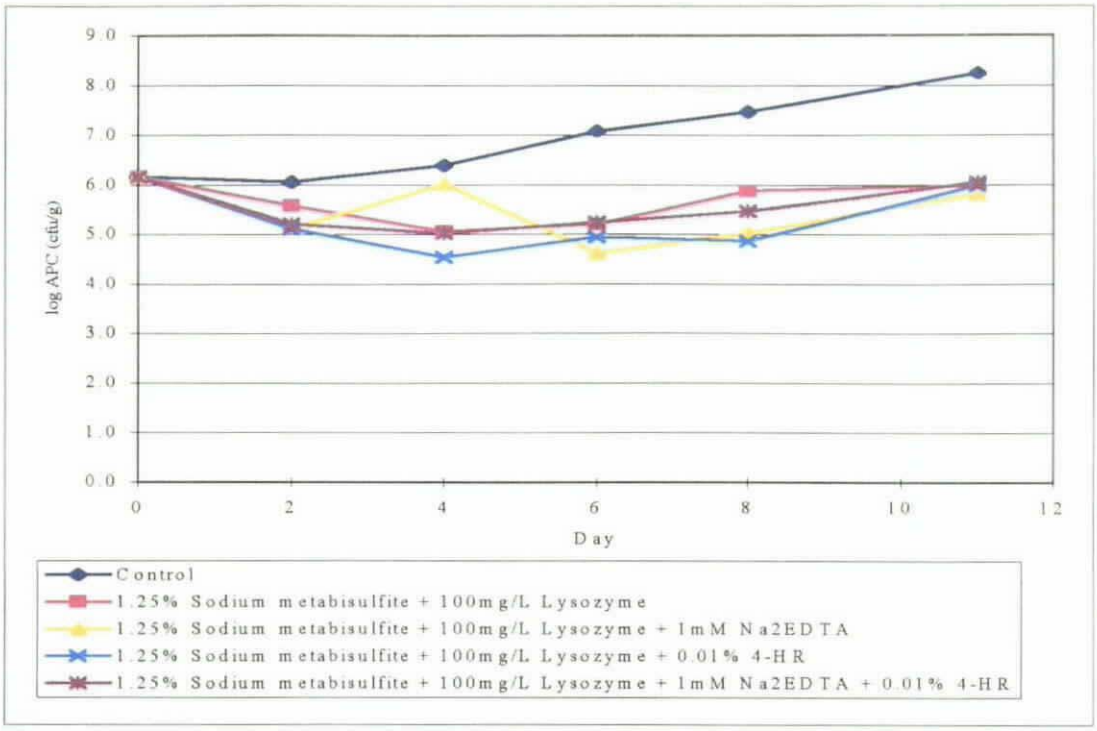


Figure 4.3.13 Change of aerobic plate count in shrimp samples during storage in Phase III experiment.

From figure 4.3.13, a slight initial decrease in APCs was observed in the curves. Such decrease was defined as the lag phase of bacterial growth. After that, the bacterial growth advanced into the log phase in which the APCs increased rapidly.

The APCs of preservative treated samples were dropped by about 1 to 1.5 log cycles in the lag phase. For 1.25% Na Metabisulphite + 100mg/L Lysozyme + 1mM Na_2EDTA treated samples, the increase in APC in day 4 during the lag phase might due to experimental error. After a comparatively short period (2 days) of lag phase, the APC of control samples increased continuously in the log phase up to more than 1 x 10^8 after 11 days of storage. On the other hand, the APC of preserved samples started to rise after about 4 days of storage. After 11 days of storage, the APC of preserved samples increased back to the original day 0 level at about 1 x 10^6. This

indicated that the preservatives were effective in delaying the lag phase of bacterial growth in the preserved samples.

The control samples were not suitable for human consumption after about 6 days of storage when the APC of which exceeded 1 x 10^7 as suggested by Farooqui B. *et al.* (1978). On the other hand, the APCs of preserved samples were only about 1 x 10^6 after 11 days of storage. Thus, the preservatives could extend the microbial shelf lives of preserved samples by at least 5 days or 83% more than that of the control.

In conclusion, the preserved samples had acceptable bacterial level at time of rejection (maximum shelf life was 9.2 days, table 4.3.1 and 4.3.2 referred).

4.4 Phase IV Experiment

This experiment was a preliminary of the modified atmosphere packaging experiments. Samples were put inside nylon bags, filled with air and then stored in refrigerator at chilling temperature of 0-4°C.

4.4.1 Sensory Evaluation

In the previous phase of experiment, we noticed that odor and melanosis of samples deteriorated much earlier than other attributes. These two attributes would be studied in this phase of experiment. Texture was also studied but not integrity as shrimps were peeled in this experiment so they have no integrities for evaluation at all. Rather, the general appearances of the samples were examined instead. The appearances included integrity for shell-on shrimps and colour for peeled shrimps. The colour of shrimps under storage did change a lot. It was found that the colour of some samples

turned blue and some were decolorized during storage. Similar discoloration can be seen in other seafood e.g. the greening of tuna (Wekell & Barnett, 1991) which might due to the oxidation of myoglobin iron. The following tables (4.4.1-4.4.5) showed the sensory evaluation results of the Phase IV experiment.

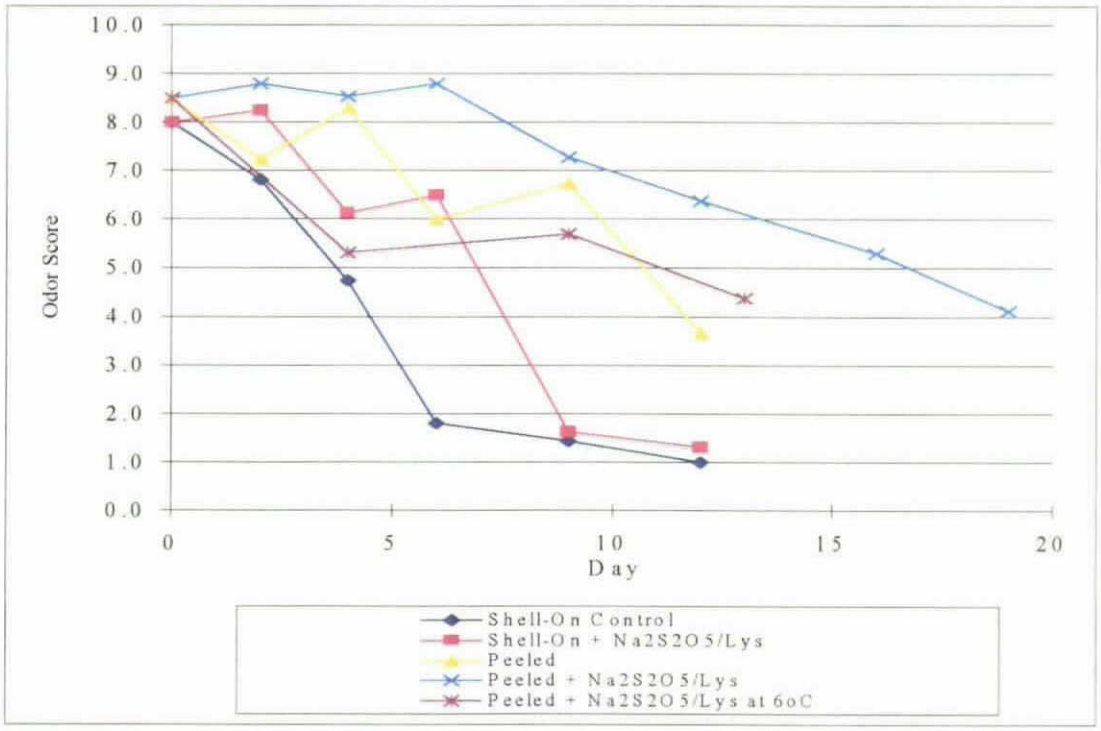


Figure 4.4.1 Change of odor scores in shell-on/peeled shrimp samples treated/not treated with 4HR/Lys during storage in Phase IV experiment.

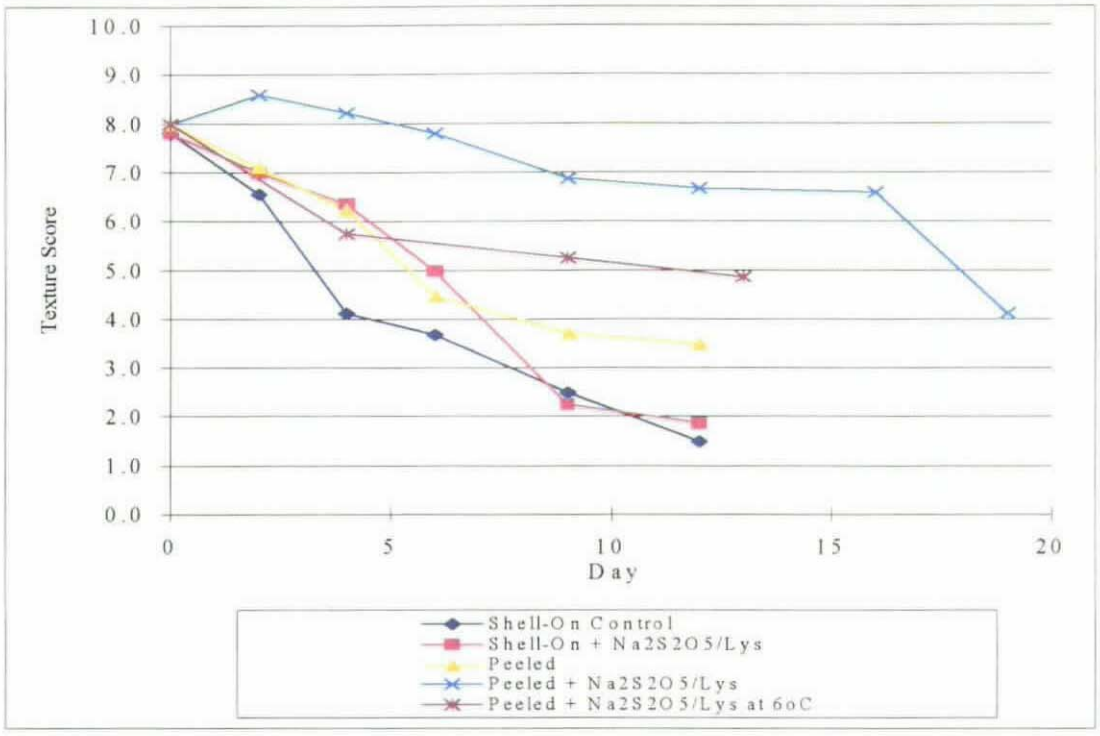


Figure 4.4.2 Change of texture scores in shell-on/peeled shrimp samples treated/not treated with 4HR/Lys during storage in Phase IV experiment.

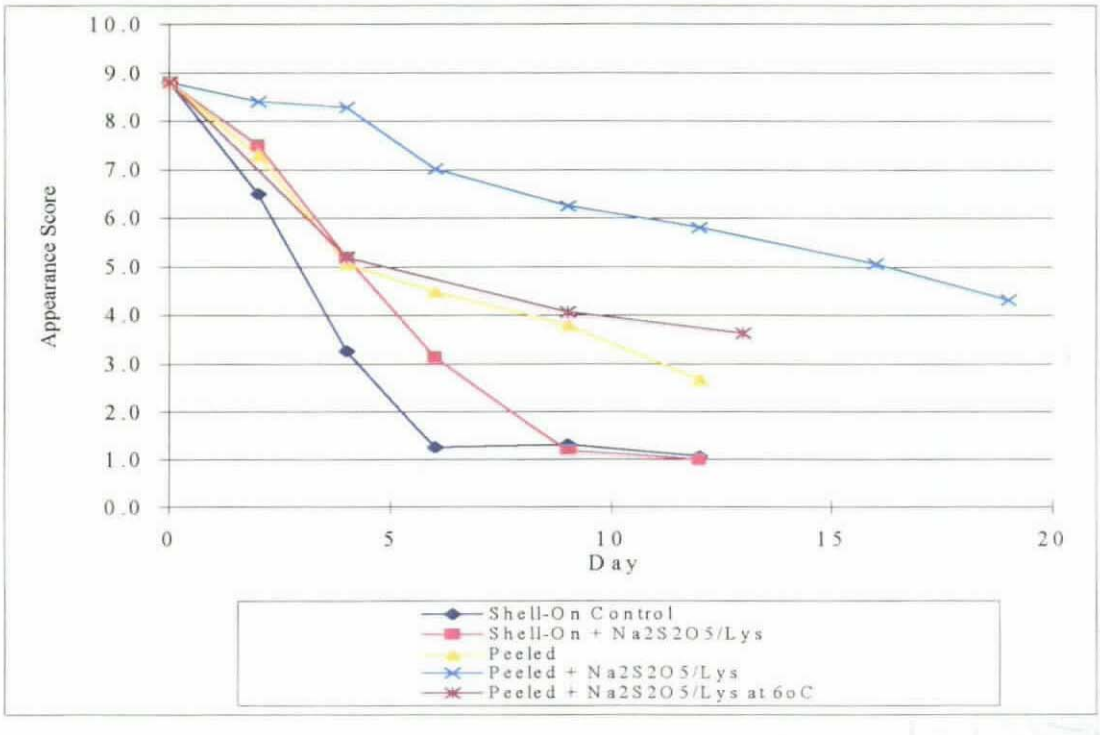


Figure 4.4.3 Change of appearance scores in shell-on/peeled shrimp samples treated/not treated with 4HR/Lys during storage in Phase IV experiment.

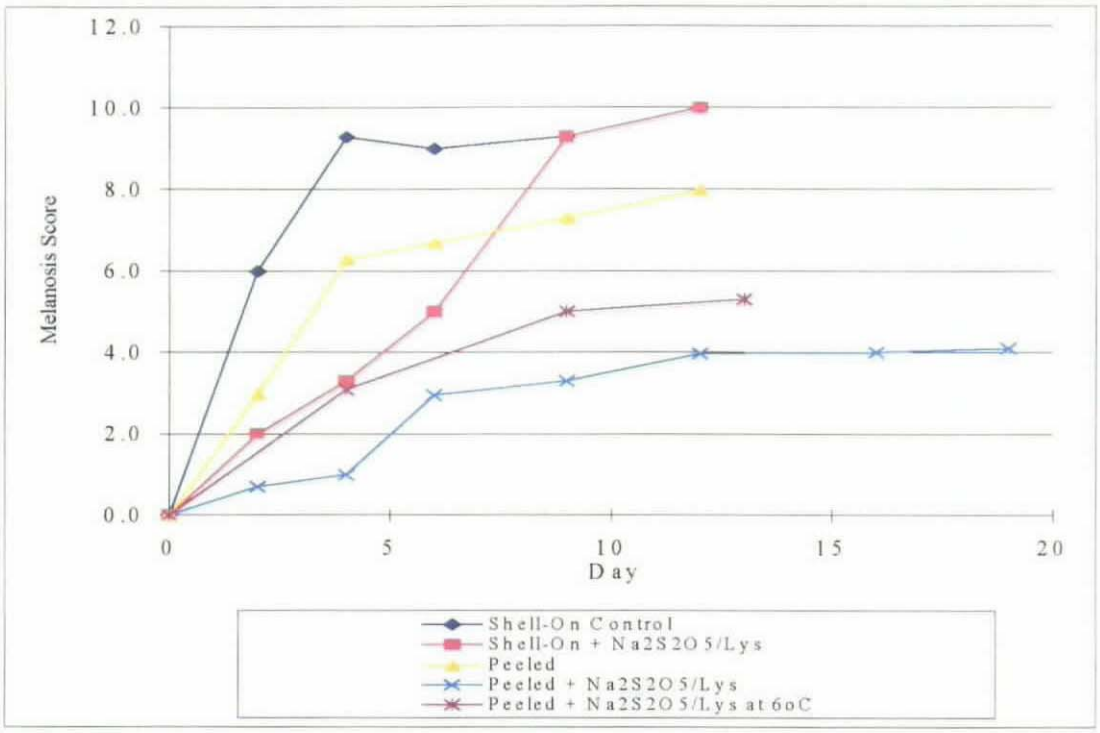


Figure 4.4.4 Change of melanosis scores in shell-on/peeled shrimp samples treated/not treated with 4HR/Lys during storage in Phase IV experiment.

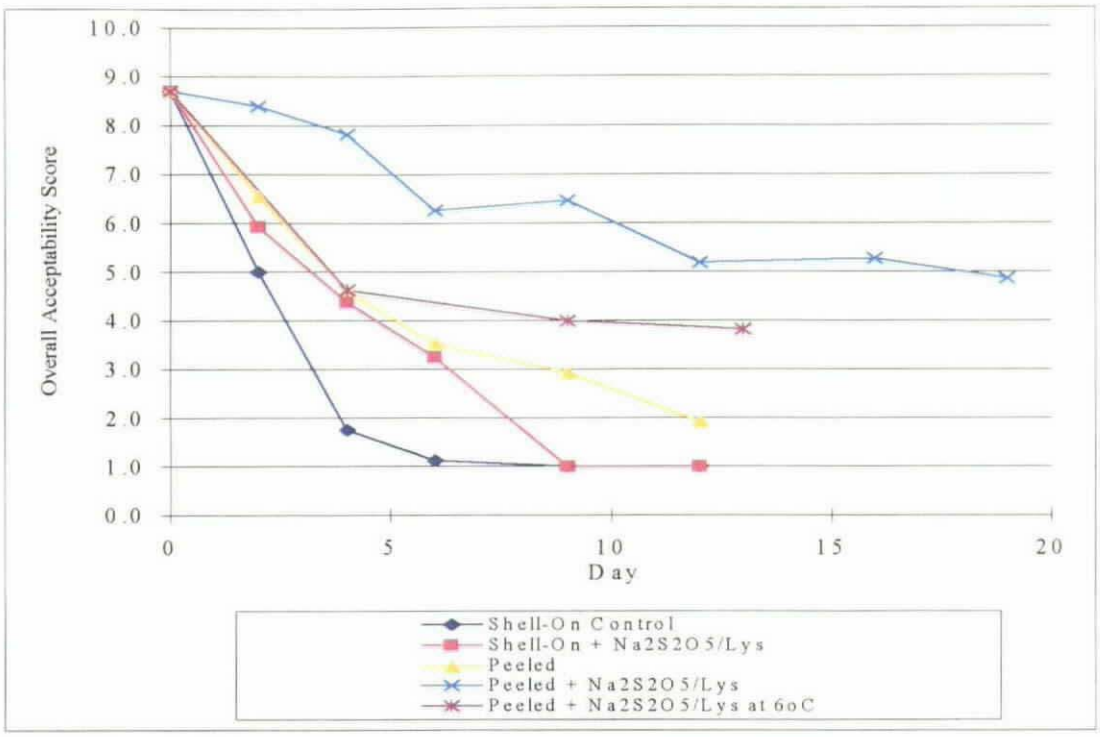


Figure 4.4.5 Change of overall acceptability scores in shell-on/peeled shrimp samples treated/not treated with 4HR/Lys during storage in Phase IV experiment.

It was observed that peeled shrimps treated with $Na_2S_2O_5$/Lys had the longest sensory-quality-attribute-shelf-lives. Other treatments could extend the shelf lives in much lesser amount. Table 4.4.1 summarized the quality attribute shell lives of samples in this phase of experiment.

Table 4.4.1 Sensory-quality-attribute-shelf-lives of shrimp samples in Phase IV experiment.

Treatment	Sensory Quality Attributes Shelf Life (day)				
	Odor	Texture	Appearance	Melanosis	Overall Acceptability
Control	4.0	3.1	2.9	1.1	2.0
Shell on + $Na_2S_2O_5$/Lys	6.9	6.0	4.2	4.8	3.5
Peeled	10.8	5.6	4.0	2.5	3.9
Peeled + $Na_2S_2O_5$/Lys	17.0	17.7	16.1	16	18.0
Peeled + $Na_2S_2O_5$/Lys at 6°C	11.1	11.7	4.8	6.3	3.9

From table 4.4.1, we observed that peeling can reduce odor in samples during storage and therefore peeled samples had longer odor shelf lives. When comparing peeled samples with the control, we found that the peeling process alone (without addition of preservatives) could increase the shelf lives to some extent; such extended shelf lives were similar to that of shell-on but preservative added samples. Furthermore, when $Na_2S_2O_5$/Lys were used, the sample's overall acceptability shelf life was remarkably increased by 9 times of that of the control. Taking into consideration that the samples stored at 6°C had overall acceptability shell lives that were only 22% of those stored at 0-4 °C, we could say temperature did affect very much the reactions involved in this system. Thus, all storage temperatures of the subsequent experiment would be fixed at 0-4°C.

4.4.2 Aerobic Plate Count

APC was performed to monitor the hygiene condition of shrimps during storage in this experiment. Figure 4.4.6 shown the results of that.

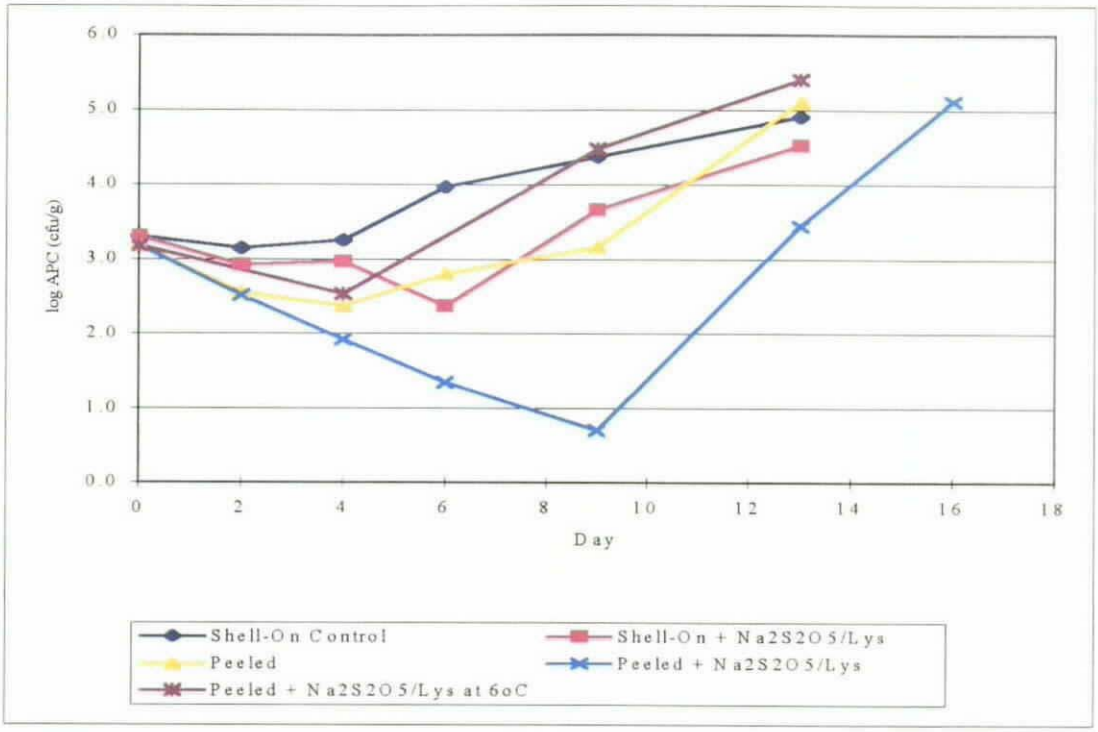


Figure 4.4.6 Change of aerobic plate count in shrimp samples during storage in Phase IV experiment.

Generally, the APCs increased from about 1 x 10^3 to about 1 x 10^5 after 13 days of storage.

The lag phase for the control samples were shortest at about 4 days and the corresponding decrease in APC was smallest. In considering the treated samples, the peeled + $Na_2S_2O_5$/Lys samples had longest lag phase and the corresponding decrease in APC was largest at about 2 log cycles. In other words, the log phase started most early in the control samples and most late in the peeled + $Na_2S_2O_5$/Lys samples. It was

obvious that the preservation system of "peeling with addition of $Na_2S_2O_5$/Lys" was the most effective method in controlling bacterial growth.

The results revealed that the peeled + $Na_2S_2O_5$/Lys samples had acceptable bacterial level (less than 10^7) when they were rejected in melanosis evaluation at day 16 of storage (table 4.4.1 referred).

4.5 Phase V Experiment

In this phase of experiment, MAP of shrimps were studied. Different mixtures of CO_2, N_2 and O_2 gases constituted the atmosphere inside the packaging material. The same preservative combination was used for all samples and the only difference between them was the atmosphere in the nylon bags.

4.5.1 Sensory Evaluation

Raw and cooked samples were evaluated by the sensory panel to study the differences between raw and cooked samples as those appeared in Phase III experiment.

4.5.1.1 Raw Shrimps

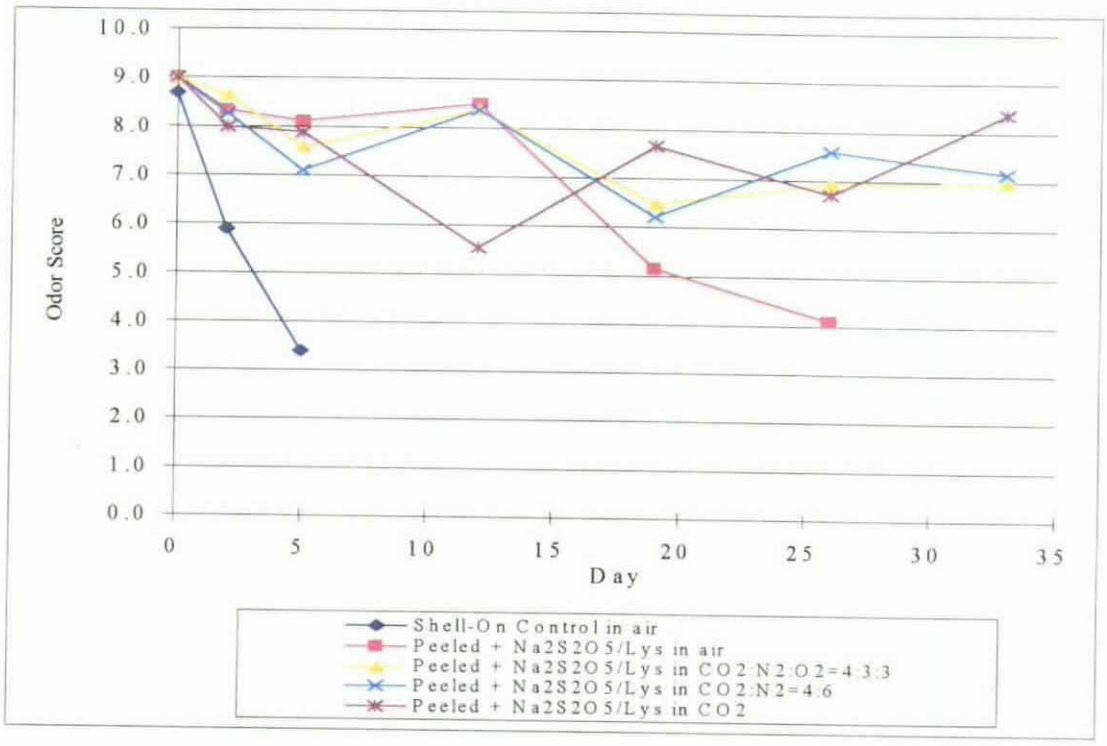


Figure 4.5.1 Change of odor scores of raw shrimp samples stored in different gas compositions in Phase V experiment.

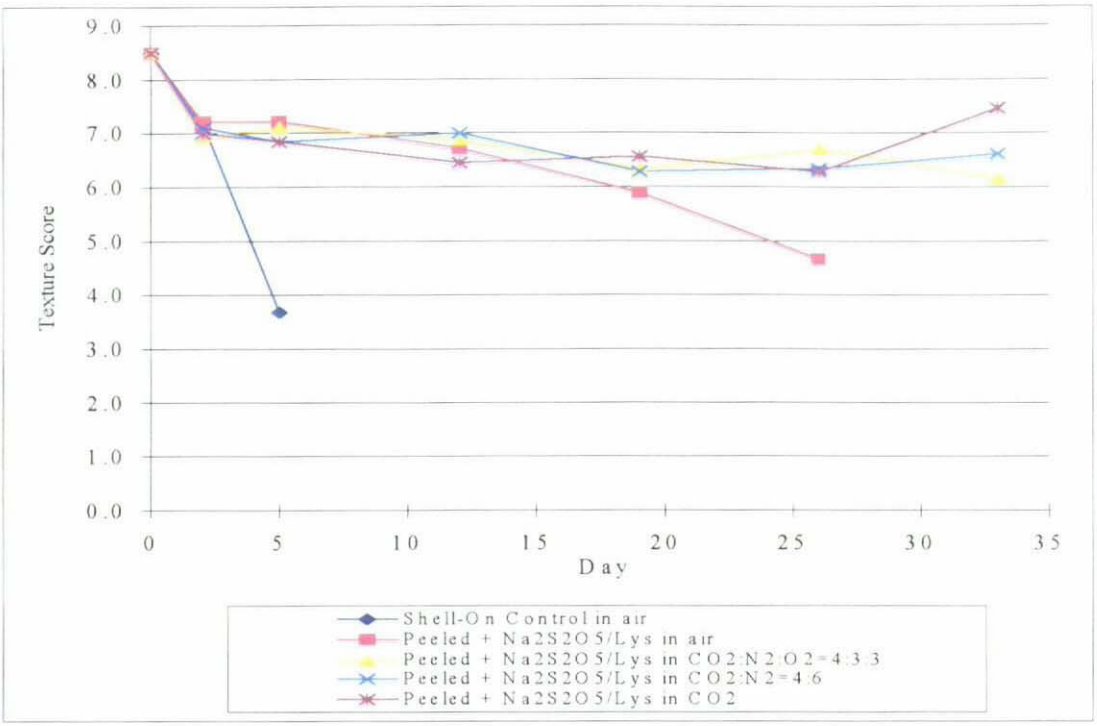


Figure 4.5.2 Change of texture scores of raw shrimp samples stored in different gas compositions in Phase V experiment.

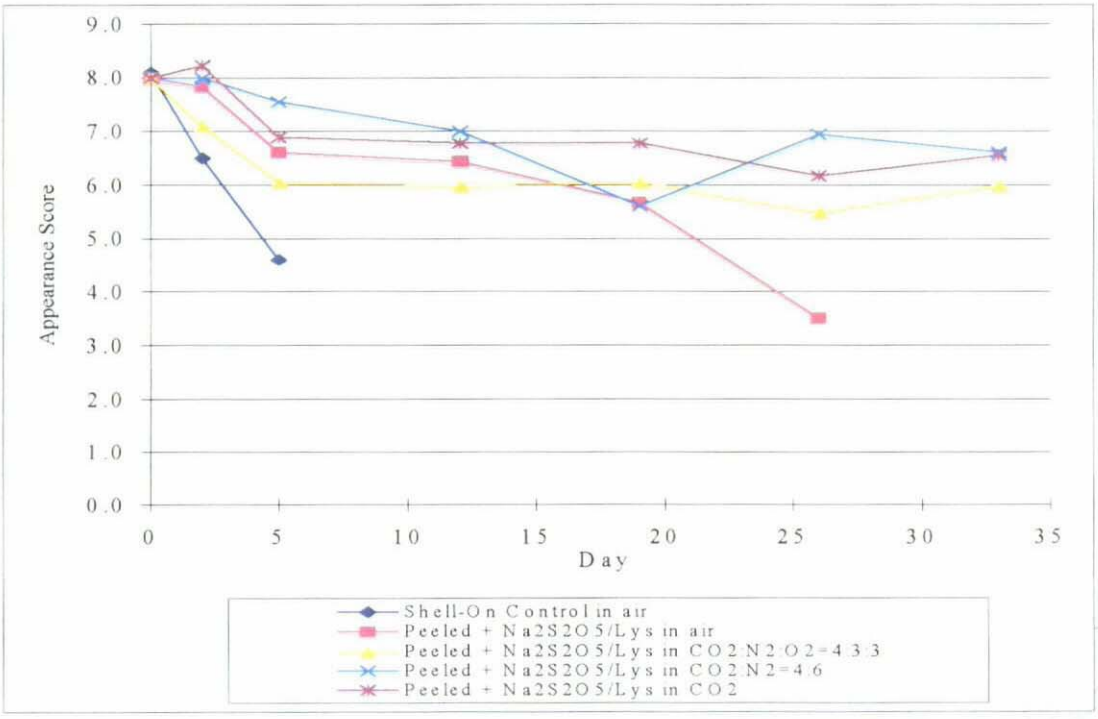


Figure 4.5.3 Change of appearance scores of raw shrimp samples stored in different gas compositions in Phase V experiment.

Results shown in figures 4.5.1 to 4.5.3 indicated that the samples, except those stored in air, were acceptable in odor, texture, and appearance after 33 days of storage.

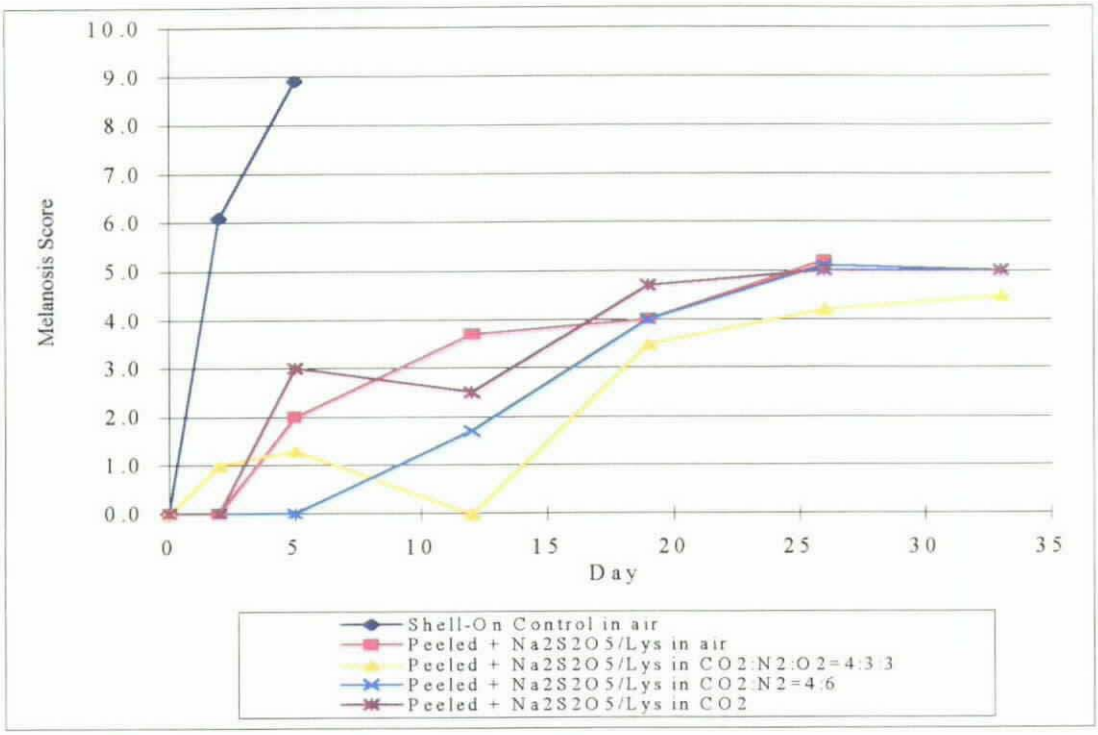


Figure 4.5.4 Change of melanosis scores of raw shrimp samples stored in different gas compositions in Phase V experiment.

All samples failed in melanosis after 24 days of storage. The melanosis shelf lives of samples were shown in table 4.5.1.

Table 4.5.1 Melanosis shelf lives of samples in Phase V experiment.

Treatment	Melanosis Shelf life (day)
Shell-On Control in air	1.2
Peeled + $Na_2S_2O_5$/Lys in air	19.0
Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2:O_2 = 4:3:3	23.8
Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6	19.0
Peeled + $Na_2S_2O_5$/Lys in CO_2	16.6

From table 4.5.1, the longest melanosis shelf life was observed in treatment of Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2:O_2 = 4:3:3. It was about 20 times more than that of the control.

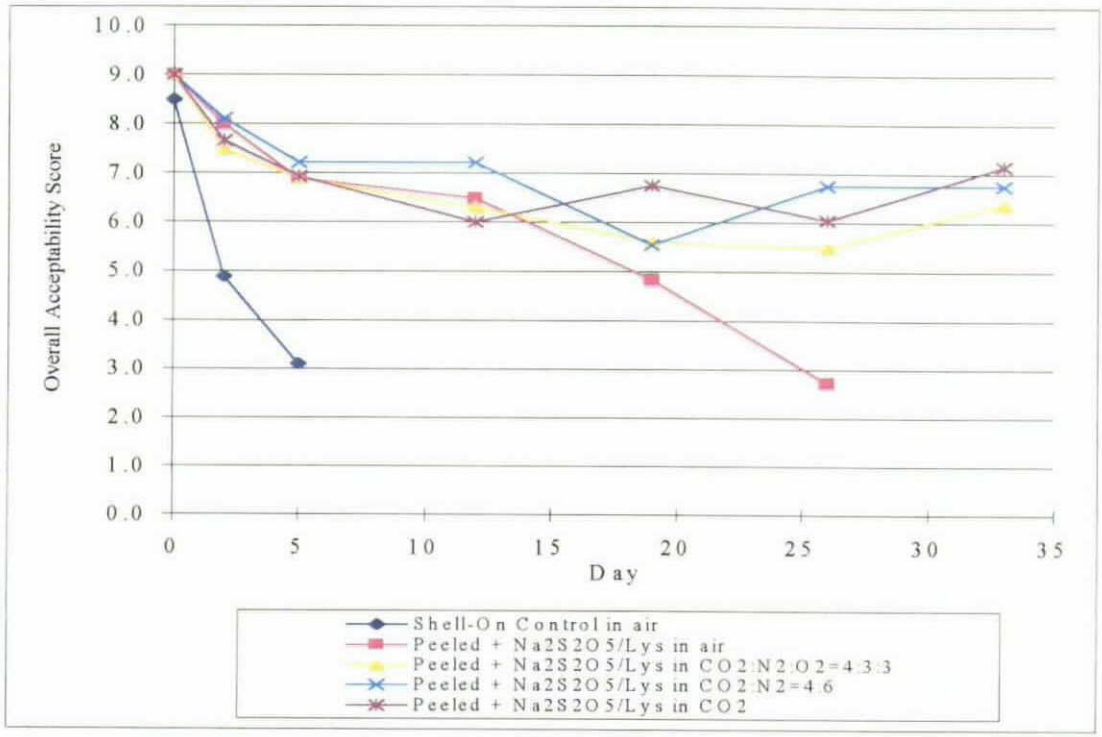


Figure 4.5.5 Change of overall acceptability scores of raw shrimp samples stored in different gas compositions in Phase V experiment.

The overall acceptability shelf lives of the control and the "Peeled + $Na_2S_2O_5$/Lys in air" samples were 2 days and 18 days respectively. All other treatments could keep samples in acceptable condition even after 33 days of storage. It seemed that all shelf lives of MAP samples were not significantly differ from each others.

4.5.1.2 Cooked Shrimps

Sensory evaluation of cooked samples were shown in figures 4.5.6 to 4.5.10.

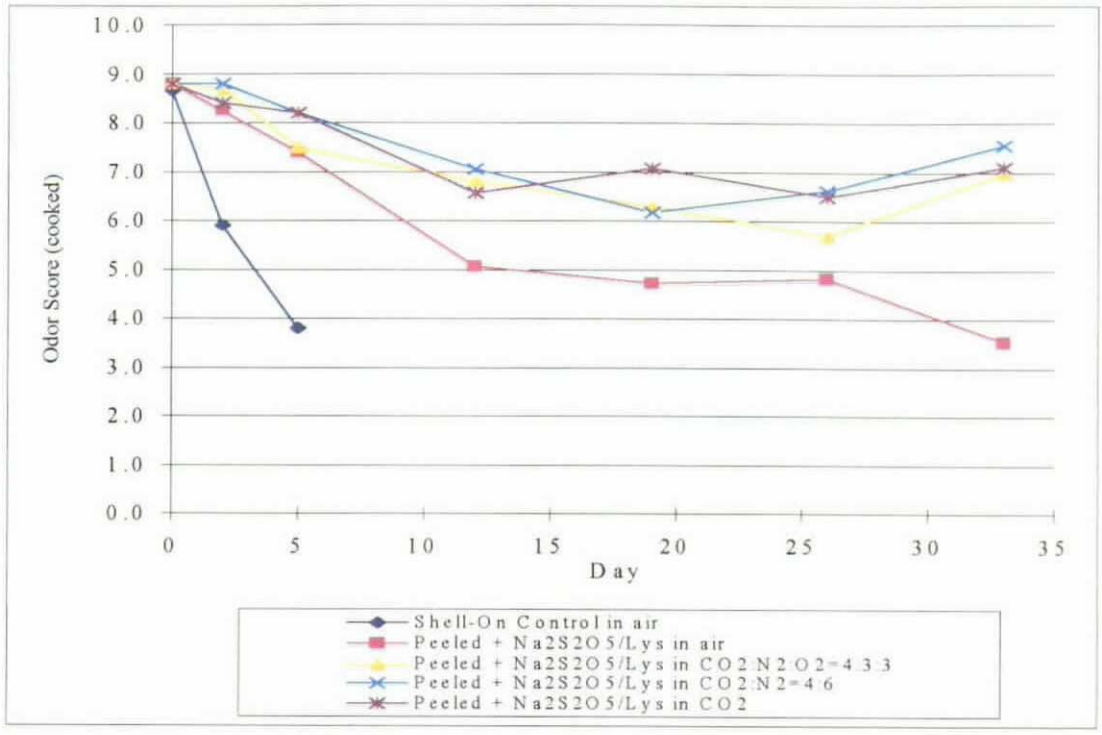


Figure 4.5.6 Change of odor scores of cooked shrimp samples stored in different gas compositions in Phase V experiment.

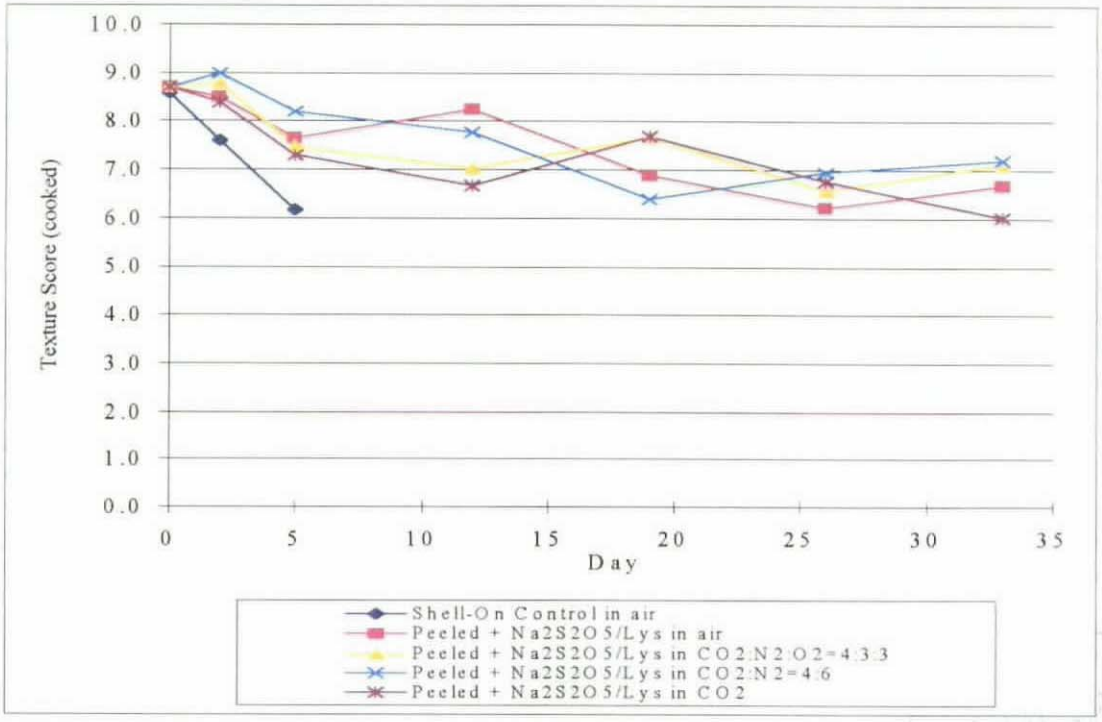


Figure 4.5.7 Change of texture scores of cooked shrimp samples stored in different gas compositions in Phase V experiment.

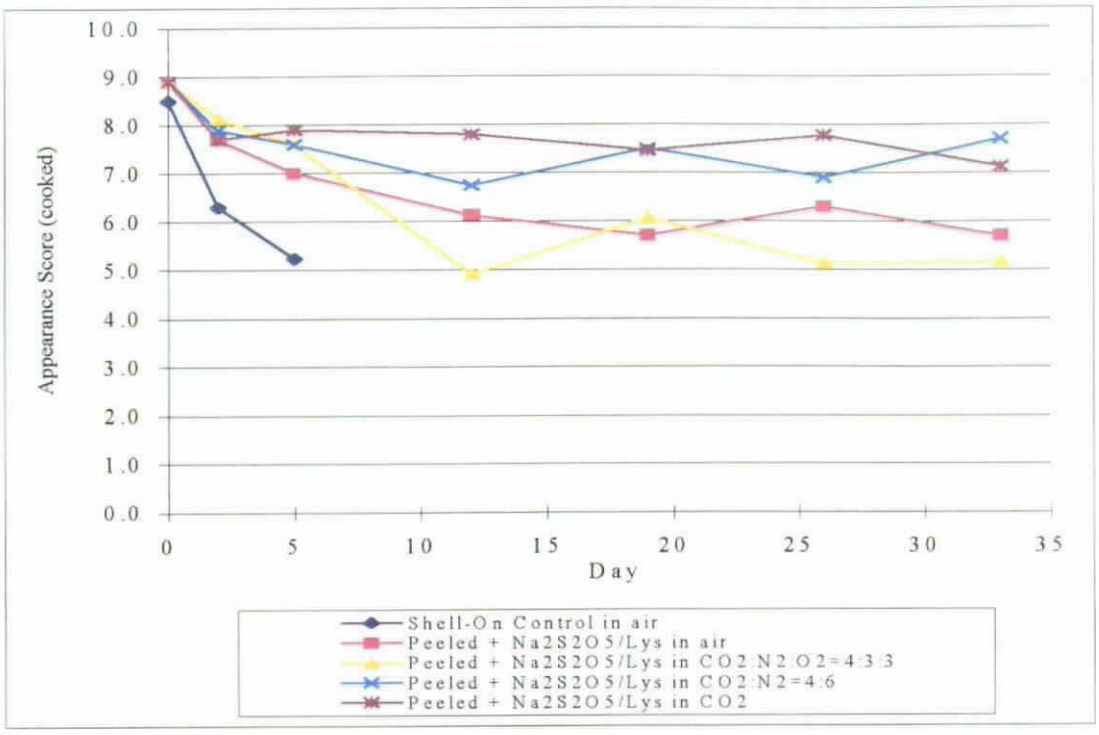


Figure 4.5.8 Change of appearance scores of cooked shrimp samples stored in different gas compositions in Phase V experiment.

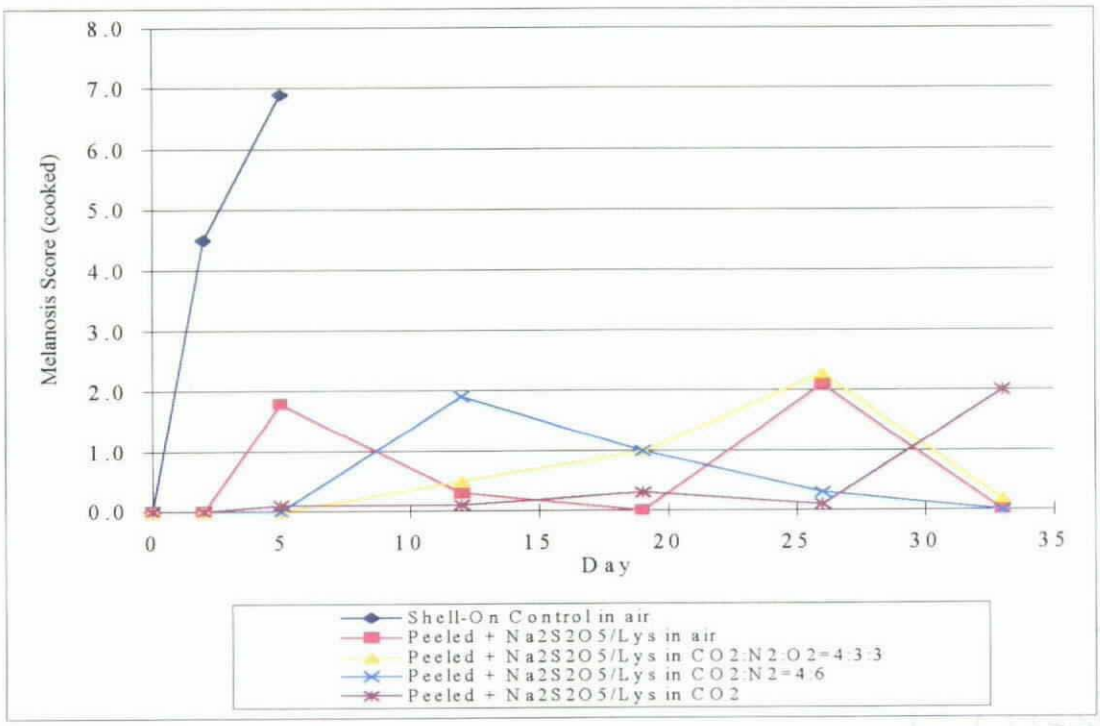


Figure 4.5.9 Change of melanosis scores of cooked shrimp samples stored in different gas compositions in Phase V experiment.

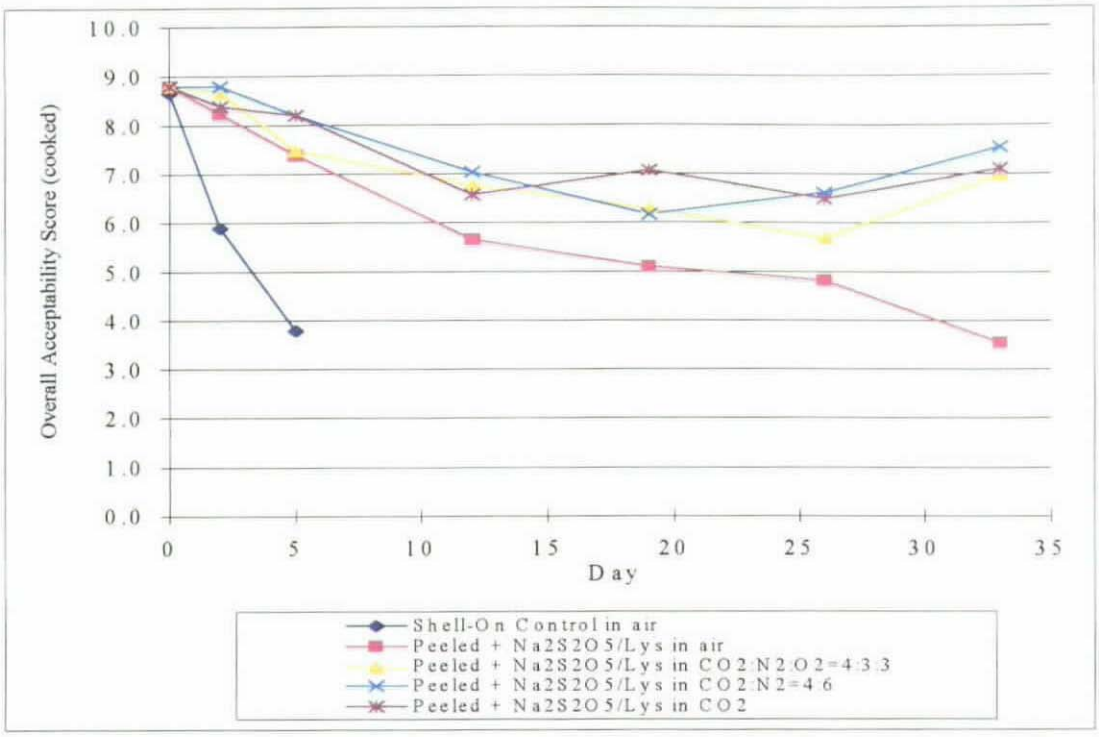


Figure 4.5.10 Change of overall acceptability scores of cooked shrimp samples stored in different gas compositions in Phase V experiment.

Similar results were observed in cooked samples as shown in the above figures. The melanosis was not conspicuous after the boiling process. All samples, except the control, were acceptable in melanosis after 33 days of storage.

The 'odor trapping' effects no longer existed in this phase of experiment. It might due to the fact that the samples in the experiment were peeled.

4.5.2 Total Volatile Basic Nitrogen Determination

TVBN was determined here and the relevant results were shown in figure 4.5.11.

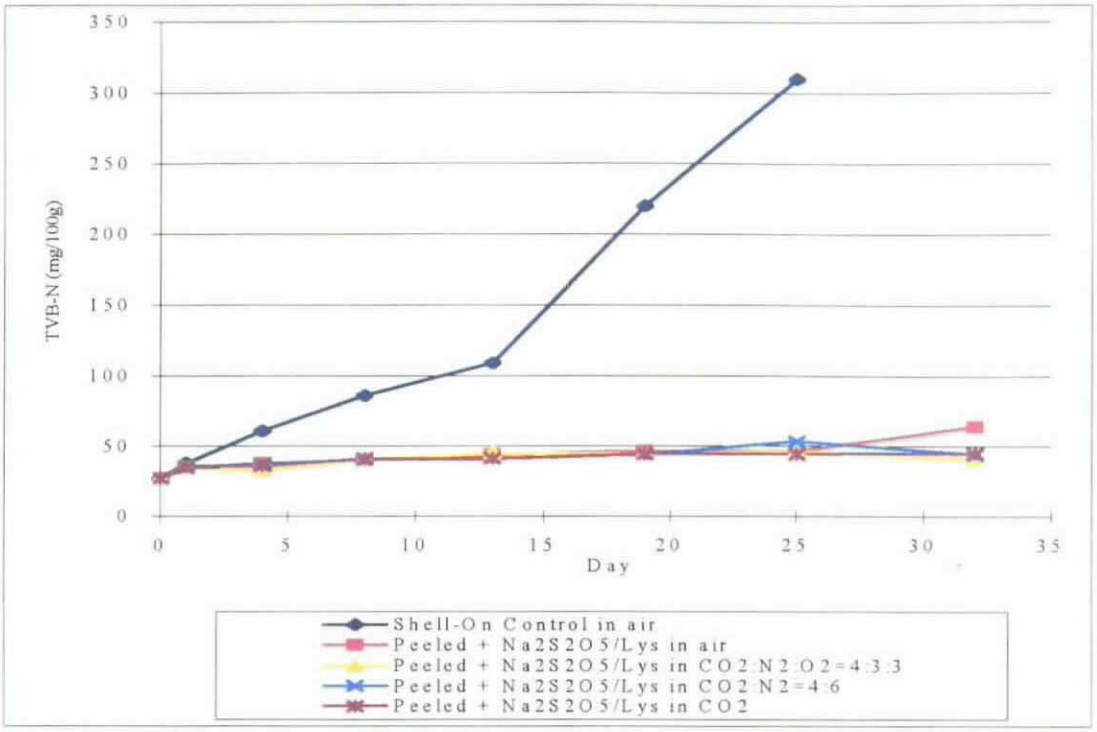


Figure 4.5.11 Change of TVBN in shrimp samples during storage in Phase V experiment.

Growth in TVBN levels in treated samples was obviously differ from that of the control samples as what we can see in figure 4.5.11. The TVBN increased rapidly up to more than 300 mg/100g in 25 days of storage. On the other hand, the TVBN level in treated samples rose slowly to about 50 mg/100g after the same period of storage.

It seemed that TVBN level could be used as a freshness indicator for our shrimp samples. Since TVBN analysis was a determination of volatile substances that constituted the core part of the odor that we can sense by our nose, it was rational to correlate the TVBN levels with odor scores as shown in figure 4.5.3. The following figure (4.5.12) showed the relationship between the two parameters in raw control samples.

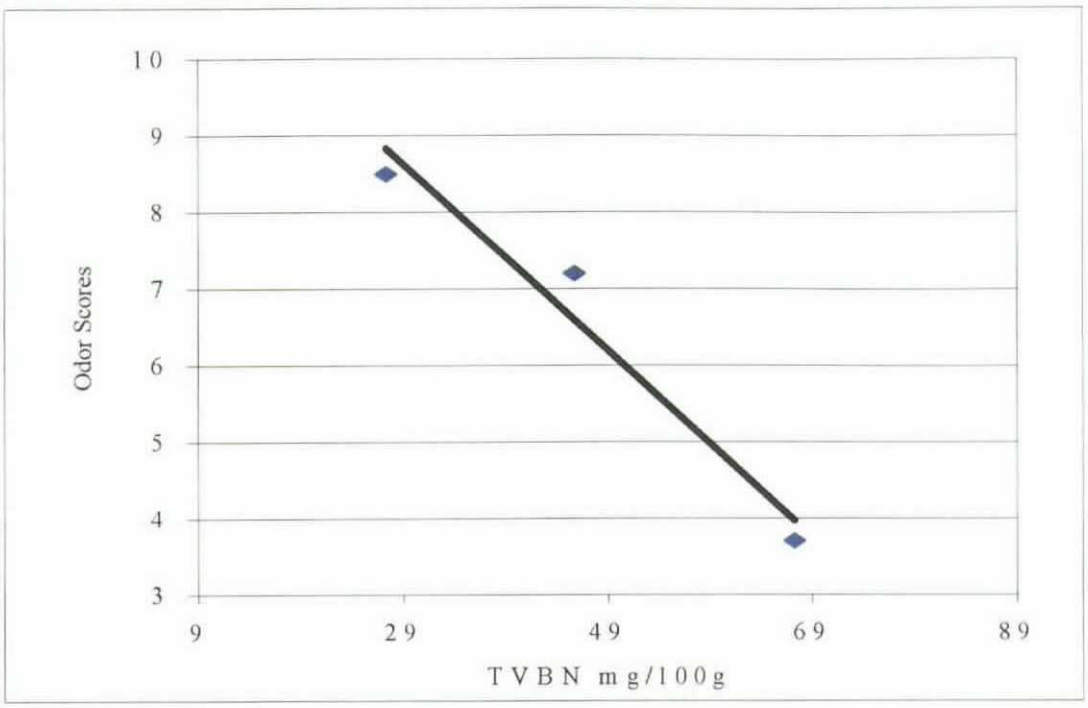


Figure 4.5.12 Correlation between odor scores and TVBN level in raw shrimp samples during storage in Phase V experiment.

The correlation coefficient for the two variables in figure 4.5.12 was 0.98 at 95% confidence level. At the time of rejection (odor score = 5), the TVBN level was 58.3 mg/100g as determined from figure 4.5.12.

The TVBN levels in fresh shrimps were determined to be 27.3 mg/100g (standard deviation = 0.84).

4.5.3 Aerobic Plate Count

APC for this phase of experiment was conducted and the results were shown in figure 4.5.13.

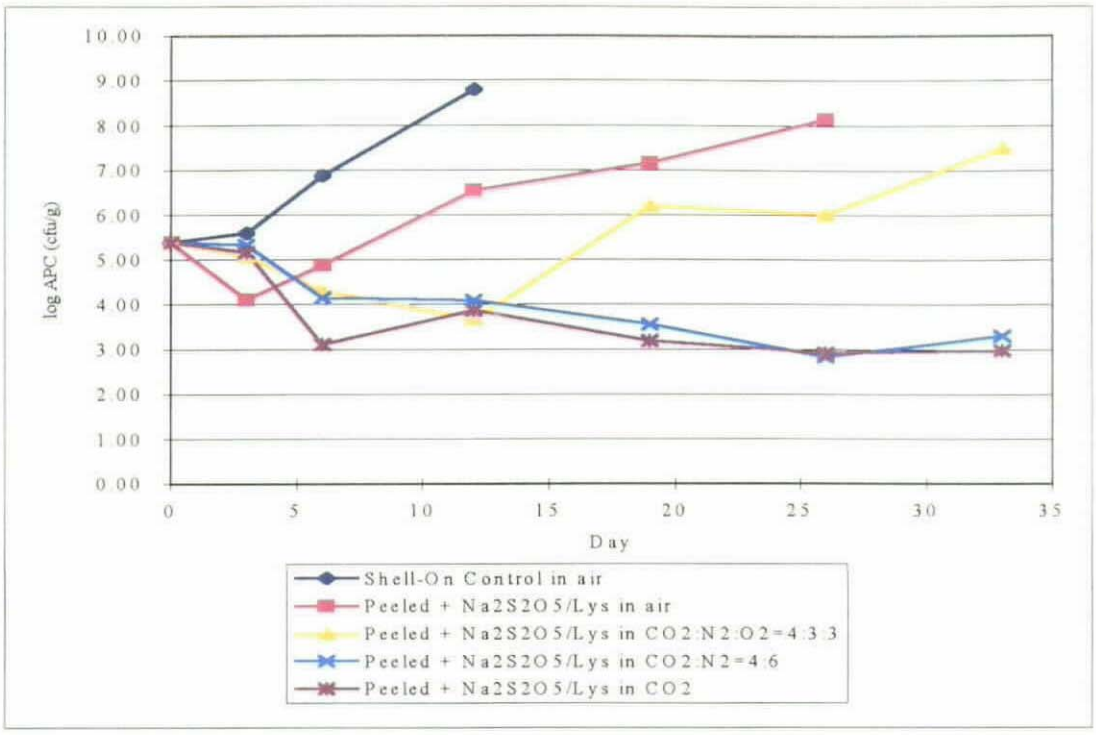


Figure 4.5.13 Change of aerobic plate count in shrimp samples during storage in Phase V experiment. (The day 0 values of peeled shrimps were assumed to be the same as the shell-on shrimps)

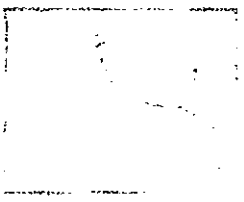
We can see in figure 4.5.12 that APC increased rapidly in the control samples after 3 days of storage. The APC in Peeled + $Na_2S_2O_5$/Lys in air samples also rose after 3 days of storage but reached 1 x 10^7 much later (~17 days) than the control(~ 7days). The increase in bacterial count was further delayed in the Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2:O_2 = 4:3:3 samples to 12 days. The increase in count after the lag phase delayed the most in CO_2:N_2 = 4:6 and pure CO_2 samples to 26 days of storage. This confirmed the findings of many other researchers that high CO_2 level prolonged the lag phase of microbes (Philips, 1996; Keizer, 1995; Daniels *et al.*, 1985).

Therefore, in the microbiological point of view, we could conclude that the atmosphere of CO_2:N_2 = 4:6 and pure CO_2 were superior in preserving our shrimp

samples. However, there was an observation that the packaging bags for pure CO_2 were shrunken and gave a 'vacuum packaging' appearance after twenty some days of storage. This phenomenon might be explained that when all carbon dioxide were dissolved in the dripped/condensed water and tissues of shrimp samples, the gas pressure inside the bag dropped and the bag collapsed. Similar shrinking of bags was also found in other samples but it will stop after about a week of storage. This might due to the fact that nitrogen inside the bags did not dissolve easily in the water and shrimp tissues.

4.5.4 Anaerobic Plate Count

Aerobic and anaerobic plate counts would be determined in the experiment. In the previous experiment we know that the APCs of samples could be kept at low level during storage and the samples were safe for human consumption in the microbiological point of view. However, the ANPCs of shrimp samples might have exceeded standard and shrimps might become hazardous to health. ANPC was, therefore, also carried out to ensure the hygiene of preserved shrimps.



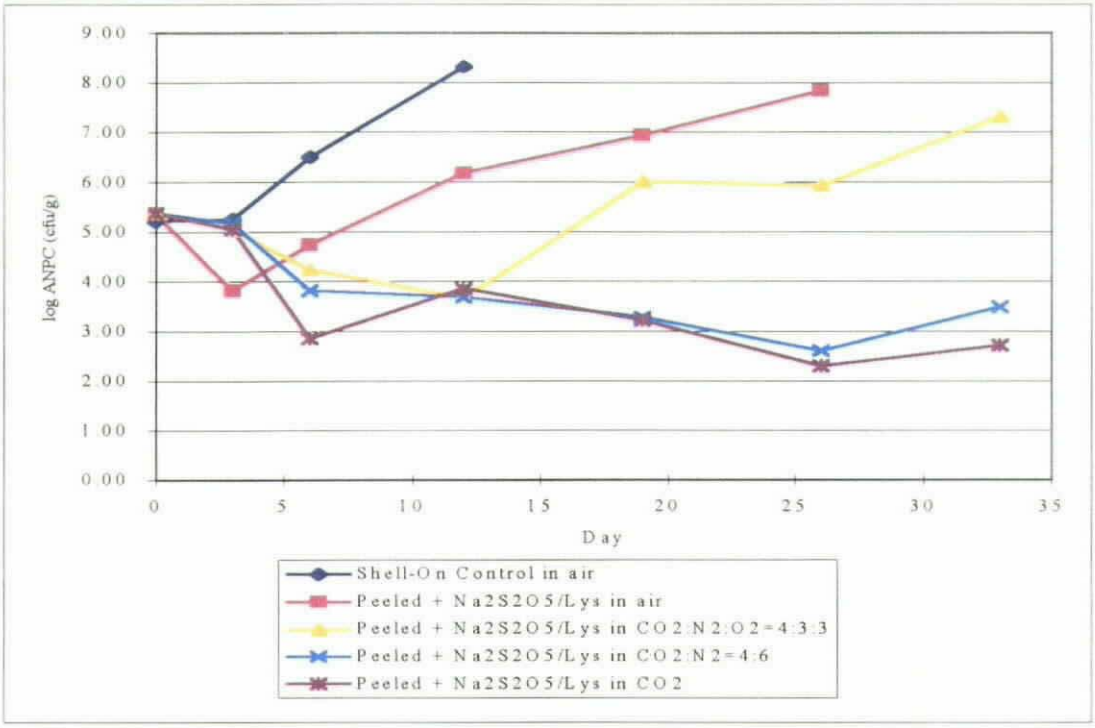


Figure 4.5.14 Change of anaerobic plate count in shrimp samples during storage in Phase V experiment.

Figure 4.5.13 indicated that the change of ANPC in samples during storage was very similar to that of APC (figure 4.5.12). In other words, the growth of anaerobes were not favored even in the absence of oxygen. This might due to the fact that elevated CO_2 level could slow down the growth rate of both aerobes and anaerobes (Keizer, 1995; Stenstrom, 1985).

4.6 Phase VI Experiment

We had already noted, from the previous experiment, that the gas composition of CO_2:N_2 = 4:6 provided the best packaging atmosphere for preserving our shrimp samples in terms of microbial control. The best performing preservation system among those studied so far was Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6.

In this system, we could take it as two components, that were 'preservatives' and 'modified atmosphere packaging'. Effects of each component on shrimps were studied in this experiment and the results would be compared with that of preservation by the whole system.

4.6.1 Sensory Evaluation

Sensory evaluations were performed for raw and cooked shrimps and the results were shown in the following subsections.

4.6.1.1 Raw Shrimps

Again, sensory evaluation for raw shrimp samples were studied and results were shown in figure 4.6.1 to 4.6.5.

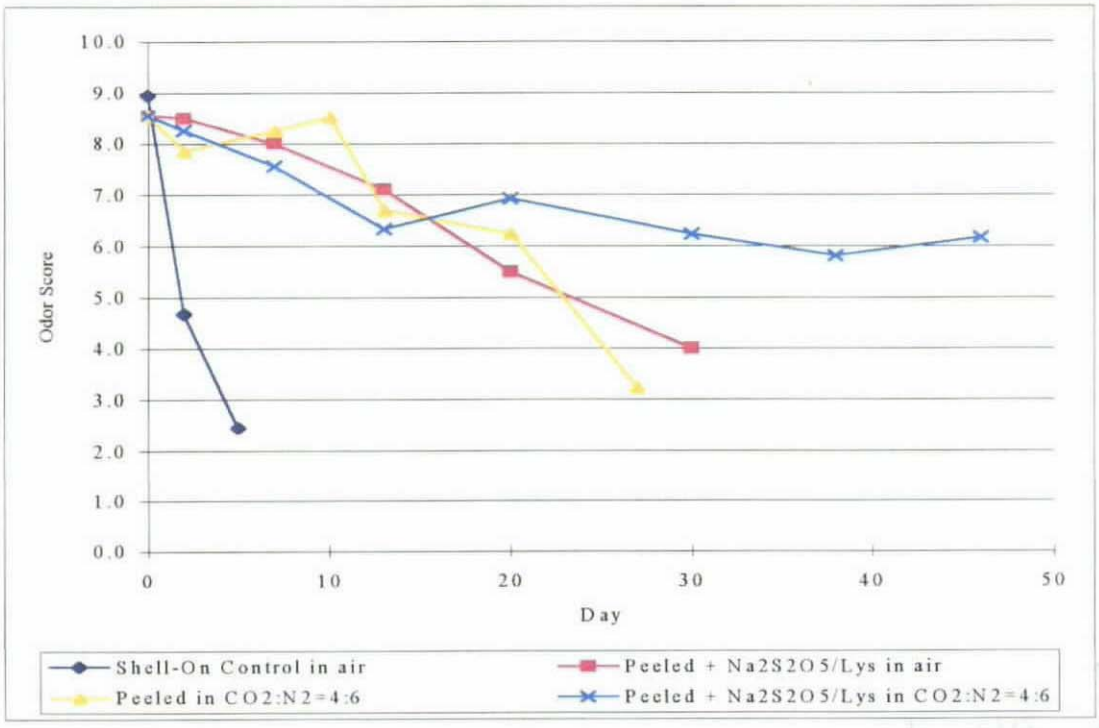


Figure 4.6.1 Change of odor scores of raw shrimp samples of Phase VI experiment.

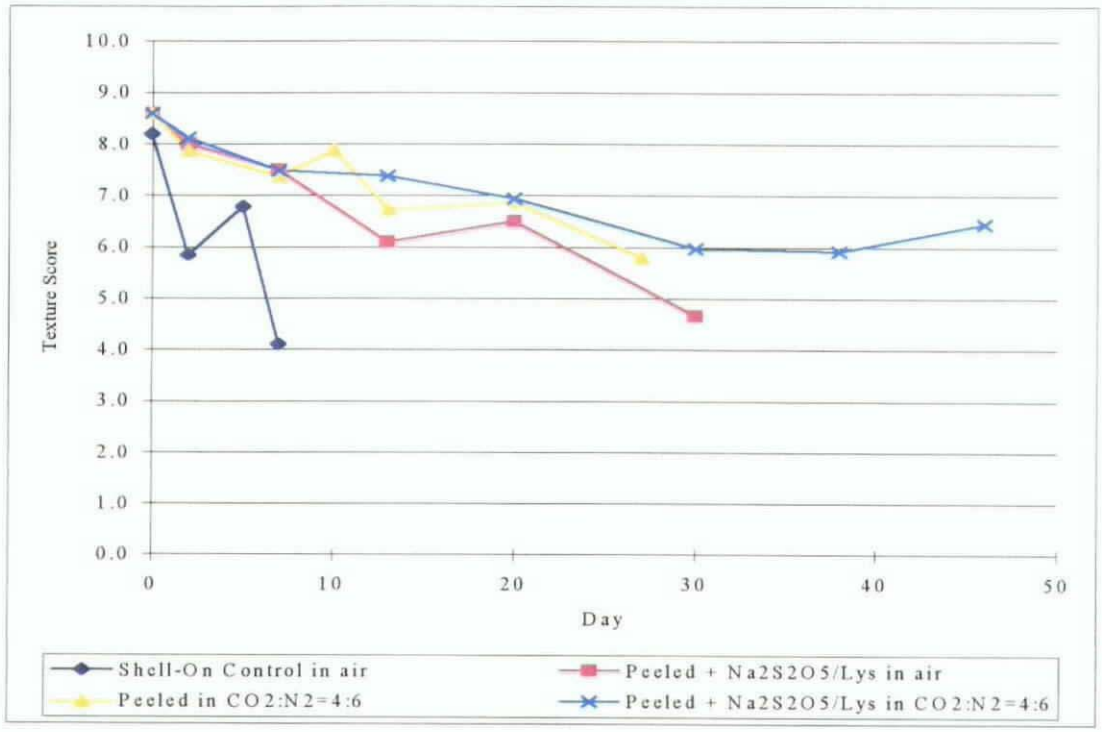


Figure 4.6.2 Change of texture scores of raw shrimp samples of Phase VI experiment.

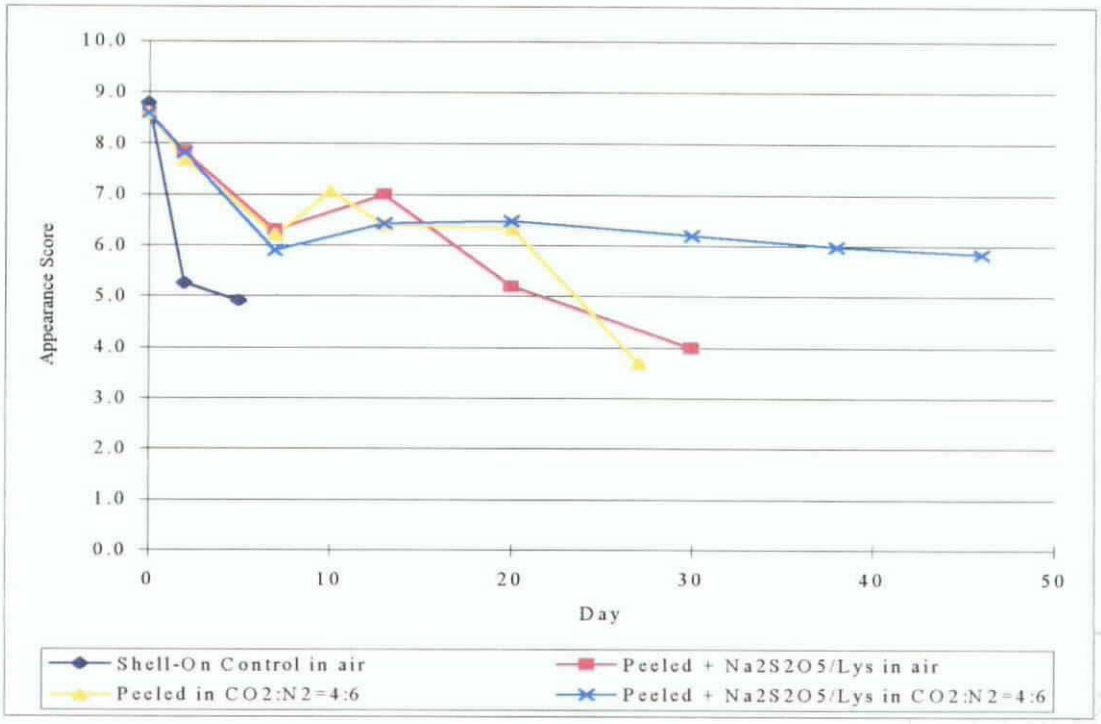


Figure 4.6.3 Change of appearance scores of raw shrimp samples of Phase VI experiment.

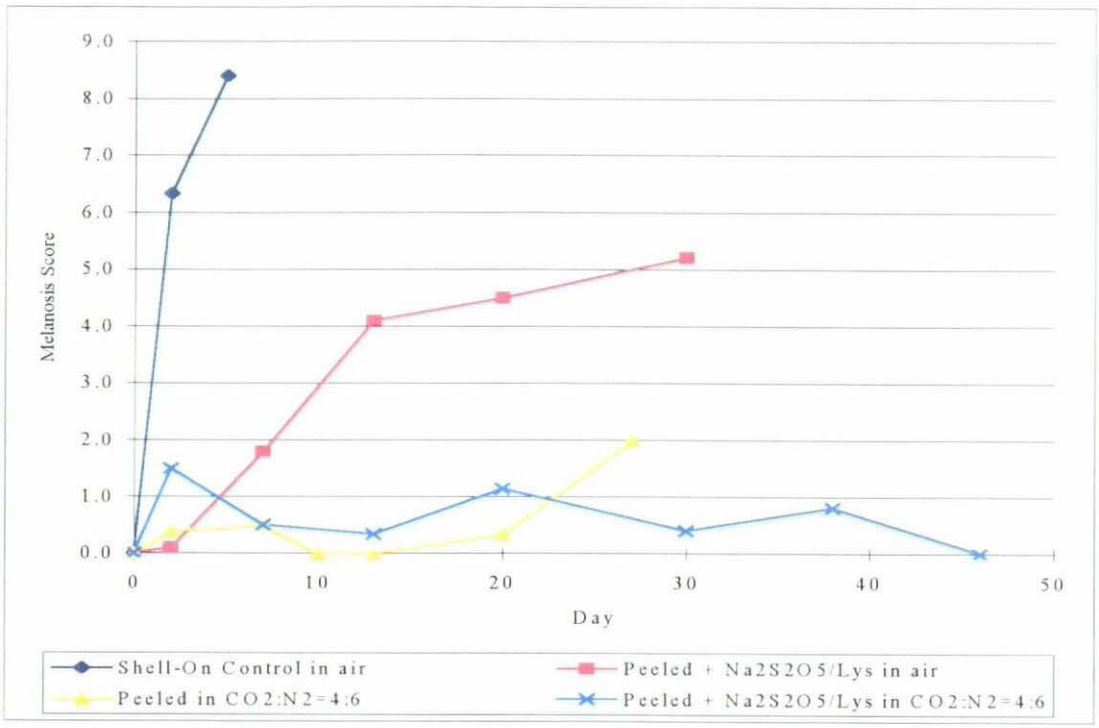


Figure 4.6.4 Change of melanosis scores of raw shrimp samples of Phase VI experiment.

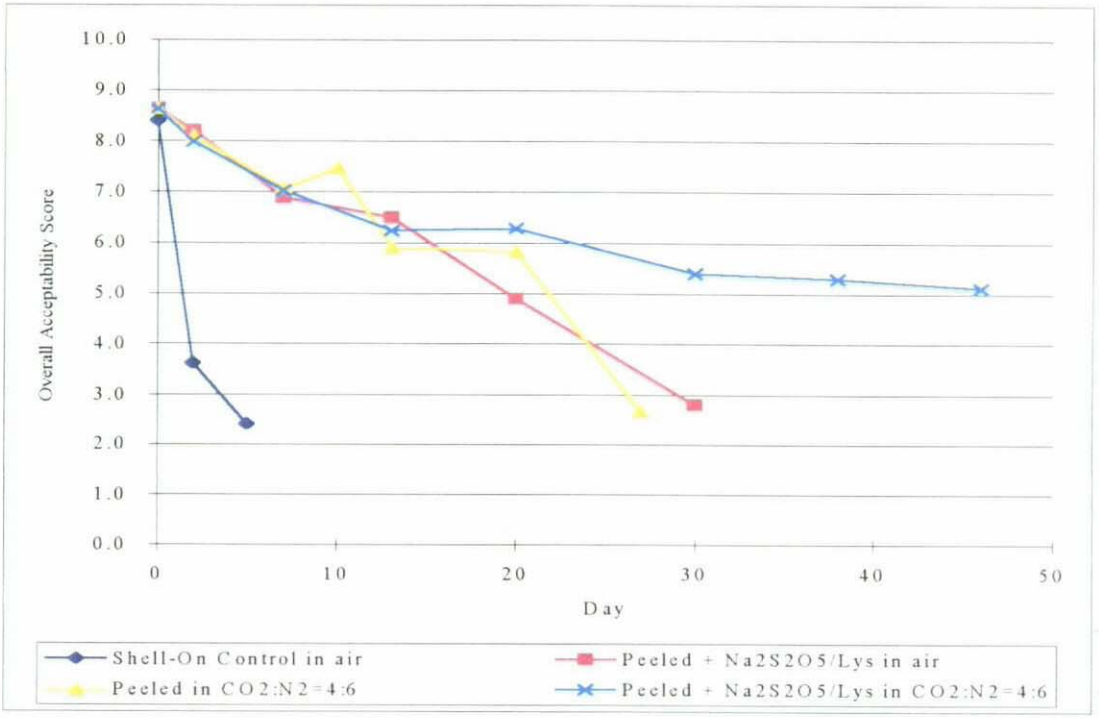


Figure 4.6.5 Change of overall acceptability scores of raw shrimp samples of Phase VI experiment.

The following table (4.6.1) summarized the attribute shelf life of samples as obtained from figure 4.6.1 to 4.6.5.

Table 4.6.1 Sensory-quality-attribute-shelf-lives of samples in Phase VI experiment.

Treatment	Sensory Quality Attributes Shelf Life (day)				
	Odor	Texture	Appearance	Melanosis	Overall Acceptability
Shell-On Control in air	1.9	6.5	4.2	1.2	1.4
Peeled + $Na_2S_2O_5$/Lys in air	23.2 (21.3)	28.0 (21.5)	21.3 (17.1)	12.8 (11.6)	19.7 (18.3)
Peeled in CO_2:N_2 = 4:6	23.0 (21.1)	>27 (>20.5)	23.1 (18.9)	>27 (25.8)	22.1 (20.7)
Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6	>46 (>44.1)	>46 (>39.5)	>46 (>41.8)	>46 (>44.8)	>46 (>44.6)

Remarks: numbers in brackets are adjusted shelf lives which equal sample shelf lives minus control shelf life.

We would like to bring your attention to the adjusted shelf lives of samples in table 4.6.1. The shelf lives of samples treated by individual components of our best performing preservative system, when added together, were less than the shelf lives of samples treated by the whole preservative system. Using overall acceptability shelf lives as examples: the shelf lives of samples treated by the two components of '$Na_2S_2O_5$/Lys' and 'CO_2:N_2 = 4:6 atmosphere' were 18.3 and 20.7 days respectively. If added together, the total shelf life would be 39 days. However, if we look at the shelf life of samples treated by the whole system of 'Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6', we could find that it was more than 44.6 days. We therefore concluded that

these two components have synergistic effects when they were combined together in preserving our shrimp samples.

4.6.1.2 Cooked Shrimps

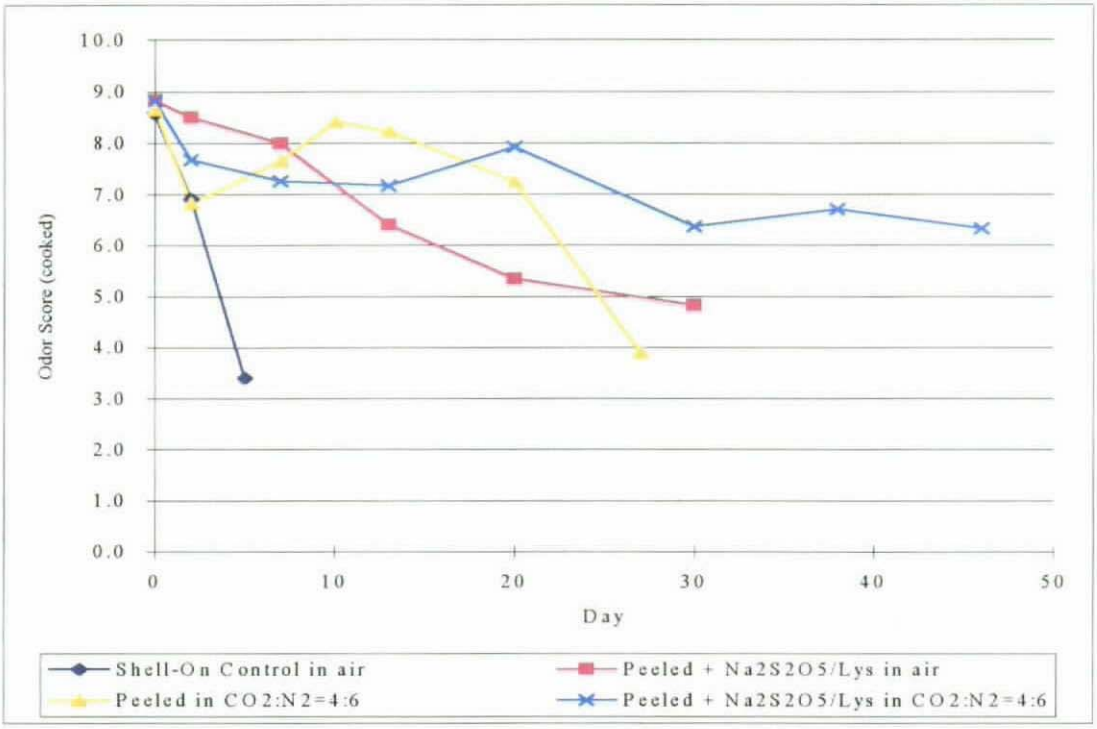


Figure 4.6.6 Change of odor scores of cooked shrimp samples of Phase VI experiment.

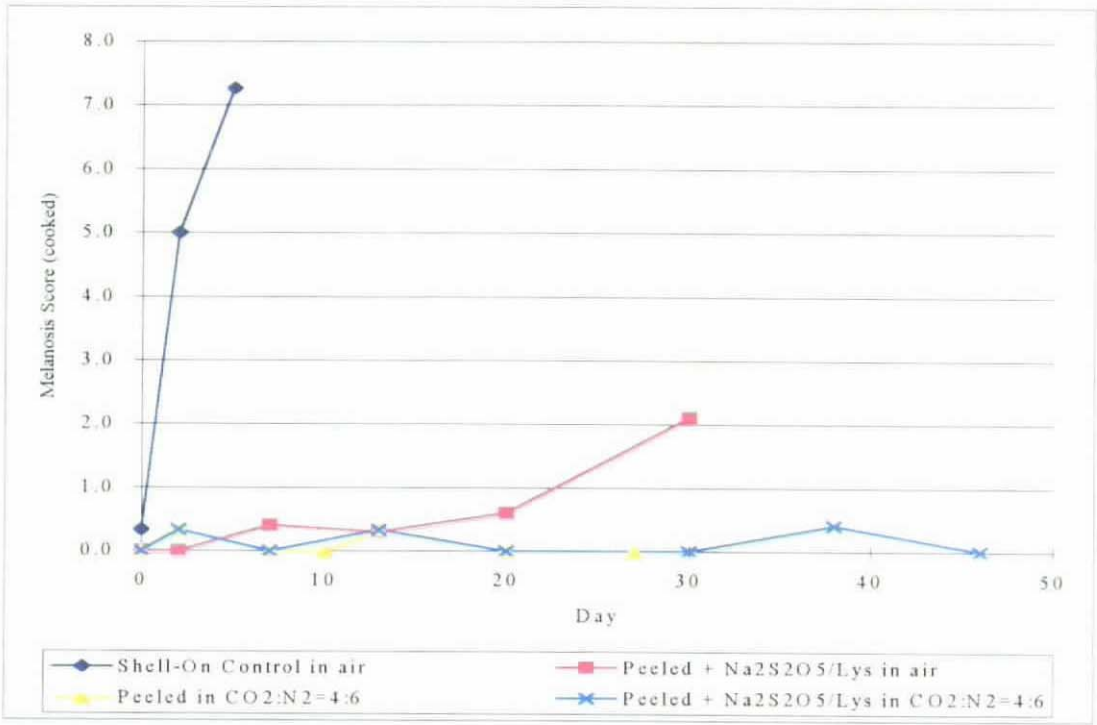


Figure 4.6.9 Change of melanosis scores of cooked shrimp samples of Phase VI experiment.

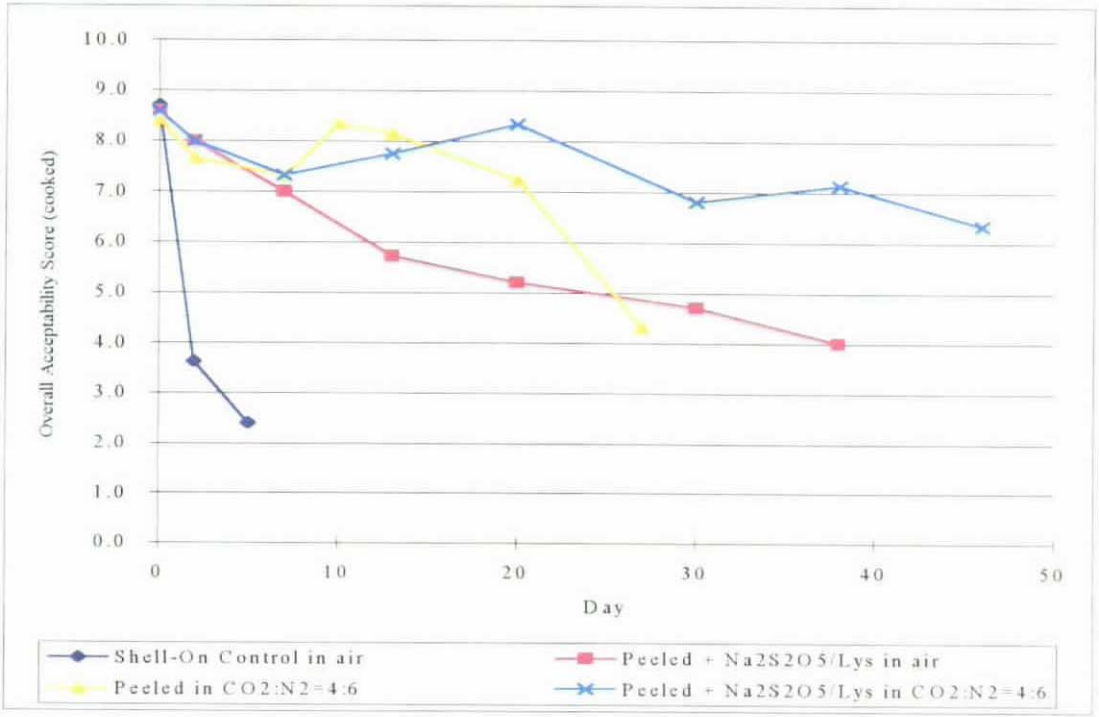
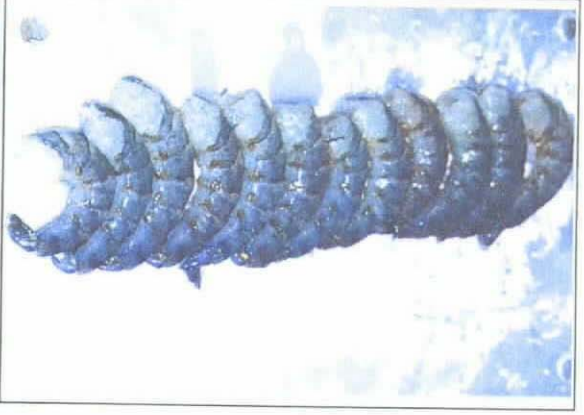
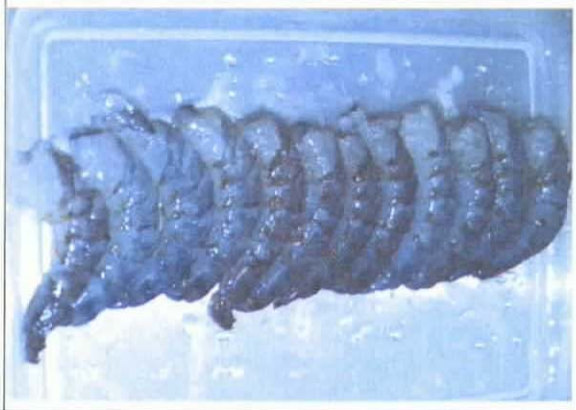


Figure 4.6.10 Change of overall acceptability scores of cooked shrimp samples of Phase VI experiment.

The results of sensory evaluation for cooked shrimp samples had similar pattern as the raw ones. The difference between them was that the cooked samples were appeared to have longer shelf lives. It might due to the fact that the odor, colour and texture of shrimps were improved by the cooking process. Thus, made it more acceptable to the sensory panel.

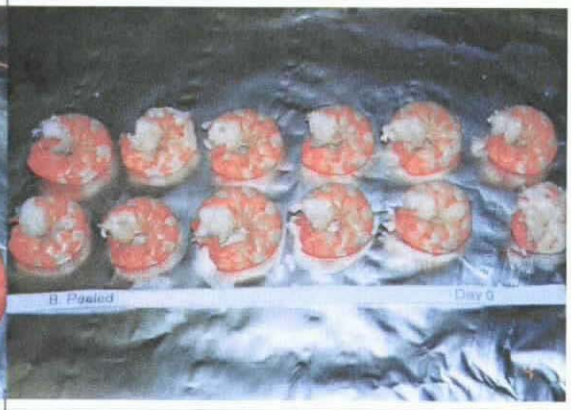
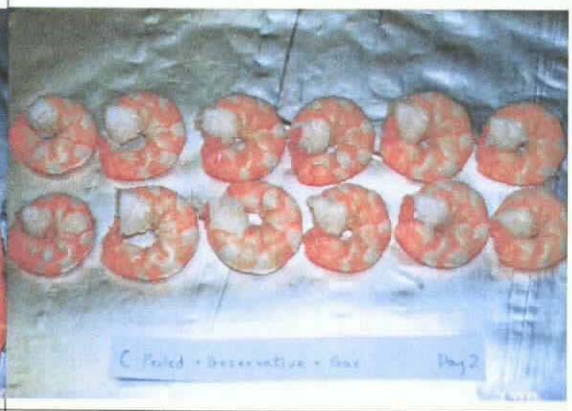
Photos for this Phase of experiment were attached below.

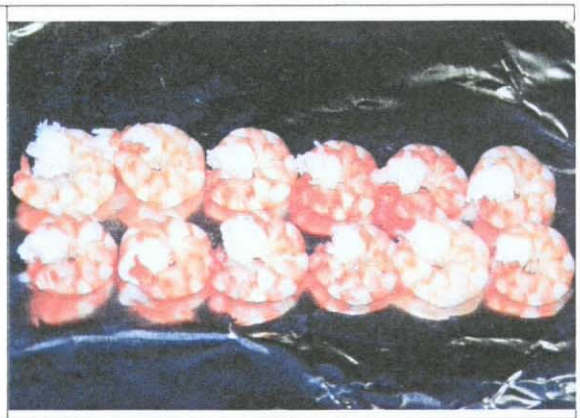
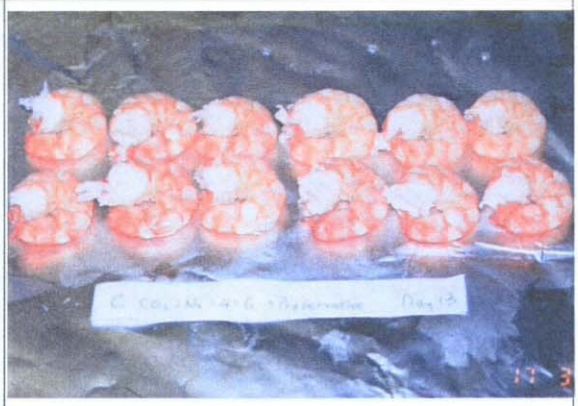
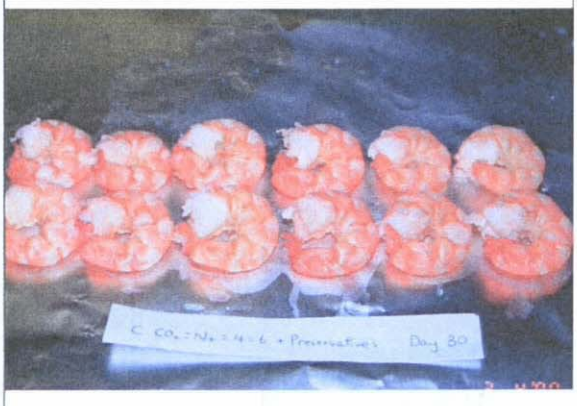
	Control	**Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6**
Day 0		
Day 2		

Day 5		**No Photo Taken**
Day 7	**End**	
Day 13		
Day 20		

Day 30		
Day 38		
Day 46		

Plate 4.6.1 Photos of raw shrimp samples taken during Phase VI experiment.

	Control	Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6
Day 0		
Day 2		
Day 5		No Photo Taken

Day 7	**End**	
Day 13		
Day 20		
Day 30		

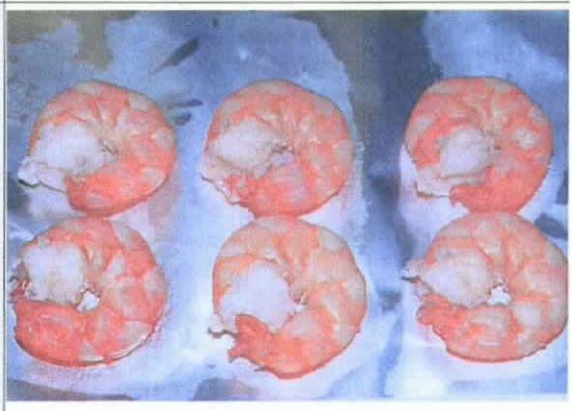
Day 38		
Day 46		

Plate 4.6.2 Photos of cooked shrimp samples taken during Phase VI experiment.

4.6.2 Total Volatile Basic Nitrogen Determination

TVBN determination was conducted and the results were shown in figure 4.6.11.

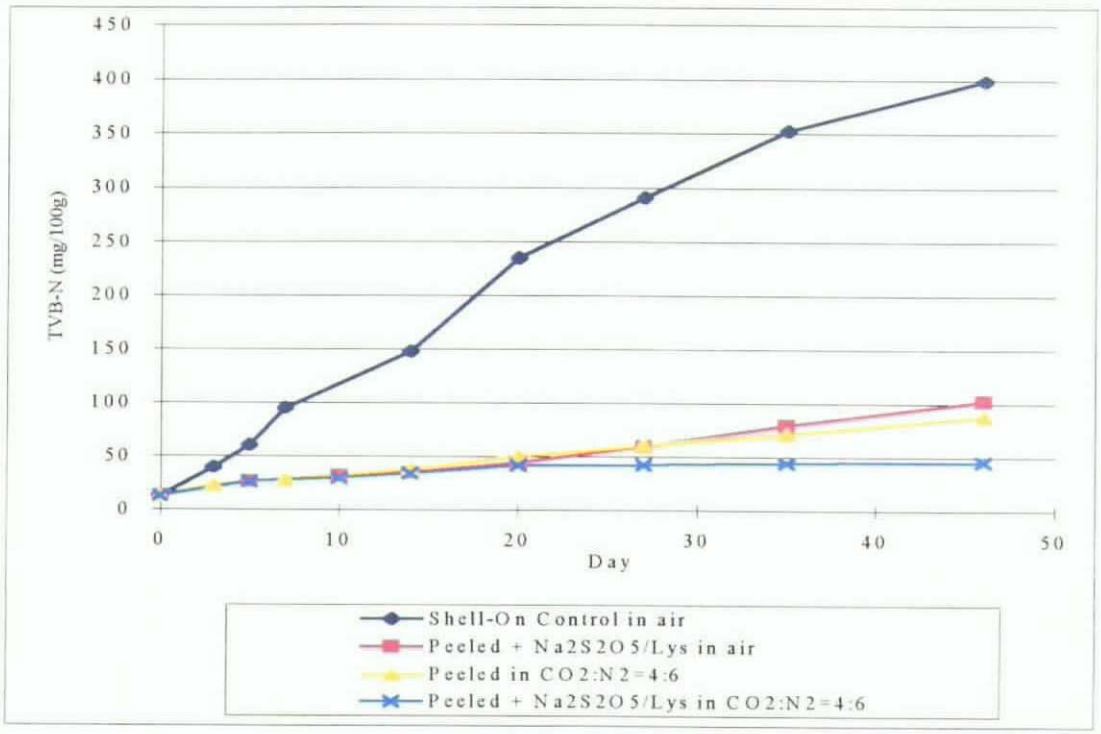


Figure 4.6.11 Change of TVBN in shrimp samples during storage in Phase VI experiment.

The pattern of curves in figure 4.6.11 was similar to that in figure 4.5.11. The TVBN level increased relatively fast in the control samples. The difference in TVBN levels of different treated samples started to become obvious after 20 days of storage. Repeating the correlation analysis in section 4.5, we had figure 4.6.12 as shown below.

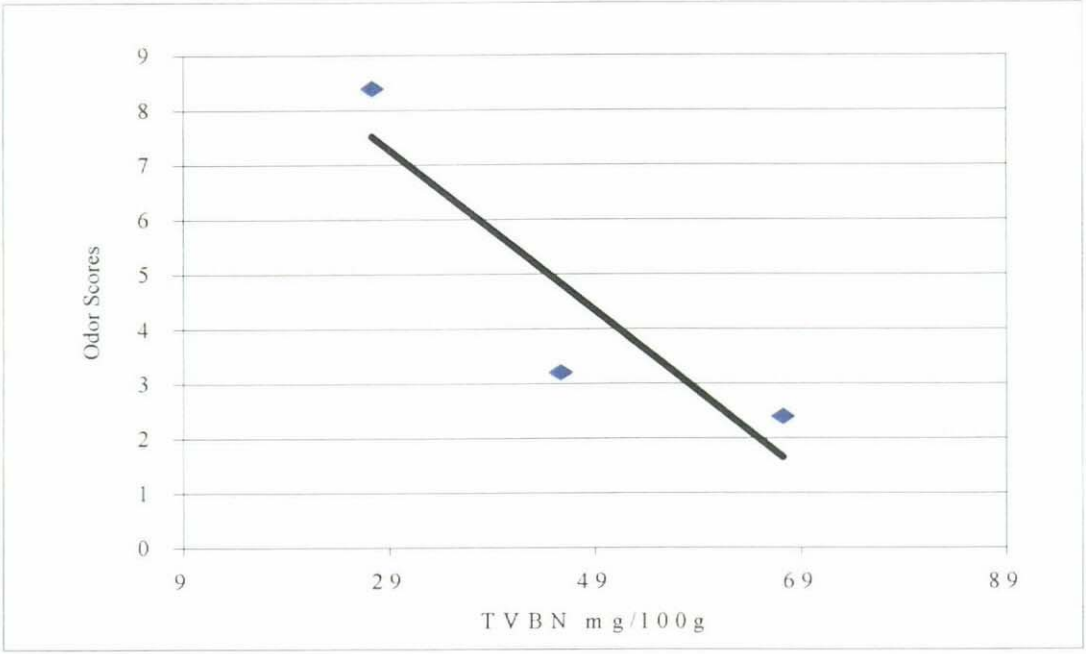


Figure 4.6.12 Correlation between odor scores and TVBN level in raw shrimp samples during storage in Phase VI experiment.

The correlation coefficient for the two variables in figure 4.6.12 was 0.90 at 95% confidence level. At time of rejection (odor score = 5), the TVBN level was 44.9 mg/100g as determined from figure 4.6.12. Together with the results obtained in Phase V experiment, the averaged TVBN level at time of odor rejection was 51.6 mg/100g.

The TVBN levels in the fresh shrimps were determined to be 13.3 mg/100g (standard deviation = 0.73). In comparison with the results in Phase V (27.3 mg/100g), the TVBN level of fresh shrimps in this phase was much lower. The difference might come from batch to batch variation, seasonal variation and sample handling time variation. The sample handling time in Phase V experiment was rather long because the sample preparation procedures were not familiarized. Therefore the spoilage of samples under room temperature was longer and this made the TVBN generation

took place more rapidly. We had paid attention in this problem and the situation was improved in Phase VI experiment.

TVBN level can therefore be a freshness indicator that we can use its determination to substitute subjective odor evaluation by human. It take about half an hour to obtain experimental results and some technical trainings are necessary for the analysis. However, the analysis require less initial capital investment when it is compared with the lightness measurement. The following table compared the differences between lightness measurement TVBN analysis and sensory evaluation of shrimps.

Table 4.6.2 Comparison between lightness measurement, TVBN analysis and sensory evaluation of shrimps.

Features	Lightness Measurement	TVBN Analysis	Sensory Evaluation
Nature	Objective	Objective	Subjective
Initial capital investment	High	Low	Low
Operating cost	Low	High	Low
Accuracy	High, Stable	High, Stable	Vary
Technical skill requirement	Low	High	Medium
Analytical time	Short	Long	Short

The primary concern about sensory evaluation is that it is subjective in nature, it's legal status is questionable. Objective evidence cannot be provided in case there are disputes between the buyers and the suppliers. Moreover, the training required for sensory evaluation is heavy before accurate evaluation can be done. Furthermore, the threshold values for detection of quality attributes were varied between different

assessors. Together with the variation in physical conditions of the assessors, sensory evaluations may vary from time to time. That is why efficient and objective analyses need to be developed by food scientists in order to substitute subjective sensory analyses.

We found that portable tri-stimulus colorimeter could provide a fast and easy to operate instrument for shrimp freshness assessment. Lightness measurement by portable colorimeter can used as a routine test for the inspectors and buyers in the seafood market to monitor their shrimp freshness.

4.6.3 Aerobic Plate Count

In the previous experiment we found that the APC and ANPC of samples were similar and therefore only APC was performed in this phase. The APC results were shown in figure 4.6.13.

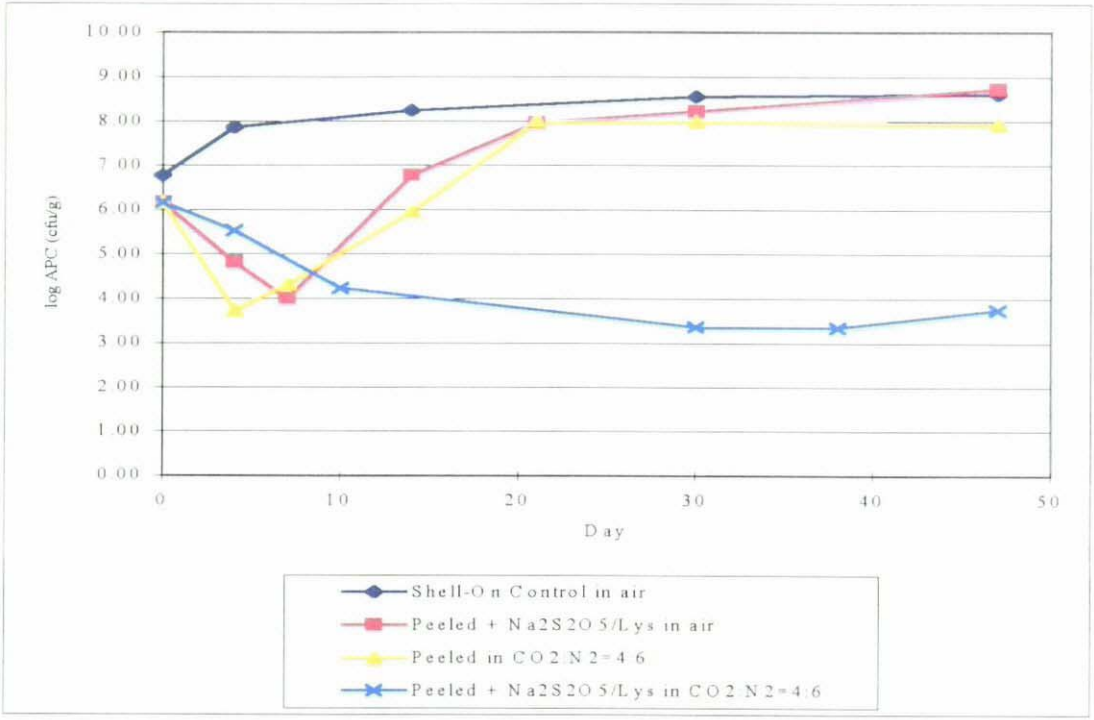


Figure 4.6.13 Change of aerobic plate count in shrimp samples during storage in Phase VI experiment.

A great difference in APC between samples was found in figure 4.6.13. Differ from the previous results, the APC in control samples grew rapidly in the initial several days of storage and there was no lag phase of bacterial growth observed in the curve. The APC of preserved samples was dropping during the initial period of storage. If we consider the preservation system of 'Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6' was comprised of two components as what we had mentioned. The two components 'preservatives: $Na_2S_2O_5$/Lys' and 'packaging atmosphere: CO_2:N_2 = 4:6" took effect

in delaying the lag phase of bacterial growth in samples by 7 and 4 days respectively. However, their effectiveness were far smaller than that of the whole system which delayed the lag phase to more than 30 days. Furthermore, the growth of bacteria in such samples during the log phase was much more slower than in other samples.

We found, in part 4.6.1, that all the sensory attributes of samples treated by 'Peeled + $Na_2S_2O_5$/Lys in CO_2:N_2 = 4:6' were still acceptable after 46 days of storage. Here in figure 4.6.13 we could confirm that such samples were still suitable for human consumption in the microbiological point of view because the APC of which was rather low (less than 10^4).

Section 5

Conclusions

SECTION 5

Conclusions

In referring to our objectives as stated before, here we drew the conclusions on the effectiveness of shrimp freshness preservation methods studied and the shrimp freshness index determined.

5.1 Shrimp Freshness Preservation Methods

The following conclusions about the best preservation system studied for shell-on and peeled shrimps were drawn. Table 5.1.1 summarized the preservation methods for shell-on and peeled shrimps.

Table 5.1.1 Suggested preservation methods for shrimps.

	Shell-On Shrimp	Peeled Shrimp
Preservatives	25% Na Metabisulphite + 100mg/L Lysozyme, 5 min. Soaking	25% Na Metabisulphite + 100mg/L Lysozyme, 5 min. Soaking
Storage Conditions	In Modified Ice Cubes, 0-2°C	MAP : 40% CO_2 + 60% N_2, 2-4°C
Shelf Lives (day)	Odor : 6.6 Overall Acceptability : 7.5 Others : >8	>46
Hygiene Condition after storage in terms of APC (cfu/g)	Fit for Human Consumption ($1x10^6$)	Fit for Human Consumption ($1x10^3 \sim 1x10^4$)

5.1.1 Shell-On Shrimps

The best preservation system for Shell-on shrimps (*Penaeus monodon)* in this research was found to be 5 minutes soaking in 1.25% Na Metabisulphite + 100mg/L Lysozyme with storage in modified (prepared with the same preservative solution) ice cubes. Although addition of 1mM Na_2EDTA or 0.01% 4-HR could help to further

extend their shelf lives but the increased amounts were insignificant. The shelf lives of cooked samples treated by this system was found to be 6.6 days at time of odor rejection and 7.5 days at time of overall acceptability rejection while other sensory quality attributes were still acceptable after 8 days of storage. The aerobic bacterial counts of these samples were acceptable at about 1 x 10^6 level at the times of their rejections.

5.1.2 Peeled Shrimps

The best preservation system for Peeled shrimps (*Penaeus monodon)* in this research was found to be 5 minutes soaking in 1.25% Na Metabisulphite + 100mg/L Lysozyme with storage in MAP (packaging material was Nylon/LLDPE) and atmosphere composition of 40% CO_2 and 60% N_2. All sensory-quality-attribute-shelf lives of raw and cooked samples treated by this system were found to be at least 46 days. The aerobic bacterial counts or these samples were acceptable at about 1 x 10^3 to 1 x 10^4 at the time of rejeotion. For samples packed with pure CO_2, similar results were obtained, but the bags shrunk seriously and gave 'vacuum packaging' appearance to the samples.

5.2 Shrimp Freshness Index Determination

All objectives tests as carried out in the research were evaluated for determining shrimp freshness index as follows.

5.2.1 Surface Lightness Measurement

Surface lightness of raw shell-on fresh shrimps (*Penaeus monodon)* as measured by portable tri-stimulus colorimeter was a good freshness indicator in terms of melanosis measurement. The results were summarized in table 5.2.1.

Table 5.2.1 Lightness as a Shrimp Freshness Index.

Index	Correlation with Melanosis Scores, r^2 (P = 0.05)	Fresh Shrimp (Batch #1)	Fresh Shrimp (Batch #2)	At Time of Rejection
Lightness	-0.92 ~ -0.99 (ave= -0.96)	17.49 (δ=1.60)	21.5 (δ=1.29)	14.8 ~ 18.1 (ave=16.5)

The correlation coefficient between surface lightness and melanosis scores were ranged from –0.92 to –0.99 (at 95% confidence level) with average of –0.96. The surface lightness of samples were determined to be ranged from 14.8 to 18.1 with average of 16.5 at time of melanosis rejection.

The averaged surface lightness of two batches of fresh shrimps (*Penaeus monodon*) were 17.49 (standard deviation = 1.60) and 21.5 (standard deviation = 1.29).

5.2.2 Total Volatile Basic Nitrogen Determination

TVBN level of raw shell-on untreated shrimps (*Penaeus monodon)* was a good freshness indicator in terms of odor measurement. The results were summarized in table 5.2.2.

Table 5.2.2 TVBN as a Shrimp Freshness Index.

Index	Correlation with Odor Scores, r^2 (P = 0.05)	Fresh Shrimp (Batch #1)	Fresh Shrimp (Batch #2)	At Time of Rejection
TVBN (mg/100g)	0.90 ~ 0.98 (ave=0.94)	27.3 (δ=0.84)	13.3 (δ=0.73)	44.9 ~ 58.3 (ave=51.6)

The correlation coefficient between TVBN level and odor scores were ranged from 0.90 to 0.98 (at 95% confidence level) with average of 0.94. The TVBN level of samples were determined to be ranged from 44.9 to 58.3 mg/100g with average of 51.6 mg/100g at time of odor rejection.

The TVBN levels of two batches of fresh shrimps (*Penaeus monodon*) were 27.3 mg/100g (standard deviation = 0.84) and 13.3 mg/100g (standard deviation = 0.73).

5.2.3 Trimethylamine Determination

The change in shrimp TMA levels during storage was similar for treated and control samples. In other words, the TMA levels of preserved samples were not significantly different from that of the control samples even though when the latter was already unacceptable in sensory evaluation. Therefore, TMA determination was not a good method for assessing freshness of shrimps (*Penaeus monodon*) and no freshness index could be established with TMA level in them.

5.2.4 Microbiological Examination

The growth of bacteria followed a sigmoid curve that included lag phase, log phase and stationary phase. The bacterial count in shrimps dropped and then rose again during storage and the results could not be correlated with sensory evaluation scores which decreased more linearly with time. Moreover, microbial examination usually took days of time to complete, this rendered it not practical for accessing freshness of shrimps which have only some days of shelf lives.

Section 6

Suggestions for Future Works

SECTION 6

Suggestions for Future Works

1. The concentrations of preservatives or gases used in this research were based on published documents on some other species of shrimps or fish. Although the present results show that preservatives at such concentrations were practical in preserving *Panaeus monodon*, there still a need to work out the optimal concentrations which could be much lower than the existing ones.

2. There was only one type of packaging material used in the research. While other packaging materials with variety of gas/moisture permeation rates were developed recently, it would be useful to explore their effectiveness in preserving seafood.

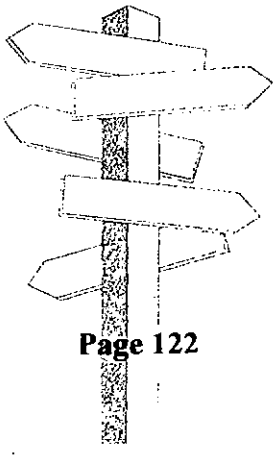
3. MAP of fish products might pose a food hazard (Eklund, 1982 & Genigeorgis, 1985). The major concern in relation to the safety of MAP fish products was the potential for growth and toxin production by *Clostridium botulinum* type E. The bacteria produced potent neurotoxins that cause botulism. Moreover, the bacteria was a strict anaerobe, can grow and produce toxins at as low as 3.3°C (Hobbs, 1976). Shrimps packed by some of MAP in this project might favored the growth of *C. botulinum* since the atmosphere was anaerobic. Conclusion were drawn from the large amount of research (Post, 1985, Garcia, 1987, Lindroth & Genigeorgis, 1986, Garcia & Genigerogis, 1987, Hussain, 1977) that the stored fish may contain botulinal toxin before any sign

of spoilage was observed. Although the incidence of *C. botulinum* type E in various seafood products appeared to be low and of a sporadic nature, for assurance of food safety, it must be assumed that all seafood products were contaminated with *C. botulinum* and their existence in MAP shrimps required to be examined (Hauschild, 1989 & Smith, 1977).

4. The modified ice cubes used in this research may be improved in size, shape and melting rate etc. It was hoped that a preservation system that require no dipping step in advance could be developed to facilitate the actual application of the system.

5. Bluing of peeled shrimps were observed during storage. Such discoloration of shrimps may be used as a shrimp freshness indicator. In other words, besides the *L* value, the a and b values of the colorimeter readings may also be used as shrimp freshness indicators.

6. The sodium metabisulphite content in shrimps were controlled by legislations in some countries, its residues in treated samples should be determined in order to monitor the residual levels. A rapid sulphite determination method, using ion-selective electrode as the main instrument, was developed recently by Gerdes *et al.* (1999).



APPENDIX I

Raw Shrimp Sensory Evaluation

Name : Sample Code :

Day :

Guildlines:

Odor	1 to 9 points, 1 = extremely bad smell, 5 = just pass, 9 = same smell as fresh
Texture	1 to 9 points, 1 = extremely soft, 5 = just pass, 9 = as firm as fresh
Integrity	1 to 9 points, 1 = extremely lose structure, 5 = just pass, 9 = as intact as fresh
Colour	1 to 9 points, 1 = extremely bad colour, 5 = just pass, 9 = same colour as fresh
Appearance	1 to 9 points, 1 = extremely bad appearance, 5 = just pass, 9 = same appearance as fresh
Melanosis	0 = not observable, 2 = noticeable in some, 4 = noticeable in most, 6 = moderate in most, 8 heavy in most, 10 = very heavy in most
Overall Acceptability	Would you buy this shrimp at resonable price? 1 = absolutely not, 5 = buy with hesitation, 9 = absolutely yes.

Please fill in the form below

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12
Odor												
Texture												
Integrity												
Colour												
Appearance												
Melanosis												
Overall Acceptability												

APPENDIX II

Cooked Shrimp Sensory Evaluation

Name : Sample Code :

Day :

Guildlines:

Odor 1 to 9 points, 1 = extremely bad smell, 5 = just pass, 9 = same smell as fresh

Texture 1 to 9 points, 1 = extremely soft, 5 = just pass, 9 = as firm as fresh

Colour 1 to 9 points, 1 = extremely bad colour, 5 = just pass, 9 = same colour as fresh

Appearance 1 to 9 points, 1 = extremely bad appearance, 5 = just pass, 9 = same appearance as fresh

Melanosis 0 = not observable, 2 = noticeable in some, 4 = noticeable in most, 6 = moderate in most, 8 heavy in most, 10 = very heavy in most

Overall Acceptability Would you eat this shrimp? 1 = absolutely not even be forced, 5 = eat with hesitation, 9 = absolutely yes.

Please fill in the form below

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12
Odor												
Texture												
Colour												
Appearance												
Melanosis												
Overall Acceptability												

REFERENCE

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