Chemical Properties and Effect of Three Different Packaging on Stability of 'Sambal Pijat'

Fatin Naimah Binti Ramli

F15A0042

A thesis submitted in the fulfilment of the requirement for the Degree of Bachelor of Applied Science (Product Development Technology) with Honours

Faculty of Agro Based Industry

UNIVERSITY MALAYSIA KELANTAN

2019

DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

_____

Student:

Name:

Date:

I certify that the report of this final year project entitled "**Chemical Properties and Effect of Three Different Packaging on Stability of 'Sambal Pijat'** " by **Fatin Naimah Binti Ramli**, matric number **F15A0042** has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

Approved by:

_____

Supervisor:

Name:

Date:

ACKNOWLEDGEMNET

In the Name of Allah, the Most Beneficent, the Most Merciful. First and foremost all praises and thanks to Allah, the Almighty, the greatest of all on His continuous grace and blessings towards the accomplishment of this thesis. Additionally, I would like to express my deepest gratitude to my supervisor, Dr. Noor Hafizoh Saidan for her willingness in accepting me as student. I am truly appreciated her patience in guiding me throughout my final year project work. Apart from that, special thanks goes to Faculty of Agro-Based Industry, Universiti Malaysia Kelantan for providing suitable environment and laboratory facilities which enable me to perform lab work effectively. Besides, I would like to give appreciation towards laboratory assistants En. Suhaimi Omar, En. Muhammad Qamal Othman, En. Nik Fakhudddin Nik Dzulkefli, ,Pn, Nor Hidayah Hamzah and Pn. Nur Aiashah Ibrahim for their technical support and help in providing adequate materials and equipments to complete my final year project. Moreover, big thanks go to En. Mohd Shahrul Ridzuan Hamil for giving clarification on how to analyse data obtained using the SPSS software. I am truly blessed for having beloved family as my backbone who never failed to support, motivates and prays for my success throughout my study. A greatest thanks goes to my parents, En. Ramli Muda and Pn. Noraisah Sulong for always being there and provide financial support. Special thanks goes to my colleagues Alya Athirah Ruziman, NorSyahira Roslan, Rahhiqin Maktuum, Rahimah Rahman and Hanisah for the moral support in completing this thesis. I am fortunate to be surrounding by supportive friends that always share knowledge and reminding each other through thick and thin. This journey truly taught me about numbers of meaningful things in real life. Last but not least, I would like to thank all parties which involved directly or indirectly in this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBEVIATIONS

%	Percent
℃	Degree celcius
LDPE	Low Density Polyethylene
HDPE	High Density Polyethylene
H_3O^+	Hydronium ions
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
H_3BO_3	Boric acid
Na_2CO_3	Sodium carbonate
PET	Polyethylene
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of variance
SD	Standard deviation
SP	'Sambal pijat'
VP	Vacuum packaging
AP	Aluminium foil pouch
AT	Air tight container

LIST OF UNITS

mL	Millilitre
mm	Millimetre
g	Gram
kg	Kilogram
h	Hour
min	Minute
s	Second
CFU/g	Colony forming unit per gram

Sifat Kimia dan Kesan Tiga Jenis Pembungkusan Terhadap Kestabilan Sambal Pijat

ABSTRAK

Sambal pijat adalah hidangan unik yang terdiri daripada rizom pepijat dan cili mata burung. Pada masa ini, hidangan ini hanya boleh didapati di Jeli, Kelantan. Ia membantu menjana sumber pendapatan bagi sesetengah peruncit di daerah ini. Walau bagaimanapun, 'sambal pijat' mempunyai jangka hayat yang lebih pendek kerana ia hanya stabil selama 2 minggu pada suhu ambien. Keadaan pembungkusan yang tidak sesuai telah menjadi salah satu faktor utama yang mengurangkan jangka hayat produk. Keadaan ini telah menyekat aktiviti perdagangan dalam memastikan kelangsungan 'sambal pijat'. Pada masa kini,tiada kajian berkaitan analisis kimia sambal pijat yang diterbitkan. Projek ini bertujuan untuk mengkaji sifat-sifat kimia dan mengenalpasti kesan keadaan pembungkusan yang berbeza pada kestabilan sambal pijat. Sehubungan dengan itu, analisis kimia, analisis fizikokimia dan ujian mikrob telah dijalankan dalam menentukan sifat kimia, kestabilan fizikokimia dan mikrobiologi sambal pijat yang disimpan dalam keadaan pembungkusan yang berlainan. Analisis fizikokimia melibatkan analisis warna dan penentuan kandungan lembapan termasuk nilai pH. Di samping itu, analisis untuk kestabilan mikrobiologi juga dilakukan untuk mengenal pasti jumlah mikroorganisma yang ditentukan melalui jumlah kiraan plat. Sambal pijat menunjukkan hasil positif dengan adanya kandungan protein dan lemak. Di samping itu, kajian menunjukkan bahawa keadaan pembungkusan memberi kesan kepada kestabilan fizikokimia 'sambal pijat' semasa penyimpanan. Selain itu, keadaan pembungkusan yang berlainan juga mempengaruhi jumlah mikrob dalam masa penyimpanan. Penemuan kajian dapat memberikan maklumat saintifik mengenai sambal pijat.

Kata kunci: sambal pijat, keadaan pembungkusan,kestabilan

Chemical Properties and Effect of Three Different Packaging on Stability of 'Sambal Pijat'

ABSTRACT

'Sambal pijat' is one of unique dish which consist of 'pepijat tree' rhizomes and bird's eye chillies. At the present time, this dish can only been found in Jeli, Kelantan. It helps to generate income sources for some retailers in this district. However, 'sambal pijat' has a shorter shelf life as it only remains stable for 2 weeks at ambient temperature. The inappropriate packaging conditions have become one the main factors which reduce the product shelf life. This situation has restricted the trade activities in ensuring the sustainability of 'sambal pijat'. Currently, there are no studies regarding chemical analysis of 'sambal pijat'. This project proposed to study the chemical properties and identify the effect of different packaging conditions on stability of this dish. Relatively chemical analysis, physicochemical analysis and microbial test were conducted in determining the chemical properties, physicochemical and microbiological stability of 'sambal pijat' that stored in different packaging conditions. The physicochemical analysis involved colour analysis and determination of moisture content including pH value. The analysis for microbiological stability also conducted to identify the total viable counts of microorganisms which indicate determination of total plate count. 'Sambal pijat' showed a positive result with presence of protein and fat content. In addition, study revealed that packaging condition does affect the physicochemical stability of 'sambal pijat' during storage. Moreover, different packaging conditions also influence the total viable count of microbes within storage time. The findings of this current study help to provide scientific information about 'sambal pijat'.

Keywords: 'Sambal pijat', packaging conditions, stability

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Ironically, every place must have its own signature dishes with their uniqueness and speciality especially to the inhabitants. Relatively, Jeli which is one of the districts in Kelantan also have a unique dish that known as ‘sambal pijat’. Basically, ‘sambal pijat’ is originated from Jeli as it rarely found at other places in Kelantan. Perhaps, this dish was named ‘sambal pijat’ due the strong aroma imparted from the crushed leaves and rhizomes of the main ingredients which is ‘pokok pepijat’ that smell like strong bed-bug (Ibrahim et al., 2009). In addition, ‘sambal pijat’ consist of rhizome and soft stem from ‘pokok pepijat’ that is blended together with chillies. ‘Sambal pijat’ can be classified under the perishable food as it cannot last longer when stored at an ambient temperature due to bio deterioration process. Bio deterioration occurs because of the packaging that might fail to give full protection or improper storage condition.

In general, packaging refers to material which enclosed or contains something. On the other hand, the basic function of packaging is to present, contain, protect, handle, and distribute goods, starting from raw materials to finished products, throughout supply chain process. Meanwhile, condition refers to the physical situation that someone or something is in and affected by. Relatively, packaging conditions indicates how the gas composition or environment inside a package can affect its

contents. There are different types of packaging system that provides different condition like modified atmosphere packaging (MAP). MAP works by removing or replacing the air surrounding food products before being enclosed with vapour barrier materials (Lee et al., 2015).

However, inappropriate packaging might shorten the shelf life of a product. This is due to the main constituent of packaging in food processing and preservatives which can influence the product shelf life (Moldovan & Pantea, 2015). This is supported by Marsh and Bugusu (2007) where package design and structure influences the food product shelf life. Thus, it is crucial to choose the right packaging materials in preserving quality and freshness of product throughout supply chain. In general, the materials that usually been used as food packaging are glass, metals ,paper and paperboards including plastics (Marsh & Bugusu, 2007). Basically, each of the packaging materials has their own speciality and limitations in shielding products.

Stability test refers to an assessment on the degradation of food properties over time (Guillet & Rodrigue, 2010). Analysis of food products and its constituents is important in ensuring their quality and safety before being consumed. This was supported by Nielsen, (2010) where all food products need to be analysed in fulfilling quality management procedure starting from manufacturing process and even until product is marketed. Other than that, stability test also need to be conducted in measuring the food ability to withstand changes in its properties for a period of time. There are three types of changes which is chemical, physical or microbiological deteriorative that may occur and effect the food composition throughout the storage period.

The purpose of this study was to perform a chemical analysis of 'sambal pijat' originated from Jeli in determining the protein and fat content. Next was to conduct a stability test on 'sambal pijat' in different packaging conditions. In achieving this goal, the effect of three packaging conditions (vacuum packaging, aluminium foil pouch and air tight container) on physicochemical stability and microbiological stability were evaluated at three different storage temperatures.

1.2 Problem Statement

Currently, 'sambal pijat' is sold by some retailers at 'Pasar Jeli' in small batches. Some villagers claimed that this dish provides health benefit. However, for this time being there is no study establish on the chemical properties of 'sambal pijat'. It is packed by using clear plastic with a rubber band or air tight container. Unfortunately, 'sambal pijat' cannot be stored for a longer period of time especially when placed at ambient temperature. This is because, it can only last long for at most 2 weeks before getting spoil. Relatively, short shelf life had become a challenge for the retailers to ensure the sustainability of this product supply. Besides, methods of packaging applied also increase the possibility of product spoilage. This shows when availability of air (oxygen) inside packaging used become the major causes for product spoilage.

1.3 Hypothesis

Hypothesis one

H_0: 'Sambal pijat' have no significant effect on protein and fat contents.

H_1: 'Sambal pijat' have significant effect on protein content and fat content.

Hypothesis two

H_0: The different types of packaging and storage conditions have no significant effect on colour analysis of 'sambal pijat'.

H_1: The different types of packaging and storage conditions have significant effect on colour analysis of 'sambal pijat'.

Hypothesis three

H_0: The different types of packaging and storage conditions have no significant effect on moisture of 'sambal pijat'.

H_1: The different types of packaging and storage conditions have significant effect on moisture content of 'sambal pijat'.

Hypothesis four

H_0: The different types of packaging and storage conditions have no significant effect on pH value of 'sambal pijat'.

H_1: The different types of packaging and storage conditions have significant effect on pH value of 'sambal pijat'.

Hypothesis five

H_0: The different types of packaging and storage conditions have no significant effect on microbiological stability of 'sambal pijat'.

H_1: The different types of packaging and storage conditions have significant effect on microbiological stability of 'sambal pijat'.

1.4 Objectives

The objectives of present study are as follows:

1. To identify protein and fat contents of 'sambal pijat'.
2. To determine the physicochemical stability (colour analysis, moisture content and pH value) of 'sambal pijat' in different packaging conditions.
3. To perform a microbial test on 'sambal pijat' in different packaging conditions during stability test.

1.5 Scope of Study

Generally, this study focussed on the chemical analysis of 'sambal pijat'. In addition, current study also focused on stability determination of 'sambal pijat' when incorporated with different packaging conditions. The samples were purchased from 'Pasar Jeli'. Initially, chemical analysis was performed to determine the protein content and fat content. The protein content was determined by Kjedahl's method whereas fat content was analysed based on soxhlet method.Then, the samples were placed in distinct types of packaging and stored at different storage conditions.

After that a stability test was conducted to analyse the physicochemical properties and microbiological characteristics of 'sambal pijat'. The methods chosen are colorimetry technique, moisture content analysis, pH value measurement and total plate count.

1.6 Significance of Study

This study helps to contribute in providing information regarding the suitable packaging condition in extending the shelf life of 'sambal pijat'. Moreover, this study also provided data regarding chemical properties of 'sambal pijat'. Besides, from the data obtained it may provide added information in the field of research activities. This is because, scientists able to postulate others researches in the future. For instance, new research can be carried out to enhance the packaging properties like applying an active packaging. Despite only focussing on the packaging, further research about the benefit of 'sambal pijat' can be established. In addition, this study offers great opportunity to any entrepreneur to set up business in commercializing 'sambal pijat'. Meanwhile, it also helps current retailers in product development activity by improving the existed packaging used.

1.7 Limitation of Study

The limitation of the study was the processing method of sample used to determine its stability in different packaging conditions. This is because, the 'sambal pijat' was purchased from a chosen retailer at Pasar Jeli. Relatively, the technique involved in preparing the sample cannot be controlled including unhygienic handling which can affect the parameter of study. Moreover, the processing method in preparing 'sambal pijat' might shorten the shelf life of the sample when incorporated into different packaging conditions. Besides, time restriction in conducting the stability study also becomes the limitation of this present.

1.8 Summary of Research Work

The summary of this study was presented in Figure 1.0. The methodologies practiced in this study were adapted from various sources of previous researches. The sample purchased was dried in oven to reduce the moisture. Some of the sample was then subjected to chemical analysis in determining the fat and protein contents. After that, sample was placed and stored in different packaging conditions. A stability test in determining the physicochemical properties and microbiological characteristics of 'sambal pijat' also were performed.

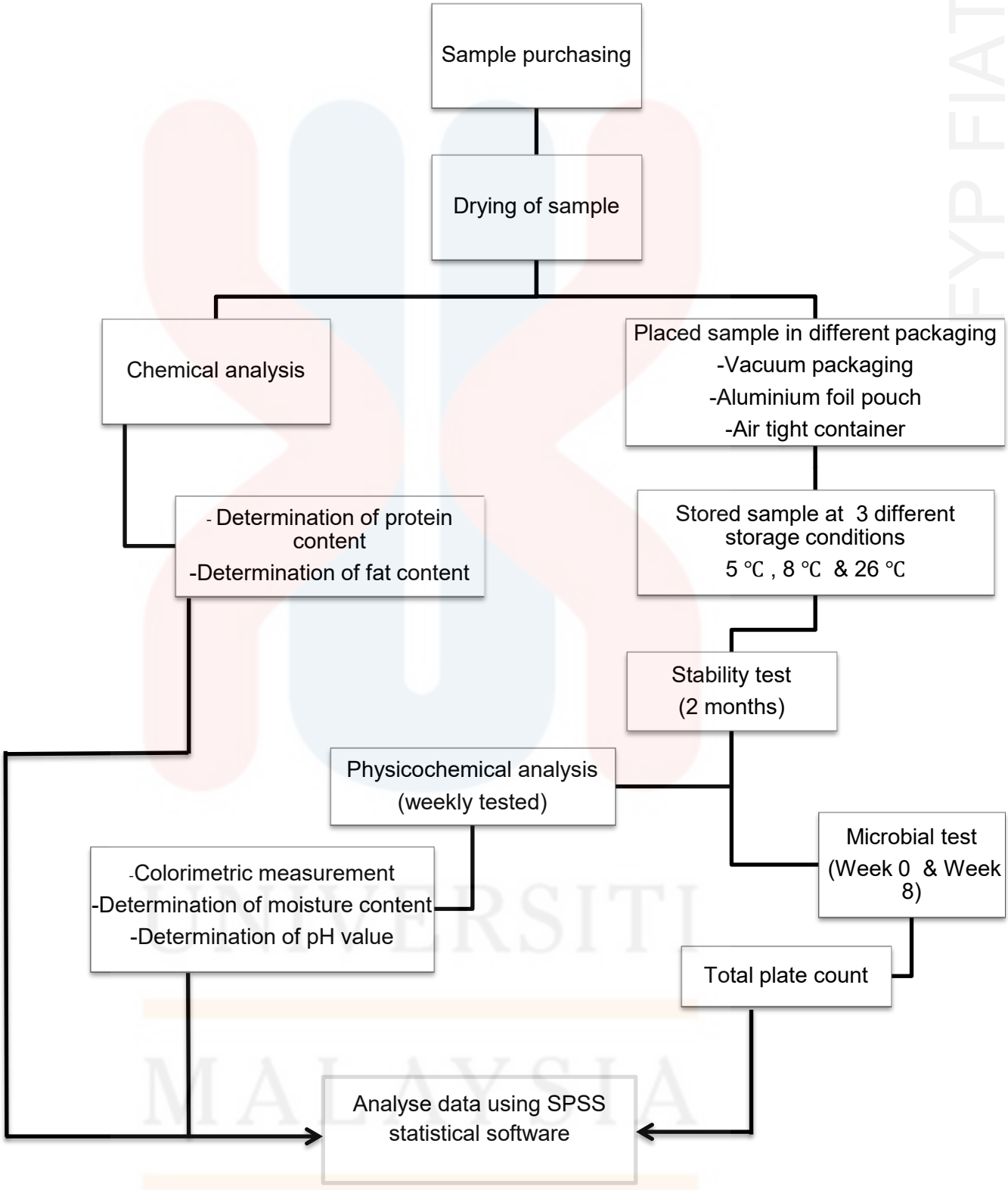


Figure 1.0: Flowchart of research work

CHAPTER 2

LITERATURE REVIEW

2.1 ‘Pokok Pepijat’

‘Pokok pepijat’ (Figure 2.0) is scientifically known as *Elettariopsis curtisii* belongs to the aromatic Zingiberaceae family under a small genus of herbaceous perennials. Based on Ibrahim et al., (2009) about 30 species of this genus available worldwide. This plant mainly grows in shady places with high humidity. Besides, *Elettariopsis curtisii* can grows up to 1 m tall with a slender rhizomes and leafy shoots (Figure 2.1) which arising from 6 to 20 cm apart including 1–5 petiolate leaves. Other than that, the rhizomes are edible and used as food flavouring. It can be an added ingredient to “curry” and can be made appetizer. This plant can be found in Malaysia and Thailand (Ibrahim et al., 2009).

Figure 2.0: ‘Pokok Pepijat (Ibrahim et al., 2009)

Figure 2.1: Rhizomes and stem

A study by Ibrahim et al., (2009) revealed that none of the oils isolated from leaves and rhizomes of 'pokok pepijat' exhibited remarkable inhibition potential against the microbes Microsporum canis, Trichophyton mentagrophytes, Trichophyton rubrum, Pseudomonas aeruginosa, Pseudomonas cepacia, Staphylococcus aureus and Staphylococcus epidermidis except for the fresh rhizome oil which showed a minimum inhibitory concentration of 0.938 mg/ mL against T. mentagrophytes.

2.2 'Sambal Pijat'

An interview was carried out in finding more information regarding 'sambal pijat' (Figure 2.2) involving the processing method and availability of this sample. According to Puan Zahrah one of the retailer who claimed that she is the pioneer of selling 'sambal pijat', this dish have been available for almost 10 years. At the present time, 'sambal pijat' is commonly available in Jeli, Kelantan as one of the dish in a meal. This dish was named after the major ingredients used in making it. Relatively the main ingredient in this dish was rhizomes and soft stem of 'pokok pepijat' that contribute to a strong aroma due to its properties. The process involved in making this dish was very simple and convenient as it only needs few ingredients with simple cooking technique. Initially, the rhizomes and soft stem were washed thoroughly before being chopped finely. Then, it was blended together with bird eye's chillies which enhance the colour of 'sambal pijat'. Basically, the amount of chillies used depends on the rate spiciness that an individual wants. After that, some seasoning and flavouring agent like salt and sugar was added in improving the bland taste.

Figure 2.2: ‘Sambal pijat’

Some retailers might add vinegar as flavouring which directly preserves the ‘sambal’. This claimed can be supported by other retailers based on their own experiences in selling ‘sambal pijat’. However, these claim was contended Puan Zahrah who indicated that vinegar resulting in shorter shelf life due to the high moisture content. Hence, she had suggested by using ‘asam gelugor’ able to prolong the shelf life of ‘sambal pijat’. ‘Sambal pijat’ only can last long for 2 weeks at ambient temperature whereas it able to remain stables for at most a month in refrigerator. The growth of mould will form upon spoilage of this dish which directly causes the colour to turn darker. In general, ‘sambal pijat’ is available every weeks depends on the sources of ‘pokok pepijat’. This is because, the sources of ‘pokok pepijat’ supplied by indigenous people who collect the tree from jungle located in Gua Musang including jungle located nearby Kelantan-Thailand border. The ‘pokok pepijat’ or also known as ‘Tepus Kesing’ is suitable to be planted in loosen soil structure under shaded areas. On the other hand, this tree can be harvested after two to three months. The longer the plant grows, the bigger the rhizomes will form. To the best of my knowledge, there is no scientific research regarding ‘sambal pijat’ for this time being. Besides, the interviewee also claimed ‘sambal pijat’ does provide health benefit.

2.3 Food Packaging

Food packaging is important as the absence of packaging may results in uncertain quality of food (Robertson, 2010). In placing more emphasis, Singh and Cadwallader (2002) stated that food packaging also compulsory part in industry because commercially processed unable to be managed and supplied smoothly without packaging. The purpose of packaging is to ensure the food quality in an ideal condition prior to manufacturing process until it is consumed.

According to Robertson (2010) packaging gives barrier from three main categories of external influences which are physical, chemical and biological. Relatively, by having protection against chemical packaging helps to reduce compositional changes due to environmental impacts like exposure to gases, moisture or light. In addition, biological protection avoids the interference of microorganisms, insects, rodents and other animals which directly prevent spoilage and illness. Meanwhile, physical protection avoids products from experiencing any mechanical damage while providing cushioning effect to any encountered shock and vibration (Marsh & Bugusu, 2007).

Other than gives protection, packaging also contributes others function like containment, preservation and identification. As stated by Robertson (2010) containment refers to the act of controlling or limiting something or someone harmful. For the context containment of food packaging, it indicates that packaging can provide an appropriate and safe container in keeping products. Moreover, containment also helps to conceal food product which directly prevent any leakage or loss throughout the supply chain. It is crucial for the producers in ensuring that contents are properly

contained especially for goods which can spread harmful effect if did not properly secured (Zuzana, 2017).

Packaging also helps to preserve certain goods such as food products, pharmaceuticals and other perishable products (as cited in Zuzana, 2017). Literally, the significance of preservation is to maintain products under a manageable condition so it stays safe to be consumed after certain times. On other hand, packaging provides a brief introduction for various types of products and manufacturers. Products can be presented differently according to its properties by using distinct design in terms of colours, size for the container, box or package.

2.4 Packaging Condition

Nowadays, food packaging has been innovate from simply a container that only contain food becoming a packaging system which able to play an active role. This can be related to existing of smart packaging which refers to intelligent and active packaging. The integration of certain compounds into packaging system indicates the definition of active packaging in extending shelf life and maintain food quality (Lee et al., 2015). Meanwhile intelligent packaging defines the usage of emerging technology that enhances packaging function by incorporating indicator inside or outside a packaging system to enhance the packaging functionality (Yam et al., 2005).

However, packaging unable to improve the quality of product but it manage to delay the occurrence of spoilage by preventing the related factors. This can be supported by Robertson (2010) as packaging can control two variables with regard to oxygen which directly effects oxidation in foods. The first variable is the availability of oxygen in a package that may retard the reaction while the concentration of oxygen in

the food is the other variable. Therefore, inappropriate packaging conditions may cause loss of product quality and affect microbiological safety which normally associated to perishable products. Moreover the spoilage of packaged foods is mainly depends on the mass and heat transfer through the package where it may experience moisture loss or gain (Robertson, 2010). In the present study, three types of packaging conditions were selected which was vacuum packaging, aluminium foil pouch and air tight container.

2.4.1 Vacuum Packaging

Vacuum packaging (Figure 2.3) is the process of removing air inside package which directly slow down the ability of aerobic microorganisms to grow and spoil the product (Pratik, 2017). Vacuum packaging is considered as one of the techniques for modified atmosphere packaging. This is because, the removal of air help to alter the gas composition inside the package which directly raised CO_2 level that resulted from microorganisms or respiring fruits and vegetables (Robertson, 2012). In recent studies by Chowdhury et al., (2017) stated that vacuum packaging indicates a static form of hypobaric storage that is commonly applied in food industry because of its efficiency in lowering oxidative reactions in the product at low cost. Besides, vacuum packaging had become a popular protection technique for refrigeration (Özpolat et al., 2014).

Figure 2.3: Example of food in vacuum packaging (Robertson, 2010)

Moreover, vacuum packaging is widely used throughout the industry for both food and non-food packaging applications. In food industry, vacuum packaging is applied in order to prolong the shelf life of fresh products without affecting the quality. This claim can be supported by Hernández- Macedo et al., (2011) that vacuum packaging has proven efficient to prolong fresh meat shelf life by preserving sensory properties of the product. Furthermore, in an observation-based study by Özpolat et al., (2014) the result have shown that by using vacuum-packing at 4±1 ℃ the shelf-life of sausage samples were extended by approximately 30 days. Commonly, vacuum packaging was applied in packing meat and products because it helps to remove the oxygen which directly reduces the microbial growth compared to conventional packaging. According to Jeremiah (2001) vacuum packaging maintains the colour of meat which is stable purple because of the formulation of deoxymyoglobin (DeoxyMb) (Li et al., 2012).

Apart from meat and meat products, vacuum packaging can be applied to a wide range of products like delicate foods, vegetables and cheese. According Chowdhury et al., (2017) shelf life of perishable products can be prolonged up to 3 to 5 times when packed with vacuum packaging as the growth of different aerobic bacteria is inhibited. Gill and Gill (2010) stated that low oxygen permeability should be used in vacuum packaging. Relatively, the permeability of package is influenced by the materials, the tightness of seals or closures and temperature (Gill & Gill, 2010). The examples of packaging materials that can be used for vacuum packaging are Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE) film and high barrier nylon polyethylene bag. LDPE have a good barrier to water and low permeability to gases as well as HDPE that have higher barrier properties toward gas and water vapour (John, 2010).

Basically, this method is suitable for powdered form products such as skimmed milk powder, tea dust, spices and others. In placing more emphasis, Gill and Gill (2010) claimed that vacuum packaging effect the physical properties of products by causing product compression including drip release. Thus, vacuum packaging techniques is not applicable for granular or bakery products as the evacuation of air from packaged can crushed the products (John, 2010).

2.4.2 Aluminium Foil Pouch

Currently, aluminium foil pouch (Figure 2.4) is widely used in food industry compared to aluminium. Aluminium can be used in raw state like in confectionary package or incorporated with another type of container such as TetraPak where one layer of package consists of aluminium. The usage of aluminium foil or film incorporating with different materials such as paper or polymer have been proved to be efficient in capitalize the benefits of aluminium including low-priced value (Brown & Williams, 2011).

Figure 2.4: Aluminium foil pouch

Foil provides an excellent protection against external influences such as air, odours and microorganisms. The properties of foil had made aluminium foil pouch act efficiently in protecting food products. It also has inert properties to acidic foods as lacquer or other protection is not required. Therefore, aluminium is suitable for a wide range of food products especially dried food. Although, aluminium is easily recyclable, foils cannot be made from recycled aluminium due to absence of pinhole formation in the thin sheets. Apart from that, "aluminium foil is the most commonly produced metal foil where it is manufactured by passing aluminium sheet between a series of rollers under pressure" (Mauer & Ozen, 2004).

2.4.3 Air Tight Container

Generally, air tight container (Figure 2.5) is not allowing something such as solids, liquids and gases under standard conditions of handling, storage and transport. When the container is intentionally to be opened more than one occasion, it must have the ability to remain air tight even re-closed. Commonly, air tight containers can be plastic, glass jars and bottles.

Figure 2.5: An air tight container

Most containers have corresponding lids that seal to maintain freshness perhaps can be frozen and applicable for dishwasher and microwave-safe (Tuan Mohd Ghazali, 2013). The examples of suitable containers are Polyethylene Terephthalate (PET), High Density Polyethylene (HDPE), Low Density Polyethylene (LDPE) and others. Even though, these packaging materials will not prolong as well as metal containers, they are economical compared to tinplate.

2.5 Food Composition Analysis

The analysis of food composition allows us to recognize the nutrition facts of the food products. In order to comply with the nutrition facts of food products there several tests, there are several test that must be conducted which are the analysis of fat content, protein content, total carbohydrate content, ash content and energy content. However, in this study, there were only two of the main constituents for food was analysed. The selected constituents to be determined were protein content and fat content.

2.5.1 Protein Content Analysis

Protein refers to polymers of 20 different amino acids that joined together by peptide bond (Deman, 1999). Other than that, proteins are polymer of amino acid which provides major constituents of food products and may affect the quality attributes of products like texture (Sehgal, 2016). Protein comes from two types of sources which are animal and plants. Studies by Pasiakos et al., (2015) claimed that animal based food provided an excellent sources of protein compared to plant based

food. This is because of the amino acid composition of animal protein that made it more biologically complete than plant protein (Ministry of Health, [MOH], 2017).

Foods composed of heterogenic materials which consist of different nutrients like protein, lipid, carbohydrates and minerals. These properties had influence procedure of food protein analysis where it may involve indirect or direct analytical methods for the determination of protein content. Relatively, protein content can be directly calculated by referring to the amino acid residues analysis. Besides, it also can be indirectly determined based on the nitrogen content or after chemical reaction with functional groups in the protein (Mæhre et al., 2018). There are some example for the food protein analysis which are Kjedahl 's method, Bradford's method and Lowry method. In this current study, protein content of 'sambal pijat' was determined based on Kjedahl method. Kjedahl method helps to determine the nitrogen content of organic and inorganic sample which indirectly measured the protein content. Furthermore, this method digests samples that contain high nitrogen with sulphuric acid to ammonium sulphate and was measured by titration with acid (Sáez-Plaza et al., 2013). Then, protein content was then calculated based on the protein-nitrogen conversion factor (Sehgal, 2016).

2.5.2 Fat Content Analysis

As stated by Sehgal (2016), fat and oils in food is analysed in order to observe the degree of food degradation like rancidity or oxidation including stability of fat. Fat and oils are the classes of lipid that soluble in organic solvent and insoluble in water.

Relatively, the fat content in food may ranging from very low to very high in animal and plant products (Deman, 1999). According to Nielsen, (2010), fat content in food usually determined by organic solvent methods.

There are some example of techniques used in analysing the fat content which are goldfish method, Soxhlet method and supercritical fluid extraction method. In this present study, Soxhlet method was chosen to determine the fat content of 'sambal pijat'. Soxhlet method is one of the examples for semicontinuous solvent extraction. This method provides the determination of fat content based on weight loss of sample or from the weight of fat removed (Nielsen, 2010). The selection of solvents must be based on certain considerations as for fat non-polar solvent was selected. Besides, selected solvents should have low boiling point in allowing low temperature evaporation that not leave any residue (AOCS Lipid Library, 2014).

2.6 Stability Test and Shelf Life Determination

"Stability test refers to progression or deterioration of product characteristics which measured over time" Guillet & Rodrigue (2010) . In accordance to this definition, stability study is different from shelf life testing. Relatively, the primary objective of shelf life determination is food safety. Besides, the data obtained for stability experiments also differ from shelf life experiments because both tests carried a distinct goal. This claim can be supported by Guillet and Rodrigue (2010) where the main interest of shelf life testing is deterioration time of a food product whereas the importance of a stability study is the assessment on degradation of a properties over time.

Relatively, chemical stability indicates the change of compounds present in food over a period of time as result of chemical or biochemical reactions such as food rancidity or browning. Meanwhile, physical stability can be defined as the movement of molecules that causes spatial distribution of the molecules present in a food with respect to time. On the other hand, biological stability refers to the change in the availability of microorganisms in a food like bacterial or fungal growth at a certain time (Alim et al., 2014). There are two types of stability testing which are real time stability test and accelerated stability test. Hence, for this study, 'sambal pijat' was analysed based on the real-time stability. For real-time stability testing, a product is recommended to be stored at ideal storage condition and monitored until it fails the specification while during accelerated stability tests, a product stored at elevated stress conditions such as temperature, humidity, and pH (Singh, & Cadwallader, 2004).

2.6.1 Physicochemical Analysis

Physicochemical analysis involves the physical and chemical evaluation of food products. These relates to the determination chemical and physical stability of food products With regard of the fact that foods are diverse, complex and active systems as microbiological, enzymatic and physicochemical reactions are simultaneously taking place, it is crucial to carry out a physicochemical analysis especially for long term storage. For this study, three tests were conducted in determining the physical and chemical stability of 'sambal pijat' which were colour analysis, moisture content and pH value.

2.6.1 (a) Colour Analysis

In general, appearance of fresh and processed food is the most important attributes that is observed by consumer. With regard to this, an appearance of food product can be judged by its colour. This can be supported by Pathare et al., (2013) which stated that colour as the main quality attribute in the food and bioprocess industries perhaps it able to influence consumer's choice and preferences. Besides, colour measurement of is of the examples for attributes in analyse food quality like flavour because it is convenient and correlates well with other physicochemical properties (Pathare et al., 2013). The colour analysis can be carried out by using visual inspection or an instrument. Commonly, in food industries there two principal of colour measurement techniques that had been used which are colour colorimetry and specthrophotometry (Kilcast, 2013).

Colorimetry technique was applied in evaluating the colour of 'sambal pijat'. The colour of foods on industry is usually been measured by L* a* b* or CIELab colour space, which is an international standard accepted by the Commission Internationale d'Eclairage (CIE) in 1976 (Kilcast, 2013). Basically, each chromacity coordinates carries different criteria as L* refers to the lightness factor and indicates the degree of brightness or darkness of the sample while a* represents the intensity of colour red(+)/green(-) and b* refers to yellow(+)/blue(-) (Granato et al., 2010) .

2.6.1 (b) Moisture Content

Moisture migration is a major cause of deteriorative physical changes in food. This is easily seen in fresh produce through moisture loss or gain in certain food products like crispy biscuits. Other than that, moisture content also can be one of the parameters in measuring food materials. In the context of microbial stability of the tendency of microorganisms to grow in foods is influence by the amount of water content (Sehgal, 2016). Therefore, most of food products are dried below some critical moisture content. According to Sehgal (2016) there are numbers of analytical techniques to determine moisture content such as oven drying method. Basically, the method is chosen based on the nature of analysed food. Oven drying is one of example for thermogravimetric analysis where sample is weighed and heated to cause the removal of moisture. The moisture content is calculated based on the difference of wet and dry weight (Appoldt & Raihani, 2017).

2.6.1 (c) pH Value Measurement

As stated by Andrés-Bello et al., (2013) "pH indicates a measurement of hydronium ions (H_3O^+) activity in a substance which become a dominant factor that determines the proceeding chemical reaction including the quality of food product,". Traditionally, pH is electrochemically measure with a pH-sensitive glass electrode and a reference electrode. Nowadays, there numbers of methods and instrument that can be used to determine pH value such as pH meter with aids of buffer solution. According to Fraser (2012) "the pH scale was developed from mathematical calculations based on the dissociation temporary breakdown of water".

The pH scales which ranging from 0 to 14 making ease of pH value determination. Generally, each number indicates difference degree of alkaline and acidity where below pH 7 are acidic and higher than pH 7 is alkaline. Besides, food also can be classified into two distinct categories where food product with pH of 4.6 or less is consider as high-acid foods while low acid foods have a pH of greater than 4.6 but less than pH 7.0 (Fraser, 2012).

2.6.2 Microbial Test

Survival and growth of microorganism in food also will affect the quality and shelf life of product. Thus, microbial testing can be used in order to analyse the food product degradation. Microbial testing is an analysis that manage to perform the qualitative and quantitative estimations of specific viable microorganisms present in samples" (Tests, 2002). This test comprises of tests for total viable count of microorganisms like fungi and bacteria including *Escherichia coli* which might presence in food. When performing the microbial testing, it is crucial to follow all the aseptic technique in avoiding any microbial contamination from the outside.

One of the examples for microbial test of food is total plate count. Total plate count (TPC) can be defined as enumeration of colony that grow under moderate temperature of 20 to 45 ℃ like aerobic and mesophillic organisms (Ara Sains, 2014). According to Mailoa et al., (2017) total plate count analysis refers to calculation of the number of bacterial colonies present in the sample with regard to dilution used. In preventing any contamination all work must be done aseptically and accuracy can be obtained by duplicating the observation. Based on Sin-bin et al. (2014) microbial count can be a quality indicator in assessing the food safety especially ready to eat food. The

acceptable colony forming unit of total viable count for cooked food is $<10^5$ CFU/g (Sin-bin et al., 2014). In general, the numbers of microbial count will be represented in the form of colony forming unit or CFU. Commonly, the acceptable count for numbers of colonies growth on a plate are in between 25 to 250 colonies (Sutton, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Materials

The chemicals and materials that had been used in this study were distilled water, buffer solution (pH 4 and pH 7) from Bendosen, nutrient agar powder from Oxoid, 70% ethanol, Kjedahl catalyst copper tablet from Fisher Chemical, sulfuric acid (H_2SO_4) from EMSURE , sodium hydroxide (NaOH) pellet from R&M Chem , hydrochloric acid(HCl) solution from HmbG Chemicals, boric acid (H_3BO_3) from Merck KGaA, indicator solution (methyl red and bromocresol green) from Bendosen, sodium carbonate (Na_2CO_3) from FRIENDEMANN SCHMIDT CHEMICAL, petroleum ether from Bendosen, potassium dihydrogen phosphate anhydrous from HmbG Chemicals, aluminium foil pouch, air tight container (PET), plastic bag (HDPE). All chemicals used were provided by FIAT's laboratory.

3.2 Equipment and Apparatus

The equipment and apparatus that were used in the research comprised of sterile glass petri dishes (≥15mm X 90mm), 1000 μL micropipette, 1000 μL sterile pipette tips, dilution bottles, incubator (37 ℃ ± 1 ℃) and 25 ^{0}C, autoclave machine, colony counter, Bunsen burner, HANNA pH meter (0.01 units accuracy), magnetic stirrer, beaker, chamber vacuum (DZ-400 Vacuum machine) , drying oven, soxhlet extractor, Foss™ Kjeltec™ 8100 Manual Distillation Unit, Foss™ Tecator™ 2508 Digestion System, Konica Minolta Chroma Meter (CR-400), chiller, refrigerator, moisture dish and lid, plastic bag, air tight container, aluminium foil pouch, stomacher machine, desiccator, , boiling flask, round bottom flask, autoclave bottle, dilution bottle, measuring cylinder, gloves, mask, thimble, burette, retort stand and clamp. All equipment were provided by FIAT's laboratory.

3.3 Experimental Design

Before sample was subjected to the stability test, two tests in for the determination of fat and protein content have been conducted. The stability test which comprises the analysis of physicochemical properties was carried out for 2 months while the evaluation will be taken off weekly. The evaluation weeks involved week 0, 1, 2, 3, 4, 5, 6,7and 8. All measurement was performed in triplicate to ensure the accuracy of results. Meanwhile, microbial test was conducted on week 0 and week 8. The samples were prepared based on experimental design of 1 x 3 x 3 x 8 which refers to 'sambal pijat' that was incorporated in three types of packaging at three different storage conditions for 8 times observations.

3.4 Sample Preparation

3.4.1 Sample Purchasing and Pre-treatment

The ‘sambal pijat’ had been purchased from the selected retailer at ‘Pasar Jeli’. About 4.5 kg of samples were used in this study. Then, ‘sambal pijat’ was put into stainless tray and dried at 50 ℃ for 24 h using a conventional oven.

3.4.2 Packaging

Three different packaging materials were used in this study which was 4.5 x 7.5 x 0.3 mm high density polyethylene plastic bag (HDPE), 5 x 9 cm aluminium foil pouch with zipper and polyethylene air tight container (PET). Each packaging was filled with 40 g of ‘sambal pijat’. Firstly, 40 g of ‘sambal pijat’ was put into HDPE plastic bags and vacuum-packed in a chamber vacuum (DZ-400 Vacuum machine) with 25 s of vacuum followed by 18 s sealing process. After that, 40 g of sample was placed into PET air tight container before being closed with the lid. Another 50 g of samples was then placed in aluminium foil pouch. In making ease of the evaluation process the packaging was labelled with an appropriate signs with regard to the packaging conditions involved. After packing, the samples was immediately stored accordingly at three different storage conditions which was 5 ℃, 8 ℃ and 26 ℃.

3.5 Chemical Analysis

3.5.1 Determination of Protein Content

3.5.1(a) Digestion

The determination of protein was carried out according to Kjeldahl's method. About 1 g of sample was placed into a 250 mL digestion tube. Then, 1 g Kjedahl catalyst copper followed by 12 mL of concentrated sulphuric acid ($H_2SO_{4)}$ was added. Two digestion tubes containing the chemical stated except sample were prepared as blank. Then, all digestion tubes that containing mixtures were placed in the digestor and was digested at 400 ℃ until a clear solution form and oxidation complete. The digestion process was continued until a clear digest solution formed. Total digestion time is approximately 1 h (Sehgal, 2016).

3.5.1(b) Distillation

A 250 mL conical flask containing 30 mL of 4% boric acid with indicator as receiver was placed on the distillation unit. Then, 80 mL of water was added gently into the digest followed by 50 mL of 50% NaOH will be poured into the digests. The conical containing the stated chemicals were attached at the dilution unit. The distillation will be continued until all ammonia release or approximately higher or equal to 150 mL distillate that will be obtained. The receiver flask will be lowered so that the delivery tube is above the liquid surface and continue the distillation for 1-2 min. Finally, the

delivery tube will be rinsed with water and allow the washings to drain into the flask. Then, receiving flask was removed from the dilution unit (Sehgal, 2016).

3.5.1(c) Titration

The distillate was titrated with the standardised 0.1 N hydrochloric acid (HCl) until the first appearance of the pink colour. Lastly, the volume of acid used was recorded to the nearest 0.05 mL (Sehgal, 2016). The percentage (%) of protein was calculated using formula as follows:

$$\text{Protein (\%)} = \frac{(X-Y) \times A \times 1.4007 \times 6.25}{W} \quad (3.1)$$

Where,

X = Volume of 0.1 N HCl used to titrate sample (mL)

Y = Volume of 0.1 N HCl used to titrate blank (mL)

A = Normality of HCl

W = Weight (g) of sample

14.007 = Atomic weight of nitrogen

6.25 = Protein-nitrogen conversation factor

3.5.2 Determination of Fat Content

The fat content was determined based on Soxhlet method. 'Sambal pijat' was dried in an oven for 5 h. Then the dried sample was grinded until form powder. About 2 g of pre dried sample was weighed into an extraction thimble. The pre dried boiling flask was weighed before pouring petroleum ether. 300 mL was used to fill up about half of the boiling flask. Boiling flask, Soxhlet flask and condenser was assembled in the fume hood. Extraction of fat occurred in Soxhlet extractor at a rate of 5 or 6 drops per second condensation for about 4 h through heating of solvent in boiling flask. Extracted fat in the boiling flask was dried using oven for 30 min at 100 ℃ and cooled in desiccator followed by weighing process (Nielsen, 2010). The percentage (%) of fat was calculated using the following formula:

$$\text{Weight of fat sample} = (\text{beaker} + \text{fat}) - \text{beaker} \quad (3.2)$$

$$\text{Fat on dry basis (\%)} = \frac{\text{Weight of fat in sample}}{\text{Weight of dried sample}} \times 100 \quad (3.3)$$

3.6 Stability Test

The stability test of 'sambal pijat' was carried out within 2 months with weekly taken off for evaluation. The test involved was determination of physicochemical analysis and microbiological analysis. The physicochemical analysis consists of colour analysis, determination of moisture content and measurement of pH value. Meanwhile microbiological analysis comprised of total plate count.

3.6.1 Colour Analysis

The intensity of the colour for 'sambal pijat' was measured with Konica Minolta Chroma Meter (CR-400). 'Sambal pijat' was placed in a clear glass petri dish to enable the chroma meters measuring head detect the colour intensity. The value for colour space was appered on the screen of data processor. By using the CIE Lab system, instrument recorded the L* value represented brightness on the scale of 0 to 100 from black to white. Then, red component or a* ranging from -60 to 60 equals to a>0 whereas a<0 indicates green component. Meanwhile, b* value from -60 to 60, (+) stands for yellow component and (-) stand refers to blue component (Hang, Chieu, Trang, & Hoa, 2014). The total colour degradation was then calculated using an equation of where $\Delta a^* = a^* - a^*0$, $\Delta b^* = b^* - b^*0$, $\Delta L^* = L^* - L^*0$ subscript ''0'' indicated initial colour on day 1 : $\Delta E = \sqrt{(\Delta a^{*2} + \Delta b^{*}2 + \Delta L^{*2})}$ (Granato et al., 2010).

3.6.2 Determination of Moisture Content

Moisture content was measured based on oven drying method. The empty moisture dish and lid was placed in oven at 105℃ to dry for an hour. Then, moisture dish was weighed using an analytical balance. About 3 g of 'sambal pijat' from each packaging was weighed into the pre dried moisture dish followed by spreading sample to the uniformity. Moisture dish containing sample was placed in the oven with the open lid at 100℃ for 5 h. After drying, the dish with partially covered lid was left cooled in the desiccator. Cooled moisture dish and its dried sample was reweighed using analytical balance. The drying process was repeated for a constant weight (Sehgal, 2016). The percentage (%) of moisture was calculated using the following formula:

$$\text{Moisture (\%)} = \frac{(W_2-W_3)}{W_2-W_1} \times 100 \qquad (3.4)$$

W_1 = Weight of the moisture dish (g)

W_2 = Weight of moisture dish + sample (before drying) (g)

W_3= Weight of moisture dish + sample (after drying) (g)

3.6.3 Determination of pH

3.6.3 (a) Calibration of pH Meter

Initially about 50 mL of 4.01 and 7.01 buffer solutions was poured into 100 mL beaker separately. Then, a beaker containing 100 mL of distilled was prepared for rinsing purposes. By using a large beaker, the electrode was rinsed with distilled water followed by rinsing with 7.01 of buffer solution in order to avoid diluting of buffer solution. Calibration menu of pH meter was entered based on the steps in choosing the 7.01 buffer. Next, pH electrode was submersed in the 7.01 buffer solution until meter indicated that it was stable and the calibration on meter need to be confirmed. Again, the electrode was rinsed with the distilled water followed by rinsing with little amount of 4.01 buffer solution to avoid diluting the buffer solution. After that, pH electrode was submersed in the 4.01 buffer solution until the meter to indicate that it was stable including confirmation of calibration on meter. The electrode was rinsed with distilled water. Then, electrode was calibrated and ready for pH measurement (Food And Drug Administration [FDA], 2017).

3.6.3 (b) Determination

The pH value was determined according to method suggested by Food and Drug Administration (FDA).The sample was dissolved with distilled water in a beaker with a ratio of 1:2. Then, the solution was stirred followed by immersion of pH electrode into solution. The pH value will be recorded (Food And Drug Administration [FDA], 2017).

3.6.4 Microbial Test

3.6.4 (a) Preparation of Nutrient Agar

About 23 g of nutrient agar powder was dissolved in distilled water. Then, the volume was made up to 1.0 L followed by mixing thoroughly of mixture. Later, the agar was poured into autoclave bottle and autoclaved for 15 min with a temperature of 121 ℃. The sterile agar was left to cool for few minutes. The melted agar was poured into petri dish until cover the bottom surface of petri dish. The lid was immediately replaced and agar plate was left to cool until formed like stiff gelatine at room temperature (Atlas, 2010).

3.6.4 (b) Determination of Total Plate Count Agar

Initially 10 g of sample was weighed into 90 mL potassium dihydrogen phosphate anhydrous diluents and the mixture was homogenized using stomacher for 2 min. By using separate sterile pipette tips with a micropipette, serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} was prepared with transferring 1.0 mL of previous dilution to

9.0 mL of diluents. The glass spreader was sterilized with 70% ethanol and Bunsen burner flame. The lid of the bottle containing sample dilution was loosen in making ease the dilution procedure. 1mL of each dilution was pipetted into separate, duplicate marked petri dishes which contained nutrient agar. Next, used pipette tips were discarded into an empty container. After that, the lid of petri dish was half opened to let the glass spreader in. The petri dish was rotated while moving the spreader from top to bottom or side to side to spread the inoculum over the surface of the agar. The agar surface was fully covered with inoculum. The petri dish was sealed and incubated promptly for 48 h ± 2 at 37 ℃ (Maturin & Peeler, 2001).

3.6.4 (c) Counting of Colonies

After incubation period, colonies were grown in all petri dish. Normal plates contained 25 colonies to 250 colonies. All colony forming units (CFU) was counted including those of pinpoint size, on selected plates. Dilutions used and total number of colonies counted was recorded. Plate with number of CFU more than 250 colonies was recorded as too numerous to count (TNTC). Meanwhile, plates with absence of CFU was reported APC as less than 1 X 10^1 which corresponding to lowest dilution used (Maturin & Peeler, 2001).

3.7 Statistical Analysis

The data was analysed by using IBM SPSS version 21.0. The data obtained in triplicate for protein and fat contents were expressed as mean ± standard deviation. The means for chemical analysis was calculated using T-test to determine the significance difference when compared to control sample. Then, means of analysis involved in determining stability of 'sambal pijat' were calculated using two way analysis of variance (ANOVA) based on multivariate test in determining the significance differences between treatments (p <0.05) for physicochemical analysis. For the microbial test data was analysed based on One Way repeated measured to obtain the significant difference in between evaluated weeks.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Positive Control

For this study, *Adabi Tradition Spicy Sambal Belacan* was introduced as positive control. This product was well packed and sealed in plastic container which contained nutritional labelling and others important information. It is comprised of several ingredients like shrimp paste (crustacean), onion, red chilli, brown sugar, salt, cooking oil, reversed osmosis water. The products able to remain stable for about 9 until 15 months based on the storage condition. This longer shelf life was able to be achieved with the availability of food conditioner and permitted preservatives. For the stability test, control was stored at different temperatures as assigned to 'sambal pijat' without changing the original packaging. Relatively, this control helps to become a guideline in observing the validity of results for each parameter tested on chosen treatments.

4.2 Chemical Analysis

According to Potter and Hotchkiss (1995), there are three major constituents that present in food which are carbohydrate, fat and protein and their derivatives. With regards to these constituents a chemical analysis was conducted to determine the chemical properties of samples listed in Table. 4.1. The percentage obtained for chemical properties tested varied significantly ($p < 0.05$) for both control and 'sambal pijat'. As can be observed in Table 4.1, protein content for control recorded 4.02 % ±0.11 with 11.10 ±0.02 fat content whereas 'sambal pijat' contains 2.25 % ±0.09 protein and 9.92 % ±0.04 fat. Protein and fat contents were the two selected constituents to be determined.

Table 4.1: Chemical properties of control and 'sambal pijat'.

Chemical properties	Control	Sambal pijat
Protein (%) (N x 6.25)	4.02 ± 0.11*	2.25 ± 0.09*
Fat (%)	11.10 ± 0.02*	9.92 ± 0.04*

- Results were presented as mean ± SD
- Symbol * defined significant difference compared to control (p <0.05) using T-test

Protein content was determined by using Kjedahl's method that involved three main steps which are digestion, distillation and titration. About 1 gram of sample was digested with concentrated sulphuric acid and Kjedahl catalyst tablets copper. The sulphuric acid was used in order to minimize foaming formation. Meanwhile, Kjedahl catalyst tablets copper aids the digestion process by making the process more efficient through increasing the boiling point of sulphuric acid. Besides, this type of catalyst is complying with the regulation as it has low toxicity and safer to be used (Buffler &

Mühleis, 2012). The digestion was assumed to be completed as the digests turned colourless which indicates that all organic matters have been breakdown. As the digests cooled, distilled water was added to dilute sample before adding sodium hydroxide. The distilled water must be added prior to base addition to avoid splashing reaction between acid and base from boiling effect of high temperature. Sodium hydroxide was used to neutralize the sulphuric acid (PanReac AppliChem, 2017).

The digests was then diluted using the dilution unit which involved a receiver solutions. The distillation with a steam power enable the conversion of ammonium ion to ammonia (Mæhre et al., 2018). The receiver solutions comprised of boric acid with indicators which are bromocresol green and methyl red that absorbed all the recovered ammonia. Sample with high protein content will exhibit a dark turquoise green colour. For sample tested, 'sambal pijat' showed a lighter turquoise colour than the control sample. The samples were titrated with standardized 0.1 N hydrochloric acid until turns pink. This method was known as indirect method because total protein in food determined by direct nitrogen measurement that was multiplied with conversion factor (Sáez-Plaza et al., 2013). For this study, a general conversion factor of 6.25 was applied where this conversion factor had been used for most foods due to insignificant amount of non-protein nitrogen (Sáez-Plaza et al., 2013).

The protein content for 'sambal pijat' and control varied significantly (p <0.05). From Table 4.1 the protein content for 'sambal pijat' recorded a lower percentage which indicates that this sample contain a small amount of protein due to the content of ingredients.'Sambal pijat' comprised of plant based ingredients which may influenced the low protein content. According to Pasiakos et al., (2015) food based on plant are less protein-dense. Meanwhile, the control which contains shrimp paste as the main ingredient yields a higher percentage of total protein content. Relatively, shrimp paste is

made up of shrimp which one of the high quality sources for protein. This directly influences the percentage of protein obtained by control sample.

Fat content was determined based on Soxhlet method which involved the usage of organic solvent. Due to the properties of fat that is only soluble in organic solvent and insoluble in water, an organic solvent have to be used to extract fat that available in a food sample. An organic solvent that selected for the determination of control and 'sambal pijat' was petroleum ether. Petroleum ether have a low boiling point and have non polar properties which unable to remove polar and bound lipid (Nielsen, 2010). Volume of solvents must be sufficient enough until immersed the whole thimble and ensured that at least half volume of flask is occupied with solvent. This is crucial in avoiding the solvents from fully evaporated and caused flask cracked. Moreover, sufficient volume of solvents can ensure the continuous flow of solvents in order to remove fat away from samples.

With regard to high moisture content of samples and the fact that moisture may restrict the dispersion of organic solvent 'sambal pijat' and control sample were dried at 100 ℃ for 5 h. The samples were grinded until formed powder to reduce the particle size and become homogenous. This helps to increase the efficiency of extraction process. Besides, extraction process was undergone until the samples turns colourless as for each sample yellow colour turned to colourless after 4 h of process. The fat content for each sample varied significantly (p <0.05) where the control recorded a high percentage of fat content and 'sambal pijat' resulted in low amount of total fat present. The presence of fat in 'sambal pijat' might result in product rancidity if purposed packaging conditions were not suitable. Based on previous study by Hasimah et al., (1993) high fat content was not lead to rancidity of 'sambal tumis bilis' as the packaging materials were suitable in maintaining the product quality.

4.3 Stability Test

For this present study, there were 9 different treatments that were tested to identify the physicochemical stability of 'sambal pijat' and 3 treatments for control sample. For vacuum packaging, HDPE plastic had been used because it is one of the recommended material that can be used for vacuum packaging due the high barrier properties toward gas and water vapour (John, 2010). A high barrier plastic toward gas helps to preserve the food quality as presence of oxygen and water vapour may cause physical or chemical degradation (Siracusa, 2012). The second packaging was aluminium foil pouch with zipper that provided a good protection from external influences like water and gas whereas PET air tight container as the third packaging has moisture barrier properties.

Each treatment was tested weekly with a triplicate measurement in ensuring the accuracy of data obtained. The storage conditions involved were chilled temperature (5 ℃ and 8 ℃) and ambient temperature which at 26 ℃. The temperature of each condition was first measured using a thermometer. For temperature at 5 ℃ three different packaging were stored in a cold storage room while 8 ℃ refers to refrigerator temperature. The ambient temperature of 26 ℃ involved storing of sample in a cupboard. Samples with treatment that involved low temperature which was 5 ℃ and 8 ℃ were left at room temperature for few minutes to enable the water vapour outside packaging evaporated before being subjected to any analysis. This is because, presence of water vapour might interfere the result obtained.

4.3.1 Physicochemical Analysis

4.3.1 (a) Colour Analysis

For colour analysis, the colour intensity of samples had been measured by CIELab colour space or coordinates L*, a* and b*. Each chromatic coordinates represent different attributes where L* indicates the degree of lightness (0-100), a* refers to red (positive value) or green (negative value) and b* shows the colour of yellow (positive value) or blue (negative value) (Granato et al., 2010).

Based on Figure 4.1 and 4.2 which proved the colour changes of sample indicated 'sambal pijat' showed that the colour observed turned darker after 8 weeks of evaluation. Based on previous study on the stability of non-dairy dessert, changes of colour in stored food was observed because of the oxidation reaction that involved enzymes, lipid and carotenoid (Granato et al., 2010).

Figure 4.1: 'Sambal pijat' on week 0

Figure 4.2: 'Sambal pijat' on week 8

Therefore, in observing the chromatic stability, 'sambal pijat' and control sample were tested based on colour intensity by measuring three coordinates of L*, a* and b*. For statistical analysis, Table 4.2 and 4.3 summarized the significant difference of colour analysis coordinates in between type of packaging within storage time.

Table 4.2: Least significant difference test on colour coordinates tested on'sambal pijat' packed in three different packaging and control at different temperature.

Storage temperature	Type of packaging	L*	a*	b*
5 ℃	C	40.27^{a}	14.16^{a}	18.87^{c}
	VP	38.56^{c}	11.5 ℃	19.95^{a}
	AP	39.54^{b}	10.91^{d}	19.14^{b}
	AT	39.67^{b}	11.69^{b}	19.83^{a}
8 ℃	C	31.74^{d}	14.16^{a}	20.62^{a}
	VP	37.23^{c}	11.4 ℃	19.94^{b}
	AP	38.50^{b}	10.91^{d}	19.49^{c}
	AT	38.75^{c}	11.57^{b}	20.07^{b}
26 ℃	C	32.65^{c}	15.12^{a}	21.79^{a}
	VP	31.21^{a}	10.87^{c}	17.99^{b}
	AP	35.94^{b}	10.99^{b}	16.91^{d}
	AT	37.14^{a}	9.29^{d}	17.37^{c}

- Means within a column of an equal storage temperature with subscript of different letter are significantly different (p <0.05) in accordance with homogenous subset defined by Two Way ANOVA.

Table 4.3: Least significant difference test on colour coordinates tested for ‘sambal pijat packed at three different storage temperatures during storage.

Storage temperature	Means for each coordinates of colour analysis tested on ‘sambal pijat during storage								
	A			B			C		
Weeks / Parameters	L*	a*	b*	L*	a*	b*	L*	a*	b*
Week 0	45.41^{a}	13.34^{a}	30.04^{a}	45.41^{a}	13.31^{a}	30.04^{a}	45.41^{a}	13.31^{a}	30.04^{a}
Week 1	43.46^{b}	13.31^{a}	25.70^{b}	42.49^{b}	13.30^{a}	26.27^{b}	40.54^{b}	12.31^{b}	23.44^{b}
Week 2	42.46^{c}	12.99^{a}	24.8 ℃	40.5^{c}	13.12^{a}	26.27^{b}	39.8^{c}	12.18^{b}	21.41^{c}
Week 3	40.32^{d}	12.46^{b}	24.32^{d}	38.64^{d}	12.53^{b}	22.84^{c}	37.95^{d}	11.4^{c}	19.45^{d}
Week 4	38.55^{e}	11.87^{c}	16.59^{e}	36.69^{e}	11.61^{c}	17.91^{d}	36.21^{e}	10.56^{d}	16.58^{e}
Week 5	37.66^{f}	10.65^{d}	14.23^{f}	36.82^{e}	10.28^{d}	14.27^{e}	35.22^{f}	9.26^{e}	12.99^{f}
Week 6	36.20^{g}	9.69^{e}	13.99^{fg}	35.65^{f}	9.64^{e}	14.17^{e}	33.18^{g}	9.00^{f}	12.21^{g}
Week 7	35.49^{h}	9.25^{f}	13.64^{gh}	34.50^{g}	9.30^{f}	13.78^{e}	31.80^{h}	8.19^{g}	10.63^{h}
Week 8	33.72^{i}	8.73^{g}	13.43^{h}	32.73^{h}	8.54^{g}	12.83^{f}	30.75^{i}	7.24^{h}	10.03^{i}

- Means within a column with subscript of different letter are significantly different (p <0.05) in accordance with homogenous subset defined by Two Way ANOVA
- A=5 ℃, B=8 ℃, C=26

Due to the nature colour of 'sambal pijat' and the value of L* (lightness) coordinate and a* (redness) coordinate were chosen to be analysed. This is because, one of the ingredients in 'sambal pijat' which are bird's eye chillies imparts red colour intensity and this sample varied in lightness over storage time. The red colour of chillies are contributed by the presence of carotenoid or specifically known as capsanthin (Arimboor et al, 2015). The analysis of L* value was presented in a line graph form as can be observed in Figure 4.3. The line graph in Figure 4.3 showed that the L* value for control increased linearly for each treatment form week 0 until week 8. The L* value for control were increased over storage time with a range of 27.21 to 40.81 which can be observed at storage condition of 26 ℃. On the other hand, control sample at 5 ℃ showed a slight increase in L* value from 27.21 to 36.41 on week 8.

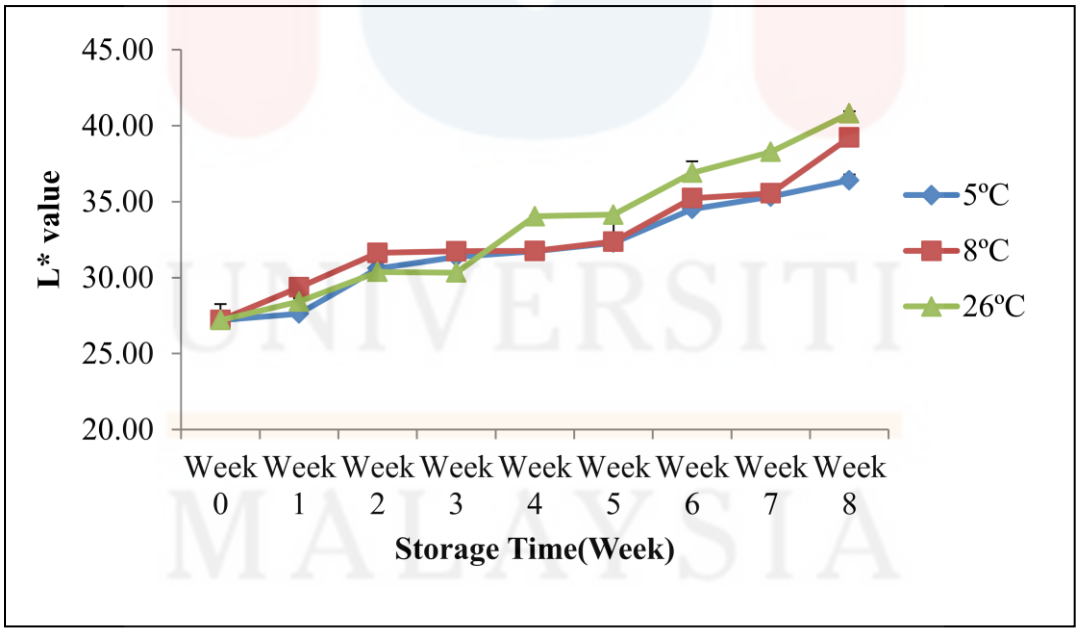


Figure 4.3: The line graph shows the lightness factor of control sample that observed for 8 weeks at different storage temperature.

For the L* value of 'sambal pijat', graph in Figure 4.4 showed that each treatment recorded a gradually decreased of L* value during the storage time observed. As can be observed in Figure 4.4 all L* coordinate were in high value on the first week of evaluation which indicated that the colour intensity represent the degree of lightness. However, the L* value recorded on week 8 as can be seen in Figure 4.4 showed a decreased in value which refers to the loss of lightness observed on 'sambal pijat'.

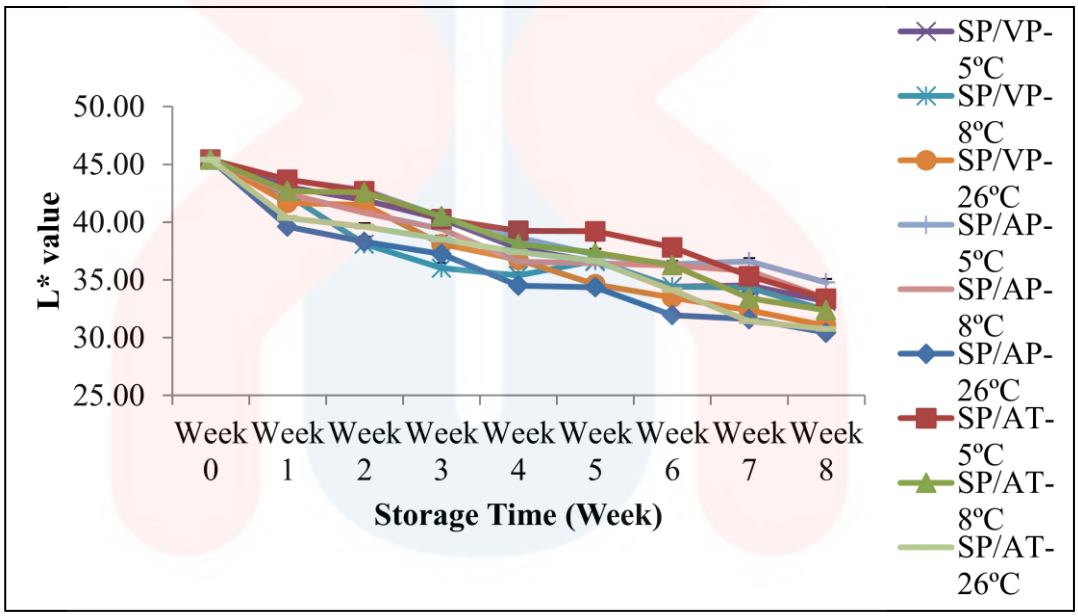


Figure 4.4: The line graph shows lightness factor of 'sambal pijat' that observed for 8 weeks in different packaging conditions.
SP='sambal pijat', VP=vacuum packaging, AP=aluminium foil pouch, AT=air tight container

Besides, the decreased in degree of lightness for 'sambal pijat' showed a statistical difference (p <0.05) (Table 4.3) in relation of storage time from week 0 to week 8. The 'sambal pijat' in aluminium foil pouch at 26 ℃ showed a high loss in degree of lightness over storage time with a value of 45.41 on week 0 and start to drop sharply at week 4 to 30.44 on week 8. The high loss in L* value might occurred due to the inability of packaging conditions to maintain the colour intensity of sample tested. 'Sambal pijat' that stored in aluminium foil pouch and vacuum packaging at 5 ℃

showed a slight loss of lightness in L* value from 45.41 to 34.79 and 33.15. The results showed that these two treatments able prevent a high loss in lightness are aluminium foil pouch stored at 5 ℃.

The second factor a* value that had been analysed was presented in Figure 4.5 for control sample. Relatively, control sample recorded a fairly same a* value for the first three week in all treatment and increased linearly starting from week 4 until 8. From Figure 4.6, a* value for each treatment for 'sambal pijat' showed a fairly decreased during the storage time. For the a* value of control as showed in Figure 4.5, it showed that all values were in positive value which represent the degree of redness (Li et al., 2012). Graph in Figure 4.5 indicated that the control which stored at 26 ℃ having a slight drop at week 2 to week 3 and the value started to increase from week 4 until end of evaluation. Meanwhile, control sample which stored at 5 ℃ and 8 ℃ showed an equal trend of a* value over storage time.

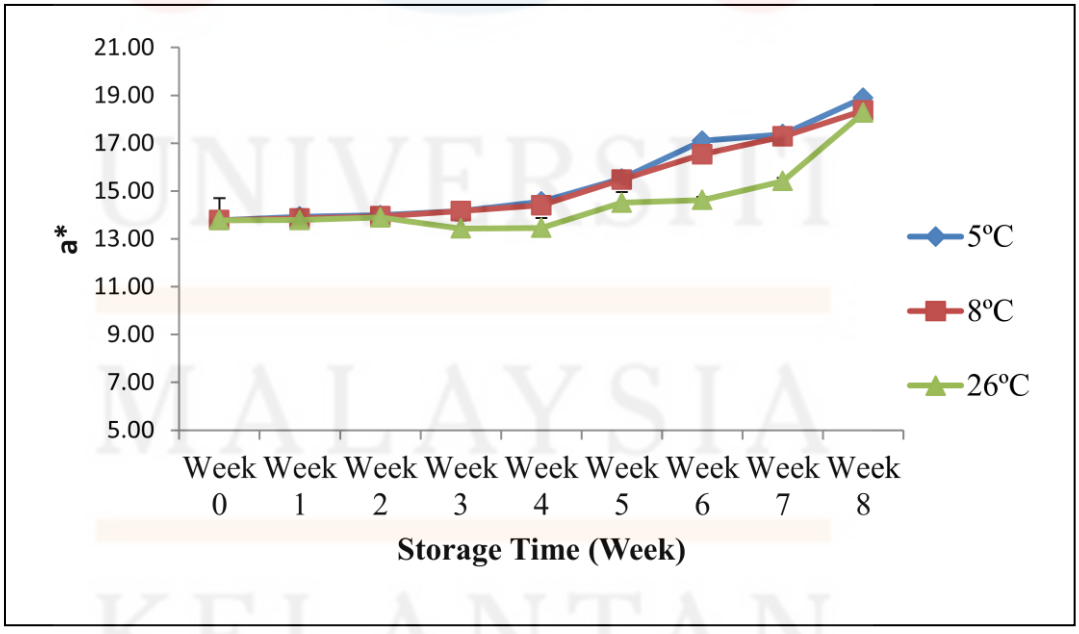


Figure 4.5: The line graph shows the redness factor of control sample that observed for 8 weeks at different storage temperature.

The decreased in a* value of 'sambal pijat' obtained a significant difference (p <0.05) compared to storage time for treatments at 5 ℃ (Table 4.3). The decrease in a* value resulted from the degradation of carotenoid. From the graph in Figure 4.6, 'sambal pijat' in air tight container stored at 26 ℃ showed a drastic changes in degree of redness as the a* value decreased from 13.31 on week 0 until 5.42 on week 8. This situation might happen due to the availability of large headspace in air tight container which increases the availability of oxygen that able to influence the degradation of carotenoid. On the other hand, other treatments on 'sambal pijat' showed a decreased in value of a* coordinates within 8 weeks of evaluation. For example, sample in vacuum packaging stored at 5 ℃ recorded a descending in a* value from 13.31 to 8.86. From the overall data, vacuum packaged sample at 5 ℃ showed a less difference when compared to the reading of a* value in week 0 which indicated that this is the recommended treatment over others in maintaining the degree of redness in 'sambal pijat'.

Figure 4.6: The line graph shows the redness factor of 'sambal pijat' that observed for 8 weeks at different storage temperature.
SP='sambal pijat',VP=vacuum packaging, AP=aluminium foil pouch, AT=air tight container

Based on observation towards the external characteristics of 'sambal pijat' during stability test the sample stored at 26 ℃ shows unfavourable properties to be consumed at certain week. For 'sambal pijat' in air tight container, observation showed that it degraded at week 4 whereas 'sambal pijat' in aluminium packaging stored at this ambient temperature degraded a week later on week 5. This is due to the growth of visible mould were detected in these packaging as can be seen in Figure 4.7 and 4.8. Thus, the L* coordinate for both packaging at ambient temperature recorded the lowest value on week 8 which was around 30.00.

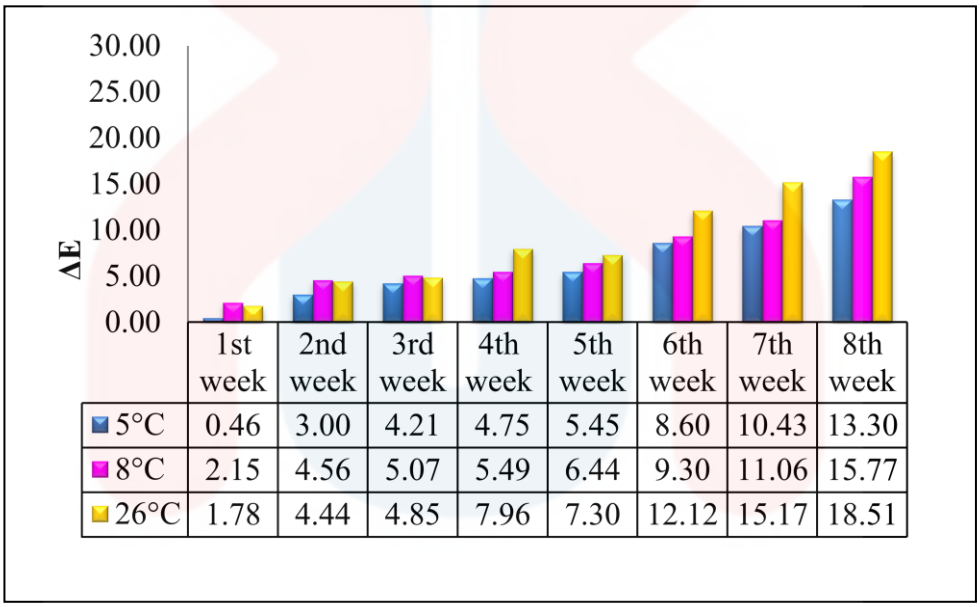
Figure 4.7: Growth of visible mold in air tight container on week 4

Figure 4.8: Growth of visible mold in aluminium foil pouch on week 5

4.3.1 (a)(i) Total colour degradation

The joint evaluation of L* a* b* coordinates were calculated based on that comprised of the differences in each coordinates on week 8 when compared to week 0. With estimation of ΔE or the total degradation for all treatments several graphs were presented as shown in Figure 4.9 to Figure 4.12. It showed that all treatments including control experienced an increase in total degradation. Relatively, each treatment recorded a linear fading in term of chromatic stability during 8 weeks of storage. Granato et al.,

(2010) stated that the increasing in ΔE during storage time may influenced by the combined effect of multiple deteriorative reactions. As for 'sambal pijat' degradation of carotenoids may affect the value of ΔE obtained. Figure 4.9 showed the total degradation experienced by control sample at three different storage conditions showed an increased over storage time. Sample that stored at 26 ℃ recorded a high value of ΔE as it started to increase at week 6 until recorded a value of 18.51 at the end of evaluation.

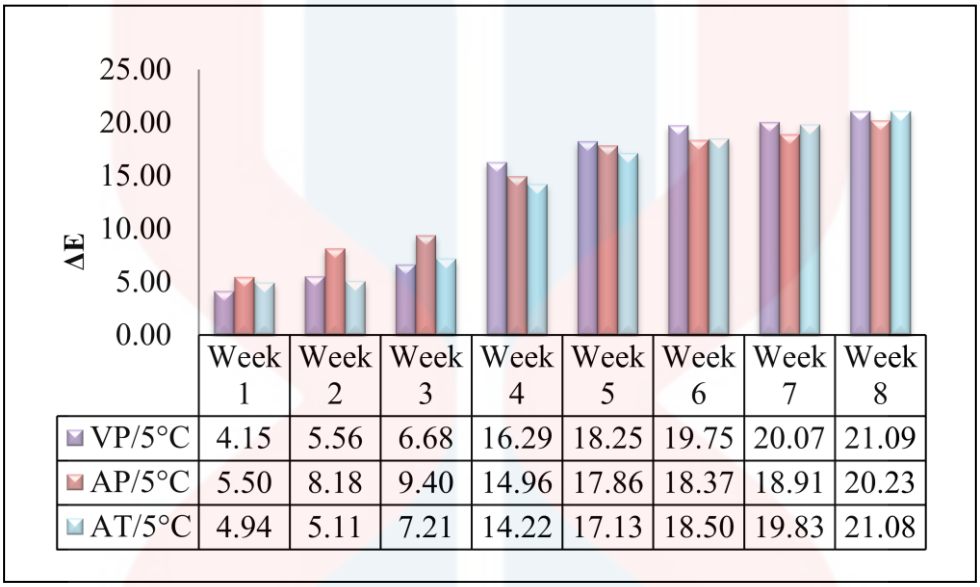


	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
5°C	0.46	3.00	4.21	4.75	5.45	8.60	10.43	13.30
8°C	2.15	4.56	5.07	5.49	6.44	9.30	11.06	15.77
26°C	1.78	4.44	4.85	7.96	7.30	12.12	15.17	18.51

Figure 4.9: ΔE of control sample.

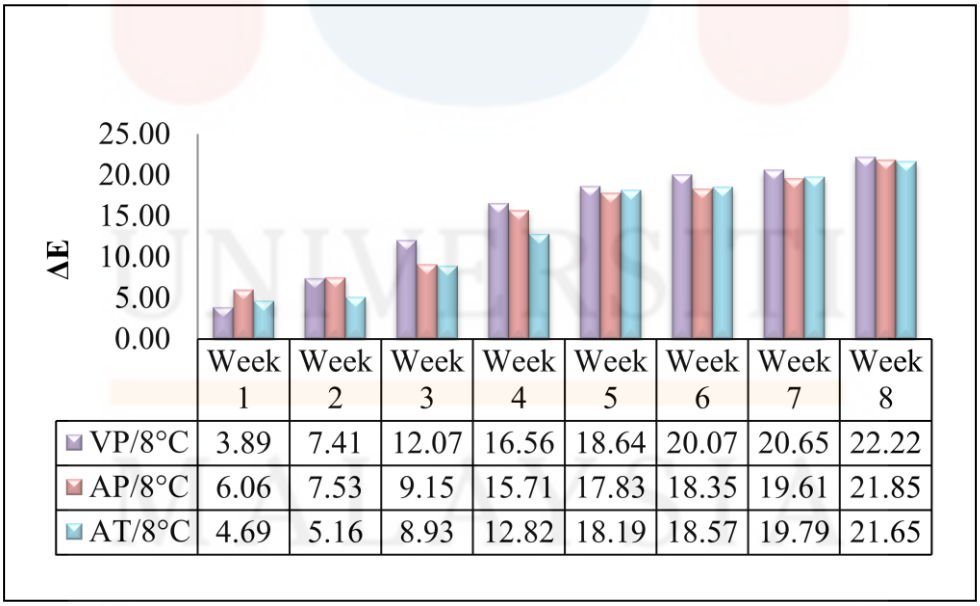
As observed in Figure 4.10 to 4.12, each treatment experienced a high changes in total degradation on week 4 which referred to half of evaluation time. For storage at 5 ℃ and 8 ℃, ΔE showed a steady increased from week 0 until week 8. This showed that chilled temperature able to preserve the quality of food over storage time. Meanwhile, all treatments applied on 'sambal pijat' at three different temperatures showed an increased in ΔE. At storage condition of 5 ℃, vacuum packaged 'sambal pijat' and air tight container recorded an equal trend of ascending in ΔE with a range of 4.15 to 21.08.

For sample stored at 8 ℃ and 26 ℃ in aluminium packaging and air tight container showed an equal rise of ΔE starting from week 3 to week 8.



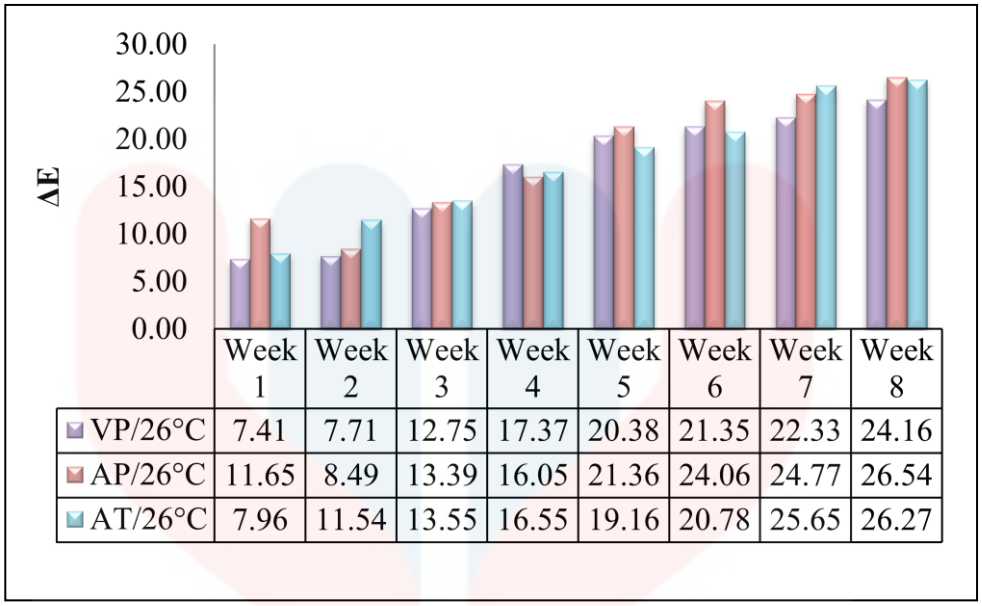
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
VP/5°C	4.15	5.56	6.68	16.29	18.25	19.75	20.07	21.09
AP/5°C	5.50	8.18	9.40	14.96	17.86	18.37	18.91	20.23
AT/5°C	4.94	5.11	7.21	14.22	17.13	18.50	19.83	21.08

Figure 4.10: ΔE 'sambal pijat' in three different packaging at 5 ℃.
VP=vacuum packaging, AP=aluminium foil pouch, AT=air tight container



	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
VP/8°C	3.89	7.41	12.07	16.56	18.64	20.07	20.65	22.22
AP/8°C	6.06	7.53	9.15	15.71	17.83	18.35	19.61	21.85
AT/8°C	4.69	5.16	8.93	12.82	18.19	18.57	19.79	21.65

Figure 4.11: ΔE of 'sambal pijat' in three different packaging at 8 ℃.
VP=vacuum packaging, AP=aluminium foil pouch, AT=air tight container



	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
VP/26°C	7.41	7.71	12.75	17.37	20.38	21.35	22.33	24.16
AP/26°C	11.65	8.49	13.39	16.05	21.36	24.06	24.77	26.54
AT/26°C	7.96	11.54	13.55	16.55	19.16	20.78	25.65	26.27

Figure 4.12: ΔE of 'sambal pijat' in three different packaging at 26 ℃.
VP=vacuum packaging, AP=aluminium foil pouch, AT=air tight container

4.3.1 (b) Determination of Moisture Content

In general, foods can be categorized as heterogeneous materials due to the existence of various form of water that bound chemically or physically and trapped in foods. Besides, within food there are different states of water that may present in gas, solid and liquid. Because of the properties of water that can presence in various molecular environments along with distinct physicochemical properties, the moisture is hardly to be measured accurately (Nielsen, 2010). Therefore, there are various methods that can be applied to determine moisture content like oven drying method, distillation method and chemical method. Moisture content of samples was determined by using oven drying method. For this method, the sample was spread to uniformity in a moisture dish before being heated at 105 ℃. The samples were heated for about 5 hours until a constant weight obtained in ensuring that all moisture within sample has been removed. Then, the loss of sample weight was used to calculate the percentage of moisture content (Kilcast et al., 2011). The significant difference within storage time and in

between type of packaging for percentage of moisture content was summarized in Table 4.4. Analysis showed that the percentage of moisture content for 'sambal pijat' was insignificantly increased over storage time.

Table 4.4: Least significant difference test moisture content of 'sambal pijat packed at three different storage temperatures during storage.

Storage temperature	Means for moisture content (%) of 'sambal pijat during storage		
	A	B	C
Weeks / Parameters	Moisture content	Moisture content	Moisture content
Week 0	67.56^{b}	$67.56b^{c}$	67.56^{c}
Week 1	68.14^{ab}	67.51^{c}	68.02^{de}
Week 2	68.57^{ab}	67.83^{abc}	69.02^{cd}
Week 3	68.75^{ab}	68.17^{abc}	69.72^{bc}
Week 4	68.65^{a}	68.29^{abc}	70.10^{abc}
Week 5	68.85^{a}	68.42^{abc}	70.23^{abc}
Week 6	68.78^{ab}	68.60^{abc}	70.69^{ab}
Week 7	68.83^{a}	68.89^{ab}	70.89^{ab}
Week 8	69.31^{a}	69.02^{a}	71.13^{a}

- Means within a column with subscript of different letter are significantly different (p <0.05) in accordance with homogenous subset defined by Two Way ANOVA
- A=5 ℃, B=8 ℃, C=26 ℃

The effect of packaging materials and storage conditions on 'sambal pijat' showed significant differences (p <0.05) when compared to control (Table 4.5). At three different temperature of 5 ℃ , 8 ℃ and 26 ℃ percentage of moisture content of 'sambal pijat' stored in three types of packaging revealed no significant differences (p >0.05) (Table 4.5) over storage time. Similarly, Saida Naik and Chetti (2017) also claimed that packaging materials and storage conditions showed no significant effect (p

>0.05) on moisture content of paddy within the storage time. However, at 26 ℃ air tight container showed a significant effect (p <0.05) on moisture content of 'sambal pijat' when compared to another two packaging. Results obtained shows that it was packaging materials and temperature not storage time that made slight significant differences in moisture content of 'sambal pijat'.

Table 4.5: Least significant difference test on moisture content of 'sambal pijat' packed in three different packaging and control at different temperature.

Storage temperature	Type of packaging	Moisture content
5 ℃	C	73.66^{a}
	VP	68.36^{b}
	AP	68.62^{b}
	AT	68.84^{b}
8 ℃	C	73.05^{a}
	VP	68.22^{b}
	AP	68.25^{b}
	AT	68.29^{b}
26 ℃	C	73.35^{a}
	VP	69.21^{c}
	AP	70.25^{b}
	AT	69.66^{c}

- Means within a column of an equal storage temperature with subscript of different letter are significantly different (p <0.05) in accordance with homogenous subset defined by Two Way ANOVA.

Data obtained for moisture content of control sample and each treatment studied on 'sambal pijat' was summarized in Figure 4.13 to 4.16. For control data as showed in Figure 4.13, the graph described that percentage of moisture content at three different temperatures decreased over storage time. The trend showed a decreased in percentage of moisture content was differ among temperature especially for chilled temperatures and ambient temperature. For storage at ambient temperature (26 ℃) results showed a gradual decreased in percentage of moisture content within 8 weeks of evaluation. Meanwhile for sample that stored at chilled temperatures at 5 ℃ and 8 ℃ graphs

resulted in an equal trend of moisture content (%) over storage time. Results showed that packaging material of control is pervious in nature where it does not enable the entering of moisture from atmosphere. However, the storage conditions of chilled temperature had influenced the forms of water within sample as control sample at ambient temperature (26 ℃) is more watery. This can be supported by moisture content of control at 26 ℃ resulted in high percentage of 72.27 % compared to 5 ℃ and 8 ℃.

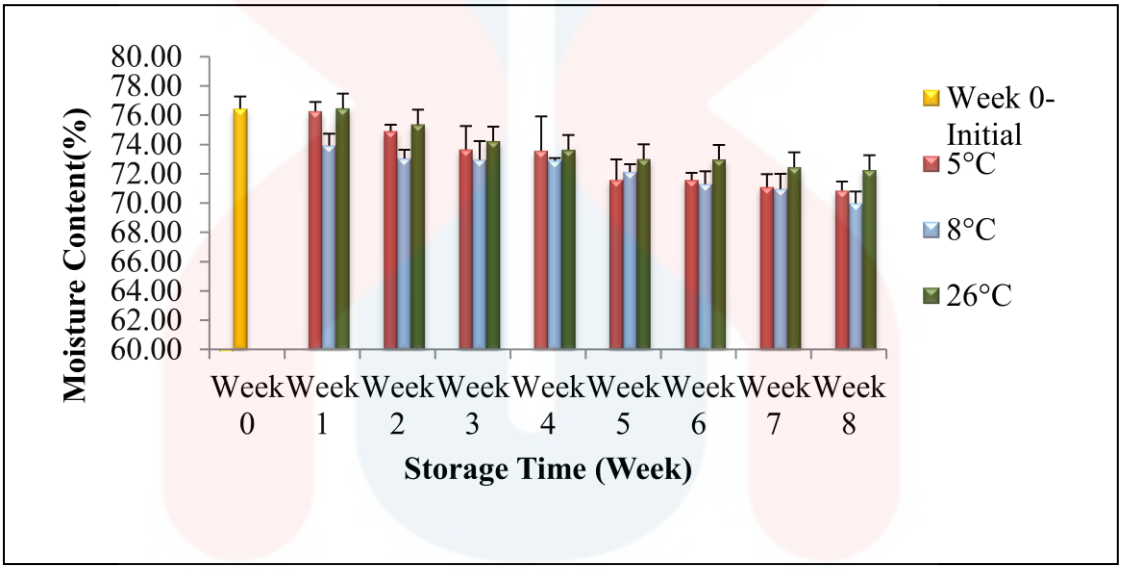


Figure 4.13: The bar graph shows moisture content of control that observed within 8 weeks at different storage temperature.

From Figure 4.14, it can be observed that the trend of graph showed that a linear increased for moisture content recorded in three different packaging at 5 ℃. When the percentage of moisture content at the end of evaluation was compared with the initial reading on week 0, it was seen that the moisture content in air tight container resulted with higher percentage of 69.61 %.This shows that PET air tight container provide a transmission of moisture from atmosphere into the package compare to another two packaging material. Even though PET material was known as moisture barrier properties, result showed that it is more pervious than vacuum packaged and aluminium foil pouch. As claimed by Siracusa (2012), vacuum packaging and foil packaging are

one of the impervious in nature where it is impermeable to moisture. Based on graph in Figure 4.14, the moisture content of 'sambal pijat' in vacuum packaging and aluminium foil pouch was not changed much within the 8 weeks of evaluation. This is because, both of packaging materials unlikely absorbed moisture from the atmosphere of chilled temperature.

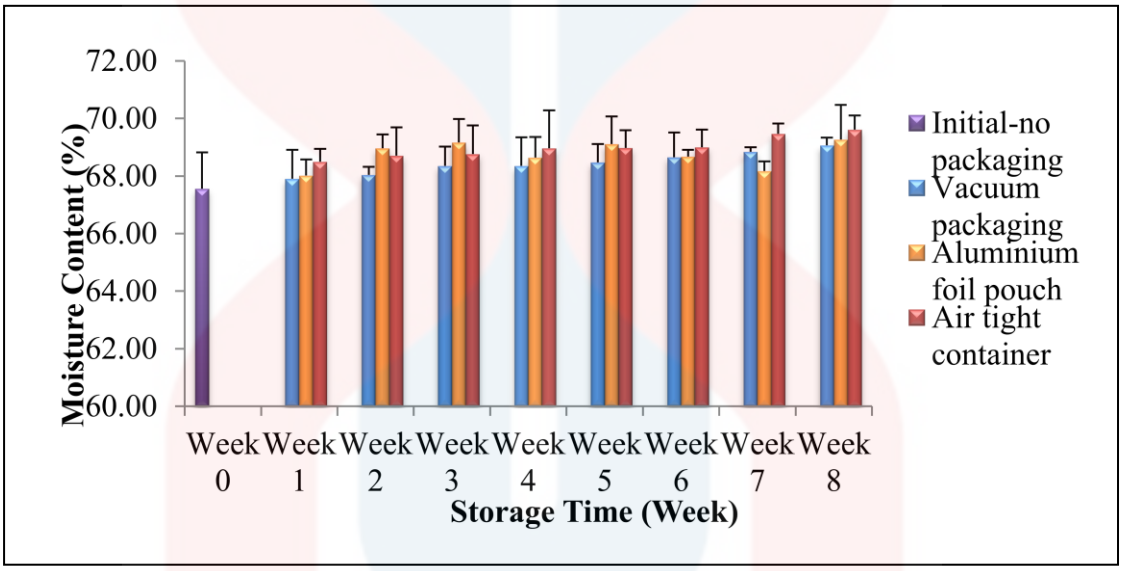


Figure 4.14: The bar graph shows the moisture content of 'sambal pijat' in three different packaging at 5 ℃ that observed for 8 weeks.

The results for moisture content in each packaging storage conditions at 8 ℃ is differ from temperature at 5 ℃ where graph in Figure 4.15, showed that the moisture content of air tight container resulted in lower percentage compared to vacuum packaging and aluminium foil pouch. This situation might occur due to the slight changes of temperature which may affect the packaging material performances in maintaining the moisture content. However, 'sambal pijat' in vacuum packaging and aluminium foil pouch remained an equal trend for percentage of moisture content. In addition, moisture content can be related to the degradation of food during storage time.

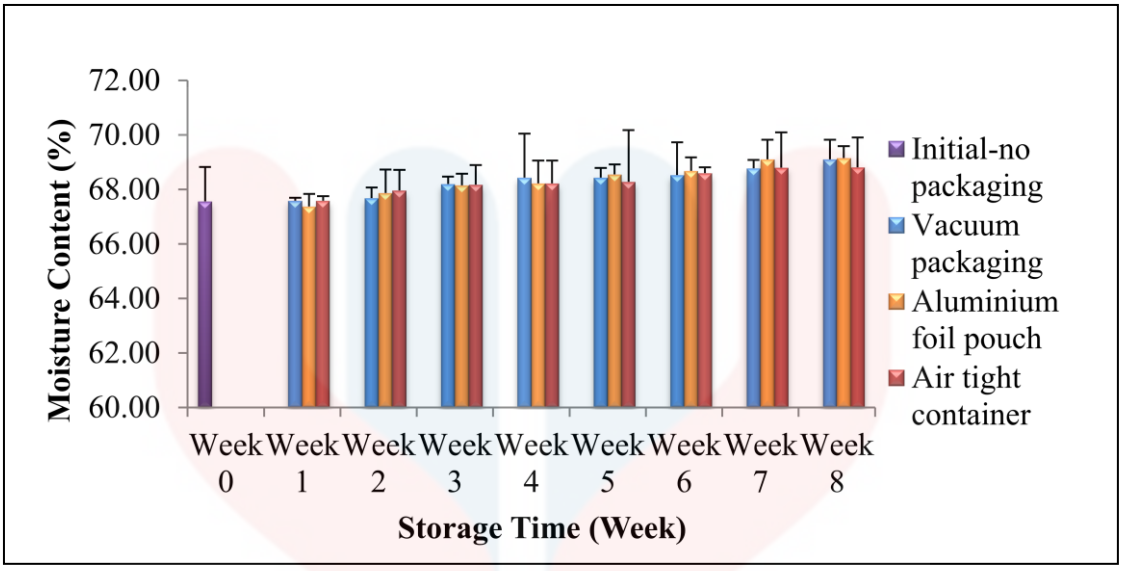


Figure 4.15: The bar graph shows the moisture content of 'sambal pijat' in three different packaging at 8 ℃ that observed for 8 weeks.

Data obtained for three different packaging was presented in Figure 4.16 showed an increased value of moisture content. It was seen that aluminium foil pouch recorded the highest result of 71.98 % at the end of evaluation week compared to another two packaging. This may cause by the properties of aluminium foil pouch which was more permeable to air. Vacuum packaged sample showed a slight change in the percentage of moisture content with a range of 66.67 % to 70.12%. Moreover, vacuum packaged sample also showed the lowest percentage of moisture content compared to another two packaging. This shows that vacuum packaging is less permeable to air than air tight container and aluminium foil pouch. For this study, 'sambal pijat' in each packaging had been observed weekly and the unfavourable changes were detected for sample that stored at ambient temperature. For 'sambal pijat' in air tight container degraded at week 4 whereas 'sambal pijat' in aluminium packaging stored at this ambient temperature degraded a week later on week 5 as the growth of visible mold present. This clearly showed that higher percentage of moisture content does resulted in degradation of food quality.

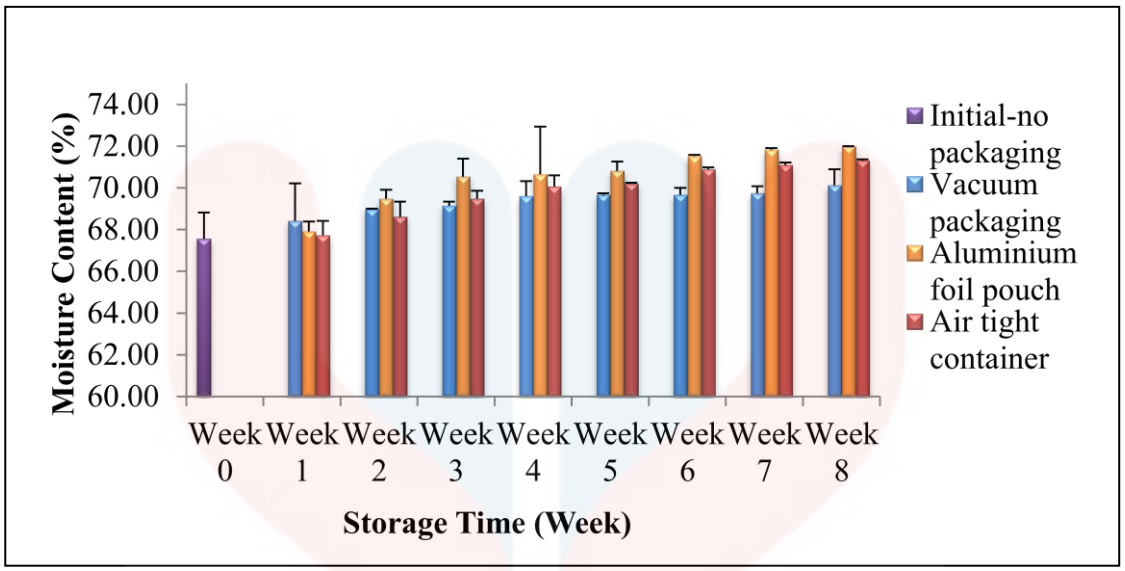


Figure 4.16: The bar graph shows the moisture content of 'sambal pijat' in three different packaging at 26 ℃ that observed for 8 weeks.

4.3.1 (c) Determination of pH Value

The measurement of pH value is crucial in determining the quality of food especially when food is subjected to longer storage time (Tamuno & Onyedikachi, 2015). This is because pH value change over time whether becomes more acidic or alkaline depends on food composition. For this current study, the samples were diluted with distilled water at ratio of 1:2 in enabling the determination of pH value. Relatively, distilled water was used as dilution because it did not greatly affect the pH readings due to insignificant amount of ions present (FDA, 2018). Before the pH electrode being submersed into sample dilution, it was calibrated with buffer solutions where buffer solution of pH solution 4 and pH 7 were used for testing of 'sambal pijat' and control. The calibration step is important to ensure the accuracy of pH meter used.

The significant difference within storage time and in between type of packaging for percentage of pH value was summarized in Table 4.6 and 4.7. The data obtained for 'sambal pijat' in three different packaging at different storage temperature of 5 ℃, 8 ℃ and 26 ℃ revealed an significant effect (p <0.05) on pH value within the storage time of 8 weeks (Table 4.6). As compared to type of packaging used the air tight container stored at 5 ℃ and 8 ℃ resulted an insignificance different (p >0.05) (Table 4.7). Meanwhile, the storage conditions of 'sambal pijat' at 26 ℃ showed a significance difference (p <0.05) when compared in between packaging (Table 4.7).

Table 4.6: Least significant difference test on pH value of 'sambal pijat packed at three different storage temperatures during storage.

Storage temperature	Means for pH value of 'sambal pijat during storage		
	A	B	C
Weeks / Parameters	pH value	pH value	pH value
Week 0	3.95^{i}	3.95^{i}	3.95^{i}
Week 1	4.04^{h}	4.03^{h}	4.38^{h}
Week 2	4.07^{g}	4.07^{g}	4.93^{g}
Week 3	4.09^{f}	4.10^{f}	5.14^{f}
Week 4	4.14^{e}	4.15^{e}	5.43^{e}
Week 5	4.18^{d}	4.18^{d}	5.49^{d}
Week 6	4.21^{c}	4.22^{c}	5.63^{c}
Week 7	4.22^{b}	4.25^{b}	5.69^{b}
Week 8	4.29^{a}	4.30^{a}	5.84^{a}

- Means within a column with subscript of different letter are significantly different (p <0.05) in accordance with homogenous subset defined by Two Way ANOVA
- A=5 ℃, B=8 ℃, C=26 ℃

Table 4.7: Least significant difference test on pH value of 'sambal pijat' packed in three different packaging and control at different temperature.

Storage temperature	Type of packaging	pH
5 ℃	C	4.65^{a}
	VP	4.13^{c}
	AP	4.13^{c}
	AT	4.14^{b}
8 ℃	C	4.48^{a}
	VP	4.14^{c}
	AP	4.13^{c}
	AT	4.15^{b}
26 ℃	C	4.26^{d}
	VP	4.81^{c}
	AP	5.26^{b}
	AT	5.43^{a}

- Means within a column of an equal storage temperature with subscript of different letter are significantly different (p <0.05) in accordance with homogenous subset defined by Two Way ANOVA.

The data for pH value of control sample and each treatment performed on 'sambal pijat' was presented in Figure 4.17 to 4.20. For control sample, graph in Figure 4.17 showed a uniform increase in pH value within 8 weeks of storage time at three different temperature. When compared in between temperature, control sample stored at 5 ℃ recorded the highest pH value throughout the evaluation weeks with a range of 4.34 to 5.04. Meanwhile, sample stored at 8 ℃ scored a slightly lowest in pH value compared to storage temperature of 26 ℃ which ranging from 4.34 to 4.88 whereas at 26 ℃ the sample recorded a pH of 4.34 to 4.98 at the end of evaluation week. From the overall results, it shows that temperature does affect the determination of pH value.

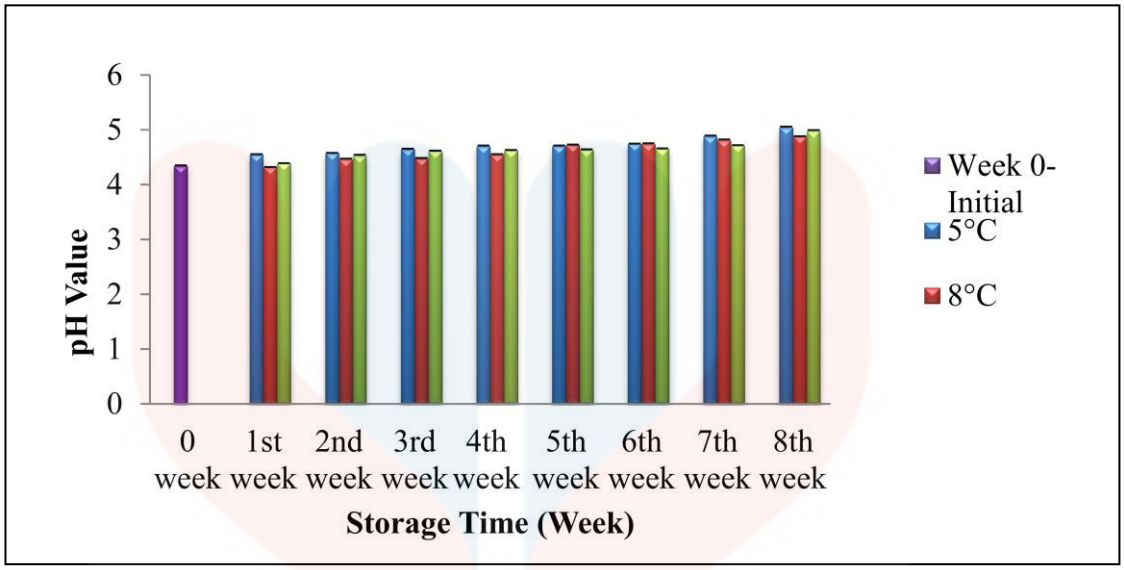


Figure 4.17: The bar graph shows pH value of control within 8 weeks of evaluation.

At three different storage conditions, pH value in three different packaging showed an increased over storage time compared to the initial reading as can be observed in Figure 4.18 to 4.20. For storage of 'sambal pijat' at 5 ℃ (Figure 4.18) it can be seen that 'sambal pijat' in air tight container resulted in higher pH value compared to aluminium foil pouch and vacuum packaging from week 1 until week. However, on the first three week of storage 'sambal pijat' in air tight container and aluminium foil pouch obtained almost an equal trend in pH value. On the other hand, at the end of evaluation which is week 8, 'sambal pijat' in aluminium pouch recorded the highest pH value of 4.34 than the other two packaging.

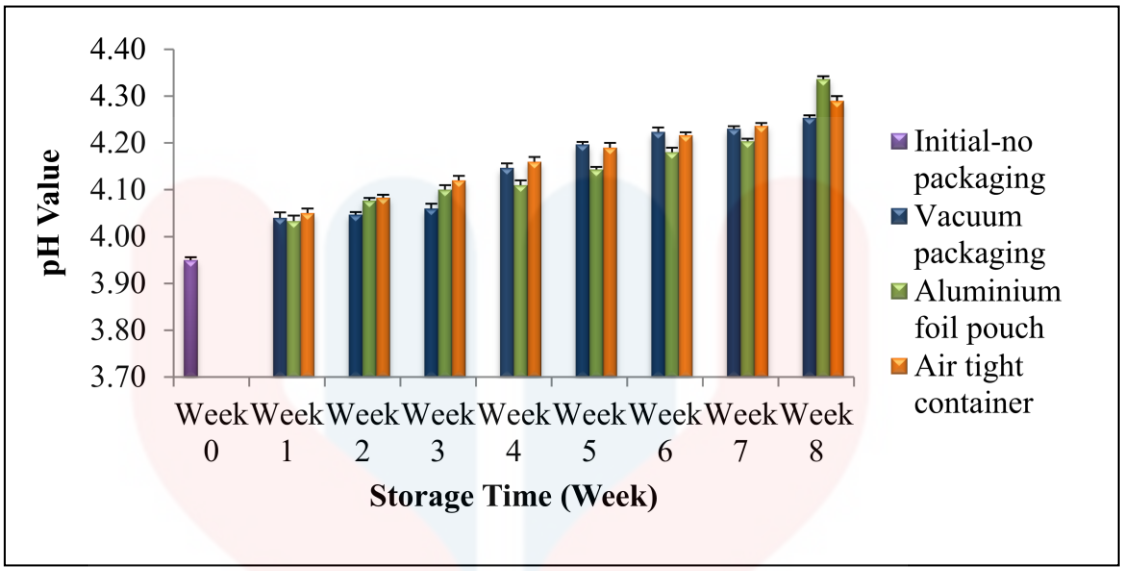


Figure 4.18: The bar graph shows pH value of 'sambal pijat' in three different packaging at 5 ℃ that observed for 8 weeks.

As can be observed in Figure 4.19, at 8 ℃ of storage condition vacuum packaged sample showed a gradual increased in pH value within 8 weeks evaluation in the range of 3.95 to 4.28. Apart from that, 'sambal pijat' stored in aluminium foil pouch resulted in higher pH value on week 8 followed by pH value of sample in air tight container. The packaging material from aluminium resulted in higher pH value compared to air tight container and vacuum packaging. This may cause by high possibility of the properties that more permeable to light and gas.

Similarly graph in Figure 4.19 which showed the pH value of 'sambal pijat' in three different packaging stored at 8 ℃ illustrated that data obtained resulted in a similar trend like pH value for samples stored at 5 ℃ as can be observed in Figure 4.18. Relatively, lower temperature storage conditions as 5 ℃ and 8 ℃ was categorized as chilled temperature (Kilcast & Subramaniam, 2011). From the results obtained, it can be concluded that lower temperature helps to maintain the quality of food product by minimizing the changes of pH value. This can be supported by Nkechi and Onyedikachi (2015), which reported that there was a slight increase of Cashew-Apple Juice pH value within packaging material stored at refrigerated temperature.

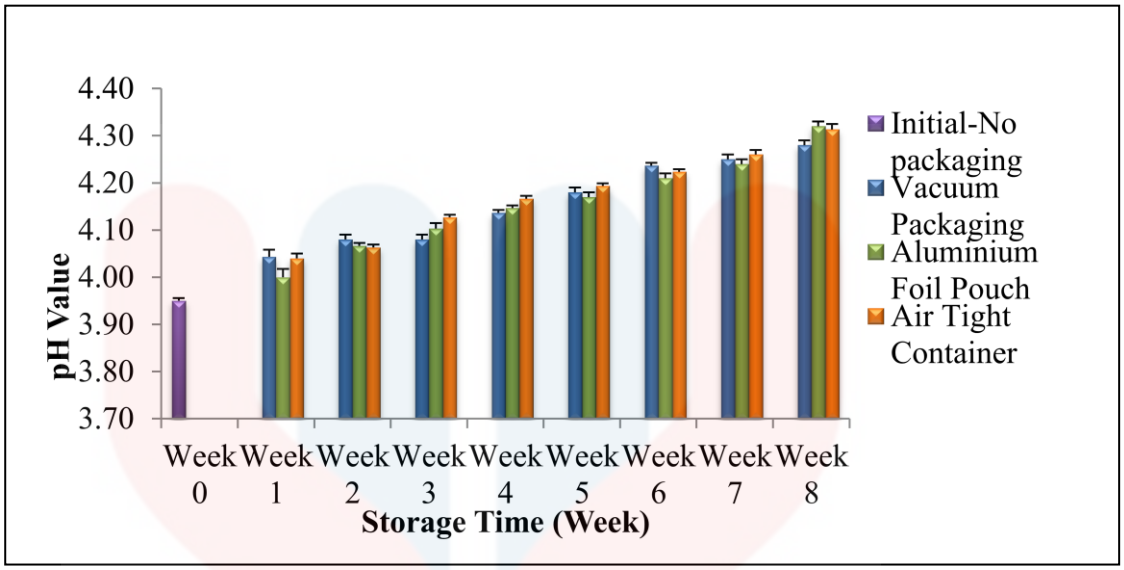


Figure 4.19: The bar graph shows pH value of 'sambal pijat' in three different packaging at 8 ℃ that observed for 8 weeks.

For sample that stored at ambient temperature (26 ℃), there was presence of visible mold growth that had been detected based on observation on the characteristics of sample along the evaluation. With regard to the pH value of 'sambal pijat' stored at 26 ℃, graph in Figure 4.20 showed a significant increased increase within all packaging materials. It can clearly be seen that pH value of sample in aluminium foil pouch and air tight container marked a sharp rise in pH value starting from week 3 until week 8.

Inversely, the samples that kept in vacuum packaging at ambient temperature of 26 ℃ showed slight changes in pH value in 8 weeks of storage time. On week 8, graph in Figure 4.20 illustrated that air tight container resulted in higher pH value compared to aluminium foil pouch and vacuum packaging. In relation to the growth of visible mold, sample stored in air tight container was detected to degrade on week 4 with a pH of 5.61 while sample in aluminium foil pouch that deteriorate a week later recorded a pH of 5.81. It can be concluded that 'sambal pijat' can be categorized as high acid foods with a pH of 3.95 and deteriorate as pH value rise to less acidic which is in between 5.61 to 6.33.

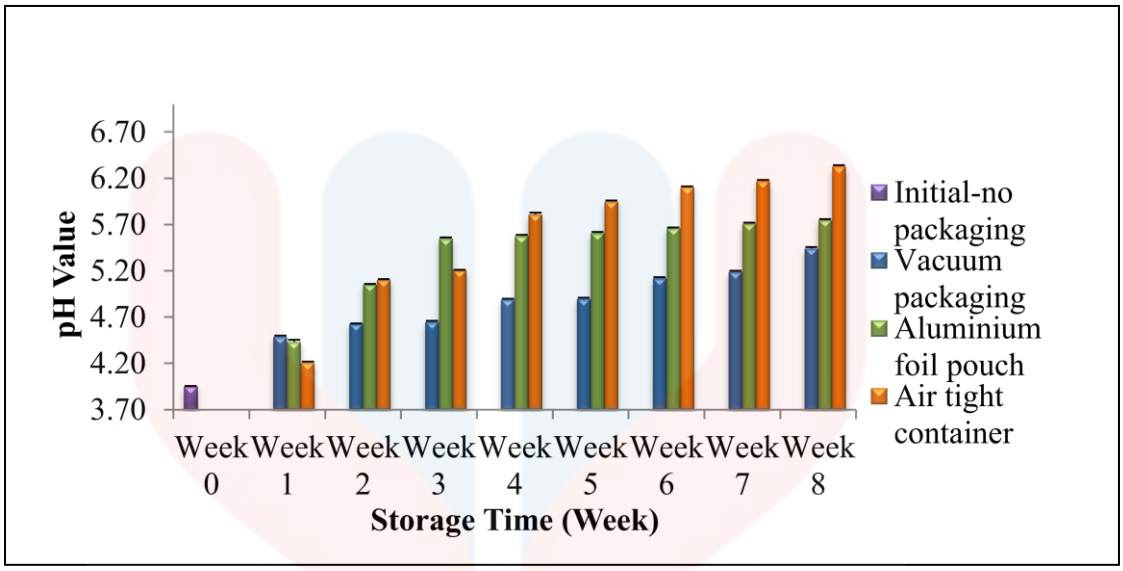


Figure 4.20: The bar graph shows pH value of 'sambal pijat' in three different packaging at 26 ℃ that observed for 8 weeks.

4.3.2 Microbial test

4.3.2 (a) Total Plate Count

In general 'sambal pijat' and control sample can be categorized as ready to eat (RTE) food as no further process need to be performed before being consumed. This can be supported by Lopašovský et al., (2016) where RTE food refers to food that can directly be eaten after purchased without further preparation or treatment. Therefore, this resulted in high risks of contamination that may cause degradation of food quality. Apart from that, RTE food is more susceptible microbial growth without a particular measure like improper packaging and storage conditions (Lopašovský et al., 2016). With regard to this issue, microbial test can be performed in evaluating the food safety to ensure safety consumption.

Relatively for this present study, the microbiological counts of samples were measured using total plate count by spreading the sample dilution on nutrient agar.

Nutrient agar acts as general medium that enable the growth of non-fastidious bacteria (Atlas, 2010). Due to heterogeneous properties of sample, it was homogenized with potassium dihydrogen phosphate anhydrous using a stomacher. Basically, potassium dihydrogen phosphate anhydrous play a role as buffered for sample dilution to promote a favourable condition for growth of microbes (Tests, 2002). The dilution factor of 10^{-1} to 10^{-6} was prepared for each samples. According to Food Act 1983 and Food Regulation Malaysia 1985 the total plate count of food product must not exceed or equal to the maximum limit 10^5 colony forming unit per gram ($\leq 10^5$ CFU/g) (Cheong Jun See, 2016).

As can be observed form Table 4.9 which outlined the microbiological counts of 'sambal pijat' in three different packaging and control at distinct storage temperature during week 0 and week 8, the total viable count was presented in colony forming unit per gram. The purposed of selecting week 0 and week 8 as the time of evaluation was to identify the effect packaging and storage conditions on sample tested. From Table 4.8, it can be seen that all treatments in week 8 obtained a significantly different (p <0.05) as compared to week 0. In addition, on week 0 'sambal pijat' which recorded 2.50×10^3 CFU/g and control (3.40×10^3 CFU/g) showed acceptable plate count that lower than the maximum limit (10^5 CFU/g).

Table 4.8: Microbiological counts of 'sambal pijat' in three different packaging and control at distinct storage temperature during week 0 and week 8.

Storage time (weeks)	Treatments (Sample/Packaging/Storage temperature)	Total viable count (CFU/g)
0	Control (C)	$2.50x10^3$
	'Sambal pijat' (SP)	$3.40x10^3$
8	C/5 ℃	$5.30x10^3$*
	SP/VP/5 ℃	$5.53x10^3$*
	SP/AP/5 ℃	$4.25x10^3$*
	SP/AT5 ℃	$6.35x10^3$*
	C/8 ℃	$5.50x10^3$*
	SP/VP/8 ℃	$6.90x10^3$*
	SP/AP/8 ℃	$6.25x10^3$*
	SP/AT/8 ℃	$7.00x10^3$*
	C/26 ℃	$1.50x10^4$*
	SP/VP/26 ℃	$7.07x10^4$*
	SP/AP/26 ℃	$1.10x10^5$*
	SP/AT/26 ℃	$2.75x10^5$*

- Symbol * defined significant difference compared to week 0 (p <0.05) using One Way repeated measure.
- VP=vacuum packaging, AP=aluminium foil pouch, AT=air tight container

Besides, at chilled temperature of 5 ℃ and 8 ℃ all treatments recorded almost an equal value for total viable count in the range of $5.30x10^3$ CFU/g to $7.00x10^3$ CFU/g. Apart from that, the total viable count measured for all treatment at ambient temperature (26 ℃) resulted in higher value with 10^4 to10^5 CFU/g compared to chilled temperature. This results is correspond to previous study by Idris & Alhassan (2010), which reported that the viable bacterial count of sample observed was significantly increased (p <0.05) when stored room temperature than cold storage within evaluation time.

Control sample showed a significantly increased in total viable count at each storage temperature compared to week 0. As can be seen from Table 4.8, sample which incorporated with aluminium foil pouch resulted in lowest viable count of $4.25x10^3$ CFU/g. Meanwhile, sample stored in air tight container recorded highest total viable

count of $6.35x10^3$ CFU/g followed by vacuum packaged 'sambal pijat' with a value of $4.25x10^3$ CFU/g.

Similarly, sample stored at 8 ℃ also resulted an equal increased and provide the same relationships among treatments for the total viable count as data obtained for treatment at 5 ℃ of storage temperature. For instance, the sample which incorporated with air tight container with a total viable count of $7.00x10^3$ CFU/g followed by vacuum packaged sample which recorded $6.90x10^3$ CFU/g. On the other hand, sample that stored in aluminium foil pouch showed the lowest total viable count with a value of $6.25x10^3$ CFU/g. The difference in total viable count of sample may cause by the possibility of packaging to have microbial contamination. Results obtained showed that aluminium foil pouch obtained lowest viable count than packaging from plastic material. This is correspond to research by Idris and Alhassan (2010) which stated that plastic container have higher microbial contamination compared to metal packaging.

Apart from storage temperature and type of packaging materials, there are others factors that can promote the growth of microbes especially for stored food. For an example, the intrinsic factors like pH value and moisture content (Hamad, 2012). As mentioned in discussion part of 4.3.1 (c) for the determination of pH value and part 4.3.1 (b) for determination of moisture content, at storage temperature of 26 ℃ 'sambal pijat' in two types of packaging showed unfavourable properties. Relatively, an observation made on external properties detected the visible growth of mold in air tight container at week 5 whereas the mold presences in aluminium foil pouch growth a week later.

Besides, sample that stored in these packaging was unacceptable to be consumed due to total viable count which correspond to the maximum limit of total plate count. In relation to pH value and moisture content, the growth of mold was

observed at higher pH value in the range of 5.61 to 6.33 whereas the moisture content was above 70%. However, there is absence of mold that observed for 'sambal pijat' which stored in vacuum packaging. This is because of the lack of oxygen gas which presence in this packaging did not promote microbes to growth.

4.4 Shelf Life Estimation

Generally, shelf life can be defined as expiring date which food product is expected to remain stable within the suggested time limit (Gill & Gill, 2010). Shelf life of 'sambal pijat' was calculated in clarifying the best treatment that correspond to storage temperature and type of packaging which can protect the product quality for longer period of time. By dividing the difference of initial value for parameter and degradation of rate constant (k), shelf life of 'sambal pijat' incorporated with three different packaging and temperature was estimated.

As can be observed in Table 4.9 which illustrated the shelf life in days for each treatment proposed, all treatments was estimated to be in good condition below than 100 days. Relatively, time estimated to ensure that all parameters within acceptance limit was in the range of 30 to 93 days. Table 4.9 showed that 'sambal pijat' in vacuum packaging and aluminium foil resulted in an equal estimated shelf life time. With regard to temperature, packed sample at chilled temperature of 5 ℃ and 8 ℃ showed an almost equal shelf life as these temperatures give insignificant effect on most of parameters tested. Meanwhile, treatment stored at ambient temperature showed a relatively shorter time for shelf life which is in between 30 to 66 days.

Table 4.9: Shelf life of 'sambal pijat' incorporated with different packaging at three storage temperature

Storage temperature	Type of packaging	k	Shelf life (days)
5 ℃	Vacuum packaging	0.06	93
	Aluminium foil pouch	0.05	93
	Air tight container	0.23	88
8 ℃	Vacuum packaging	0.06	88
	Aluminium foil pouch	0.05	93
	Air tight container	0.06	88
26 ℃	Vacuum packaging	0.26	66
	Aluminium foil pouch	0.38	37
	Air tight container	0.39	30

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As a conclusion, this study was carried out in determining the chemical properties of ‘sambal pijat’ and effect of different packaging conditions on it stability. The presences of protein and fat contents were determined based on the proposed method. Apparently, ‘sambal pijat’ does contain major chemical constituents with the presence of fat and protein even in a small amount. This study showed that the incorporation of different packaging at suggested temperature give affect the total viable count. Additionally, different packaging conditions do affect the physicochemical stability of ‘sambal pijat. Relatively, the physicochemical stability in each packaging revealed a significantly difference within storage time. Based on estimated shelf life which corresponds to evaluate parameter on physicochemical stability of ‘sambal pijat’ the time of 93 days showed the highest acceptable limit for sample to remain stable. In a nutshell, the recommended treatments to maintain the quality of ‘sambal pijat’ was incorporation of vacuum packaging and aluminium foil pouch at chilled temperature which most preferable at 5 ℃. Therefore, it can be concluded that all objectives were achieved. Lastly, this current study helps to provide information about ‘sambal pijat’ in a scientific ways.

5.2 Recommendation

From this present study, it showed that further research must be conducted on evaluating the proximate composition of 'sambal pijat'. This is because, an analysis on proximate composition of food product helps to revealed the nutritional benefit. Furthermore, proximate analysis refers to the determination of micronutrients in food sample that comprises of protein, fat, moisture, ash, fiber and carbohydrates. These six components will be declared as nutritional as nutritional facts for finished products that help to guide consumers. Apart from that, an antioxidant activity also can be evaluated in order to study the beneficial influence of 'sambal pijat' on human health. Besides, with regard to low acid properties of 'sambal pijat' with 3.95 pH value, an enumeration of yeast and mold should be performed in proving the microbiological stability of this product during storage time. This is due to the fact that yeast and mold tend to grow at an environment with low pH value. Moreover, a sensory evaluation needs to be carried out among potential consumer other than people in Jeli district. Relatively, sensory evaluation helps to reveal consumer's satisfaction and judgement on their acceptance of this unique product.

REFERENCES

Alim, I., Rizvi, A. F., Khan, S., & Rafi, M. (2014). Purity testing of the raw and processed plant origin drugs for phytoformulation. *Nature & Environment*, *19*(20), 151–157.

Andrés-Bello, A., Barreto-Palacios, V., García-Segovia, P., Mir-Bel, J., & Martínez-Monzó, J. (2013). Effect of pH on color and texture of food products. *Food Engineering Reviews*, *5*(3), 158–170.

AOCS Lipid Library. (2014). Crude fat methods-considerations. *Lipid Library* 1-6.

Appoldt, Y., & Raihani, G. (2017). Determining moisture content. *Food Quality & Safety*. Wiley Company *1*,1-3.

Ara Sains. (2014). Total plate count. *Indonesia Leading Supplier of Scientific & Engineering products*.

Arimboor, R., Natarajan, R. B., Menon, K. R., Chandrasekhar, L. P., & Moorkoth, V. (2015). Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: analysis and stability-a review. *Journal of Food Science and Technology*, *52*(3), 1258–1271.

Atlas, R. M. (2010). *Handbook of microbiological media*. 4th Edition.Boca Raton, Fla 1403,1412,1413.

Brown, H., & Williams, J. (2011). *Packaged product quality and shelf life. food and beverage packaging technology*: 2nd Edition, (1993), 59–83.

Buffler, M., & Mühleis, A. (2012). *Decode the mysteries of the Kjeldahl tablets*.1-11.

Cheong Jun See-Universiti Tunku Abdul Rahman. (2016). *Staphylococcus aureus counts of randomly selected street foods in Kampar, Perak*.Universiti Tunku Abdul Rahman 1-59.

Chowdhury, S., Nath, S., Biswas, S., & Dora, K. C. (2017). Vacuum packaging extend shelf life of sea basis fillet. *Journal Exp.Zool. India 20*(2), 1241-1245.

D Saida Naik, & M B Chetti. (2017). Influence of packaging and storage conditions on the moisture content and its effect on fungal load of paddy. *Research Journal of Agricultural Science*, 370–374.

Deman, J. M. (1999). *Principles of food chemistry*. Lipids proteins carbohydrates - principles of food chemistry 3rd Edition, 1-84.

Food and Drug Administration (2018). Protein serving size 1 package (272g) servings per container 1-2.

Food And Drug Administration. (2017). CFR - Code of Federal Regulations, *2*(21), 1-4.

Fraser, A. (2012). Food Safety education , for educators ,competencies ,general bacteria | explain what pH is and how it relates to bacterial growth. *Food and Packaging Science*.

Gill, A. O., & Gill, C. O. (2010). *Food packaging and shelf life: a practical guide*. CRC PRESS, Taylor and Francis Group 259–288.

Granato, D., Masson, M. L., João, R., & Freitas, S. De. (2010). Stability studies and shelf life estimation of a soy-based dessert, 797–807.

Guillet, M., & Rodrigue, N. (2010). *Shelf life testing methodology and data analysis*. CRC PRESS, Taylor and Francis Group. 31-53.

In G. L. Robertson (2010). *Food packaging and shelf life: A practical guide*. CRC PRESS, Taylor and Francis Group, 31–53.

Hamad, S. H. (2012). *Factors affecting the growth of microorganisms in food*. In progress in food preservation. Oxford, UK: Wiley-Blackwell. 405–427

Hang, N. T., Chieu, N. D., Trang, N., & Hoa, K. (2014). Process development for production of smoked fish floss products from atlantic mackerel. *UNU-Fisheries Training Programme*, *1*, 1–39.

Hasimah, H. A., Noraini, M. K., & Hasanah, M. Y. R. (1993). Shelf-life study of freeze dried "sambal tumis bilis",*Malaysian Agricultural Research and Development Institute*, *21*(2), 157–162.

Hernández- Macedo, M. L., Barancelli, G. V., & Contreras-Castillo, C. J. (2011). Microbial deterioration of vacuum-packaged chilled beef cuts and techniques for microbiota detection and characterization: A review. *Brazilian Journal of Microbiology*, *42*(1), 1–11.

Ibrahim, H., Syamsir, D. R., Aziz, A. N., Awang, K., Nor Azah, M. A., Mastura, M., & Mat Ali, R. (2009). Essential oils of elettariopsis curtisii (zingiberaceae) and their antimicrobial activities. *Journal of Essential Oil Research*, *21*(5), 464–466.

Idris, Y. M. A., & Alhassan, I. H. (2010). Effect of Packaging material on microbiological properties of sudanese white cheese. *International Journal of Dairy Science*, *5*(3), 128–134.

John, P. J. (2010). *A handbook on food packaging*. Delhi: Daya Publishing House. 1st edition, 324-360.

Kilcast, D. (2013). *Instrumental assessment of food sensory quality : a practical guide*. Woodhead Publishing.

Kilcast, D., & Subramaniam, P. (2011). *The stability and shelf-life of food*. 1st edition. Edited by. Woodhead Publishing. Series In Food Science Technology And Nutrition.

Lee, S. Y., Lee, S. J., Choi, D. S., & Hur, S. J. (2015). Current topics in active and intelligent food packaging for preservation of fresh foods. *Journal of the Science of Food and Agriculture*, *95*(14), 2799–2810.

Li, X., Lindahl, G., Zamaratskaia, G., & Lundström, K. (2012). Influence of vacuum skin packaging on color stability of beef longissimus lumborum compared with vacuum and high-oxygen modified atmosphere packaging. *Meat Science, 92*(4), 604–609.

Lopašovský, Ľ., Terentjeva, M., Kunová, S., Zeleňáková, L., & Kačániová, M. (2016). Microbiological quality of ready-to-eat foods produced in Slovakia. *Journal of Microbiology, Biotechnology and Food Sciences, 5*(1), 31–35.

Mæhre, H. K., Dalheim, L., Edvinsen, G. K., Elvevoll, E. O., & Jensen, I. (2018). *Protein determination—method matters. Foods, 7*(1), 5.

Mailoa, M. N., Marthina Tapotubun, A., & Matrutty, T. E. A. A. (2017). Analysis total plate counte (tpc) on fresh steak tuna applications edible coating caulerpa sp during stored at chilling temperature analysis total plate count (TPC) on fresh steak tuna applications edible coating caulerpa sp during stored at chilling temperature, *IOP Conference Series: Earth and Environmental Science* (89), 1-7.

Marsh, K., & Bugusu, B. (2007). Food packaging - Roles, materials, and environmental issues: Scientific status summary. *Journal of Food Science, 72*(3), 1-18.

Maturin, L., & Peeler, J. T. (2001). *Laboratory methods - BAM: aerobic plate count*. Center for Food Safety and Applied Nutrition.

Mauer, L. J., & Ozen, B. F. (2004). *Food packaging*.University of Szeged. 1-196.

Ministry of Health. (2017). Protein-recommended nutrient intakes for Malaysia 2005. *5*(54) ,1-14.

Moldovan, L., & Pantea, G. (2015). Development of innovative biodegradable packaging system to improve shelf life, quality and safety of high fresh products, *Journal of EcoAgriTourism 11*(1), 24–25.

Nielsen, S. S. (2010). Food Analysis. In S. S. Nielsen (Ed.), Food analysis 3rd Edition, Kluwer Academic/ Plenum publishers. *Journal of Food Analysis, 184*,116–121.

Nkechi Juliet Tamuno, E., & Onyedikachi, E. C. (2015). Effect of packaging materials, storage conditions on the vitamin c and ph value of cashew apple (*Anacardium occidentale L.*) Juice. *Journal of Food and Nutrition Sciences, 3*(4), 160.

Özpolat, E., Patır, B., Guran, H. Ş., & Gul, M. R. (2014). Effect of vacuum-packing method on the shelf – life of Capoeta umbla sausages. *Iranian Journal of Fisheries Science, 13*(1), 178–184.

PanReac AppliChem. (2017). *Nitrogen determination by Kjeldahl method*.ITW Reagents 1-12.

Pasiakos, S. M., Agarwal, S., Lieberman, H. R., & Fulgoni, V. L. (2015). Sources and amounts of animal, dairy, and plant protein intake of US adults in 2007–2010. *Nutrients, 7*(8), 7058–7069.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour measurement and analysis in fresh and processed foods: A review. F*ood and Bioprocess Technology, 6*(1), 36–60.

Piergiovanni, L., & Limbo, S. (2016). Food packaging materials, *Food Reviews International 31*.172-193.

Potter, N. N., & Hotchkiss, J. H. (1995). *Constituents of foods: properties and significance*. Springer Boston, MA. 24–45.

Robertson, G. L. (2010). *Food packaging and shelf life: a practical guide*. In G. L. Robertson (Ed.), 1st Edition, Singapore: CRC PRESS, Taylor and Francis Group 1–15.

Sáez-Plaza, P., Navas, M. J., Wybraniec, S., Michałowski, T., & Asuero, A. G. (2013). An overview of the kjeldahl method of nitrogen determination. part ii. sample preparation, working scale, instrumental finish, and quality control. *Critical Reviews in Analytical Chemistry, 43*(4), 224–272.

Sehgal, S. (2016). *A laboratory manual of food analysis*. New Delhi: I K International Publish.16–23.

Sin-bin, C., Ng, M., Craig, D., & Xiu-mei, L. (2014). *Microbiological guidelines for food for ready-to-eat food in general and specific food items*. Centre for Food Safety, Food and Environmental Hygiene Department, 46.

Singh, T. K., & Cadwallader, K. R. (2004). *Understanding and measuring the shelf-life of food*. In R. Steele (Ed.). Woodhead Publishing. 165–183.

Siracusa, V. (2012). Food packaging permeability behaviour: a report. *International Journal of Polymer Science*, 2012, 1–11.

Sutton, S. (2006). Microbiology network - counting colonies | The microbiology network: Dedicated to the improvement of regulatory science and compliance. *PMF Newsletter*.

Tests, B. G. (2002). Microbial limit tests. *Microbiological Tests, 1814*(61), 1814–1823.

Tuan Mohd Ghazali, T. N. (2013). *Packaging methodologies and processing techniques to extend shelf life of longan fruits (dimocarpus longan).* University Malaysia Kelantan.

Yam, K. L., Takhistov, P. T., & Miltz, J. (2005). Intelligent Packaging: concepts and applications. *Journal of Food Science, 70*(1), 1–10.

Zuzana, S. (2017). *Food packaging materials- comparison of materials used for packaging purposes*.Helsinki Metropolia University of Applied Sciences. 1-50.

APPENDIX A: Biographical of Interviewee

Figure A.1 shows one of the retailers in Pasar Jeli who sell ‘sambal pijat’. Puan Siti Zahrah Binti Kasim was born in 1958 and currently living in Jeli, Kelantan. She is a single mother who lives with her children. Apart from being housewife, Puan Siti will spend her time selling vegetables every Saturday, Tuesday and Thursday. She is one of the pioneers in selling ‘sambal pijat’ where she had already sold this ‘sambal’ for almost 10 years. Relatively, Puan Siti follows the ancient recipes of her family in order to produce a good quality of ‘sambal pijat’.

Figure A.1 Puan Siti Zahrah Binti Kasim

APPENDIX B: Chemical properties of sample

B.1Triplicate measurement of percentage or protein and fat content.

Protein content (%)					
Sample	Reading 1	Reading 2	Reading 3	Average	Standard deviation
Control	4.1400	4.0000	3.9300	4.0233	0.1069
Sambal Pijat	2.2500	2.3400	2.1600	2.2500	0.0900
Fat content (%)					
Sample	Reading 1	Reading 2	Reading 3	Average	Standard deviation
Control	11.0830	11.1390	11.1060	11.1093	0.0281
Sambal Pijat	9.9000	9.8850	9.9650	9.9167	0.0425

APPENDIX C: Physicochemical properties

C.1 Triplicate measurement of colour coordinate of L* for control at three different temperature.

Colour analysis-L*															
Control															
Temperature	5°C					8°C					26°C				
Readings/ L*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	26.27	27.00	28.35	27.21	1.0553	26.27	27.00	28.35	27.21	1.0553	26.27	27.00	28.35	27.21	1.0553
1st week	27.41	27.85	27.64	27.6333	0.2201	29.23	29.66	29.20	29.36	0.2574	28.20	28.62	28.45	28.42	0.2113
2nd week	30.62	30.22	31.00	30.6133	0.3900	31.43	31.74	31.75	31.64	0.1819	30.13	30.05	30.96	30.38	0.5039
3rd week	31.44	31.20	31.46	31.3667	0.1447	31.78	31.73	31.70	31.74	0.0404	30.35	30.35	30.31	30.34	0.0231
4th week	31.82	31.45	31.97	31.7467	0.2676	31.95	31.70	31.63	31.76	0.1682	34.03	34.05	34.04	34.04	0.0100

Table C.1 Cont.

5th week	33.17	30.66	33.06	32.2967	1.4185	32.42	32.54	32.18	32.38	0.1833	34.18	34.12	34.14	34.15	0.0306
6th week	34.43	34.66	34.47	34.5200	0.1229	35.20	35.19	35.32	35.24	0.0723	37.76	36.62	36.35	36.91	0.7484
7th week	35.11	35.26	35.64	35.3367	0.2732	35.48	35.74	35.44	35.55	0.1629	38.18	38.34	38.32	38.28	0.0872
8th week	36.38	36.06	36.80	36.4133	0.3711	39.12	39.35	39.20	39.22	0.1168	40.69	40.97	40.78	40.81	0.1429

C.2 Triplicate measurement of colour coordinate a* for control at three different temperature.

Colour analysis-a*															
Control															
Temperature	5°C					8°C					26°C				
Readings/ L*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	13.17	13.33	14.84	13.78	0.9215	13.17	13.33	14.84	13.78	0.9215	13.17	13.33	14.84	13.78	0.9215
1st week	13.91	13.96	13.91	13.93	0.0289	13.73	13.89	13.94	13.85	0.1097	13.63	13.75	13.99	13.79	0.1833
2nd week	13.98	13.99	14.00	13.99	0.0100	13.96	13.90	13.97	13.94	0.0379	13.88	13.81	13.99	13.89	0.0907
3rd week	14.15	14.16	14.18	14.16	0.0153	14.12	14.18	14.19	14.16	0.0379	13.53	13.70	13.06	13.43	0.3315
4th week	14.41	14.69	14.57	14.56	0.1405	14.70	14.49	14.03	14.41	0.3427	13.56	13.81	13.00	13.46	0.4148
5th week	15.61	15.69	15.25	15.52	0.2344	15.42	15.75	15.26	15.48	0.2499	14.87	14.65	14.02	14.51	0.4412
6th week	17.14	17.15	17.01	17.10	0.0781	16.91	16.43	16.27	16.54	0.3331	14.56	14.54	14.78	14.63	0.1332
7th week	17.27	17.21	17.65	17.38	0.2386	17.29	17.29	17.27	17.28	0.0115	15.56	15.36	15.33	15.42	0.1250
8th week	18.79	18.90	19.01	18.90	0.1100	18.36	18.24	18.51	18.37	0.1353	18.18	18.42	18.22	18.27	0.1286

C.3 Triplicate measurement of colour coordinate b* for control at three different temperature.

Colour analysis-b*															
Control															
Temperature	5°C					8°C					26°C				
Readings/ L*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	18.17	19.37	17.60	18.38	0.9035	18.17	19.37	17.60	18.38	0.9035	18.17	19.37	17.60	18.38	0.9035
1st week	18.31	18.26	18.28	18.2833	0.0252	18.30	18.48	18.42	18.40	0.0917	19.75	19.81	19.51	19.69	0.1587
2nd week	18.35	18.98	18.49	18.6067	0.3308	19.76	19.23	19.39	19.46	0.2718	21.55	21.34	21.59	21.49	0.1343
3rd week	18.99	18.86	18.77	18.8733	0.1106	20.52	20.75	20.60	20.62	0.1168	21.38	22.45	22.43	22.09	0.6121
4th week	19.26	19.70	19.66	19.5400	0.2433	21.90	21.06	21.19	21.38	0.4521	22.30	22.65	22.48	22.48	0.1750
5th week	19.24	19.26	19.26	19.2533	0.0115	22.02	21.85	21.60	21.82	0.2113	23.88	23.81	13.91	20.53	5.7361
6th week	21.35	21.98	21.07	21.4667	0.4661	22.17	22.31	22.03	22.17	0.1400	25.42	25.90	25.45	25.59	0.2689
7th week	23.98	23.80	23.72	23.8333	0.1332	24.64	24.71	24.88	24.74	0.1234	28.79	28.71	28.37	28.62	0.2230
8th week	26.42	26.47	26.62	26.5033	0.1041	27.63	27.66	27.24	27.51	0.2343	30.27	30.55	30.11	30.31	0.2227

C.4 Triplicate measurement of colour coordinate L* for ‘sambal pijat’ in vacuum packaging at three different temperature.

Packaging	Sambal pijat- Vacuum packaging														
Temperature	5°C					8°C					26°C				
Readings/ L*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	45.02	45.58	45.64	45.41	0.3420	45.02	45.58	45.64	45.41	0.3420	45.02	45.58	45.64	45.41	0.3420
1st week	43.12	43.14	42.87	43.04	0.1504	42.65	42.01	42.37	42.34	0.3208	41.71	41.56	41.74	41.67	0.0964
2nd week	41.93	41.84	41.99	41.92	0.0755	38.13	38.14	38.03	38.10	0.0608	41.52	41.48	41.46	41.49	0.0306
3rd week	40.30	40.12	40.40	40.27	0.1419	36.50	35.59	35.95	36.01	0.4583	38.13	38.14	38.03	38.10	0.0608
4th week	37.81	37.74	37.64	37.73	0.0854	35.67	35.15	35.31	35.38	0.2663	36.53	36.98	36.70	36.74	0.2272
5th week	36.79	36.43	36.53	36.58	0.1858	36.39	36.74	36.86	36.66	0.2442	34.75	34.59	34.52	34.62	0.1179
6th week	34.52	34.37	34.37	34.42	0.0866	34.39	34.37	34.51	34.42	0.0757	33.86	33.15	33.44	33.48	0.3570
7th week	34.36	34.71	34.55	34.54	0.1752	34.36	34.25	34.37	34.33	0.0666	32.12	32.14	32.87	32.38	0.4274
8th week	33.16	33.13	33.17	33.15	0.0208	32.12	32.14	32.87	32.38	0.4274	31.03	31.08	31.00	31.04	0.0404

C.5 Triplicate measurement of colour coordinate L* for ‘sambal pijat’ in aluminium foil pouch at three different temperature.

Packaging	Sambal pijat- Aluminium foil pouch														
Temperature	5°C					8°C					26°C				
Readings/ L*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	45.02	45.58	45.64	45.41	0.3420	45.02	45.58	45.64	45.41	0.3420	45.02	45.58	45.64	45.41	0.3420
1st week	43.57	43.76	43.66	43.66	0.0950	42.90	42.46	41.99	42.45	0.4551	39.55	39.71	39.58	39.61	0.0850
2nd week	42.74	42.85	42.78	42.79	0.0557	40.60	40.96	40.85	40.80	0.1845	38.00	38.05	38.82	38.29	0.4597
3rd week	40.84	40.17	40.26	40.42	0.3636	39.79	39.12	39.28	39.40	0.3499	37.07	37.19	37.45	37.24	0.1943
4th week	38.80	38.92	38.29	38.67	0.3345	36.88	36.45	36.42	36.58	0.2574	34.40	34.74	34.36	34.50	0.2088
5th week	37.09	37.18	37.31	37.19	0.1106	36.32	36.96	36.05	36.44	0.4674	34.38	34.25	34.49	34.37	0.1201
6th week	36.06	36.32	36.66	36.35	0.3009	36.26	36.52	35.99	36.26	0.2650	32.00	32.05	31.82	31.96	0.1210
7th week	36.27	36.77	36.77	36.60	0.2887	35.73	35.81	35.75	35.76	0.0416	31.55	31.71	31.58	31.61	0.0850
8th week	34.99	34.44	34.93	34.79	0.3017	33.34	33.31	33.64	33.43	0.1825	30.93	30.38	30.02	30.44	0.4583

C.6 Triplicate measurement of colour coordinate L* for ‘sambal pijat’ in air tight container at three different temperature.

Packaging	Sambal pijat- Air tight container														
Temperature	5°C					8°C					26°C				
Readings/ L*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	45.02	45.58	45.64	45.41	0.3420	45.02	45.58	45.64	45.41	0.3420	45.02	45.58	45.64	45.41	0.3420
1st week	43.76	43.59	43.67	43.67	0.0850	42.66	42.61	42.79	42.69	0.0929	40.44	40.32	40.32	40.36	0.0693
2nd week	42.70	42.38	42.96	42.68	0.2905	42.38	42.95	42.43	42.59	0.3156	39.73	39.28	39.83	39.61	0.2930
3rd week	40.22	40.14	40.43	40.26	0.1498	41.01	40.29	40.20	40.50	0.4440	38.16	38.65	38.74	38.52	0.3121
4th week	39.76	39.61	38.40	39.26	0.7457	38.47	38.48	37.36	38.10	0.6438	37.25	37.16	37.80	37.40	0.3465
5th week	38.89	39.44	39.30	39.21	0.2858	37.74	37.13	37.16	37.34	0.3439	37.82	37.78	34.36	36.65	1.9862
6th week	37.69	37.82	37.96	37.82	0.1350	37.25	35.89	35.71	36.28	0.8420	34.03	34.02	34.29	34.11	0.1531
7th week	35.65	35.28	35.05	35.33	0.3027	33.07	33.42	33.78	33.42	0.3550	31.55	31.71	31.01	31.42	0.3657
8th week	33.12	33.14	33.87	33.38	0.4274	32.12	32.14	32.87	32.38	0.4274	30.84	30.76	30.70	30.77	0.0702

C.7 Triplicate measurement of colour coordinate a* for ‘sambal pijat’ in vacuum packaging at three different temperature.

Packaging	Sambal pijat-Vacuum packaging														
Temperature	5°C					8°C					26°C				
Readings/ a*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	13.03	13.56	13.34	13.31	0.2663	13.03	13.56	13.34	13.31	0.2663	13.03	13.56	13.34	13.31	0.2663
1st week	13.33	13.38	13.49	13.40	0.0819	13.75	13.66	13.74	13.72	0.0493	13.02	13.03	13.07	13.04	0.0265
2nd week	13.18	13.56	13.38	13.37	0.1901	13.78	13.71	13.62	13.70	0.0802	13.00	12.98	12.99	12.99	0.0100
3rd week	12.91	12.89	12.06	12.62	0.4851	13.05	12.83	13.00	12.96	0.1153	12.23	12.56	12.67	12.49	0.2290
4th week	11.81	11.74	11.64	11.73	0.0854	11.00	11.15	11.31	11.15	0.1550	11.05	11.09	11.07	11.07	0.0200
5th week	10.64	10.75	10.44	10.61	0.1572	10.10	10.60	10.58	10.43	0.2831	9.37	9.41	9.33	9.37	0.0400
6th week	9.99	9.28	10.82	10.03	0.7708	9.69	9.50	9.95	9.71	0.2259	9.00	9.08	9.31	9.13	0.1609
7th week	9.00	9.92	9.73	9.55	0.4857	9.05	9.15	9.02	9.07	0.0681	8.90	8.47	8.65	8.67	0.2159
8th week	9.07	8.57	8.94	8.86	0.2594	8.23	8.47	8.70	8.47	0.2350	7.63	7.79	7.78	7.73	0.0896

C.8 Triplicate measurement of colour coordinate a* for ‘sambal pijat’ in aluminium foil pouch at three different temperature.

Packaging	Sambal pijat-Aluminium foil pouch														
Temperature	5°C					8°C					26°C				
Readings/ a*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	13.03	13.56	13.34	13.31	0.2663	13.03	13.56	13.34	13.31	0.2663	13.03	13.56	13.34	13.31	0.2663
1st week	12.53	12.28	12.09	12.30	0.2207	12.53	12.28	12.09	12.30	0.2207	12.38	12.78	12.77	12.64	0.2281
2nd week	12.04	12.14	12.12	12.10	0.0529	12.04	12.14	12.12	12.10	0.0529	12.54	12.75	12.38	12.56	0.1856
3rd week	11.39	11.22	11.38	11.33	0.0954	11.39	11.22	11.30	11.30	0.0850	11.60	11.50	11.60	11.57	0.0577
4th week	11.20	11.30	11.16	11.22	0.0721	11.20	11.30	11.16	11.22	0.0721	11.39	11.49	11.45	11.44	0.0503

Table C.8 Cont.

5th week	10.31	10.13	10.31	10.25	0.1039	10.31	10.13	10.31	10.25	0.1039	9.79	9.50	9.95	9.75	0.2281
6th week	9.69	9.48	9.74	9.64	0.1380	9.69	9.48	9.74	9.64	0.1380	9.56	9.37	9.51	9.48	0.0985
7th week	9.25	9.44	9.30	9.33	0.0985	9.25	9.44	9.30	9.33	0.0985	9.51	9.64	9.60	9.58	0.0666
8th week	8.78	8.77	8.71	8.75	0.0379	8.78	8.77	8.71	8.75	0.0379	8.49	8.44	8.78	8.57	0.1836

C.9 Triplicate measurement of colour coordinate a* for 'sambal pijat' in air tight container at three different temperature.

Packaging	Sambal pijat-Air tight container														
Temperature	5°C					8°C					26°C				
Readings/ a*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	13.03	13.56	13.34	13.31	0.2663	13.03	13.56	13.34	13.31	0.2663	13.03	13.56	13.34	13.31	0.2663
1st week	14.30	14.20	14.43	14.31	0.1153	13.57	13.99	14.05	13.87	0.2615	11.15	11.29	11.29	11.24	0.0808
2nd week	13.37	13.48	13.64	13.50	0.1358	13.49	13.60	13.54	13.54	0.0551	11.00	10.98	11.03	11.00	0.0252
3rd week	13.40	13.45	13.47	13.44	0.0361	13.63	13.26	13.05	13.31	0.2937	10.16	10.15	10.14	10.15	0.0100
4th week	12.52	12.86	12.63	12.67	0.1735	12.45	12.47	12.49	12.47	0.0200	9.11	9.19	9.19	9.16	0.0462
5th week	11.00	11.13	11.18	11.10	0.0929	10.10	10.15	10.26	10.17	0.0819	8.82	8.78	8.36	8.65	0.2548
6th week	9.25	9.39	9.59	9.41	0.1709	9.46	9.45	9.77	9.56	0.1819	8.43	8.27	8.43	8.38	0.0924
7th week	8.84	8.73	9.03	8.87	0.1518	9.63	9.47	9.42	9.51	0.1097	6.68	6.24	6.03	6.32	0.3317
8th week	8.44	8.67	8.69	8.60	0.1389	8.05	8.27	8.88	8.40	0.4300	5.54	5.42	5.31	5.42	0.1150

C.10 Triplicate measurement of colour coordinate b* for 'sambal pijat' in vacuum packaging at three different temperature.

Packaging	Sambal pijat- Vacuum packaging														
Temperature	5°C					8°C					26°C				
Readings/ b*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	30.03	30.08	30.01	30.04	0.0361	30.03	30.08	30.01	30.04	0.0361	30.03	30.08	30.01	30.04	0.0361
1st week	26.47	27.19	26.26	26.64	0.4877	29.95	26.22	26.89	27.69	1.9885	23.64	23.65	23.65	23.65	0.0058
2nd week	26.65	26.36	25.93	26.31	0.3623	28.97	28.80	28.87	28.88	0.0854	23.43	22.98	23.82	23.41	0.4204
3rd week	26.36	25.75	25.24	25.78	0.5607	22.48	22.52	22.44	22.48	0.0400	19.59	19.60	19.71	19.63	0.0666

4th week	15.76	15.66	15.66	15.69	0.0577	17.05	17.08	17.00	17.04	0.0404	15.10	15.14	15.23	15.16	0.0666
5th week	14.15	14.26	14.04	14.15	0.1100	13.97	13.70	13.82	13.83	0.1353	13.07	13.30	13.23	13.20	0.1179
6th week	14.01	14.06	13.49	13.85	0.3156	13.71	13.65	13.56	13.64	0.0755	12.74	12.87	12.89	12.83	0.0814
7th week	13.70	13.44	13.63	13.59	0.1345	12.86	12.72	13.85	13.14	0.6160	12.40	12.48	12.66	12.51	0.1332
8th week	13.27	13.75	13.40	13.47	0.2483	12.43	12.83	12.85	12.70	0.2369	11.50	11.53	11.29	11.44	0.1308

C.11 Triplicate measurement of colour coordinate b* for ‘sambal pijat’ in aluminium foil pouch at three different temperature.

Packaging	Sambal pijat- Aluminium foil pouch														
Temperature	5°C					8°C					26°C				
Readings/ b*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	30.03	30.08	30.01	30.04	0.0361	30.03	30.08	30.01	30.04	0.0361	30.03	30.08	30.01	30.04	0.0361
1st week	24.96	24.86	24.98	24.93	0.0643	24.52	24.94	25.09	24.85	0.2955	22.62	22.22	22.49	22.44	0.2040
2nd week	22.61	22.04	22.42	22.36	0.2902	23.89	24.71	24.02	24.21	0.4407	20.55	20.56	20.34	20.48	0.1242
3rd week	21.82	22.01	22.71	22.18	0.4687	23.62	23.22	23.49	23.44	0.2040	19.60	19.50	19.60	19.57	0.0577
4th week	16.75	17.04	16.71	16.83	0.1801	17.20	17.16	17.31	17.22	0.0777	18.53	18.18	18.55	18.42	0.2081
5th week	14.38	14.25	14.32	14.32	0.0651	14.93	14.94	14.96	14.94	0.0153	12.10	12.18	12.05	12.11	0.0656
6th week	14.08	14.46	14.54	14.36	0.2458	14.65	14.51	14.52	14.56	0.0781	10.48	10.41	10.55	10.48	0.0700
7th week	13.86	13.72	13.78	13.79	0.0702	13.45	13.44	13.43	13.44	0.0100	9.99	9.38	9.63	9.67	0.3066
8th week	13.33	13.63	13.37	13.44	0.1629	12.50	12.12	12.39	12.34	0.1955	8.94	8.94	8.95	8.94	0.0058

C.12 Triplicate measurement of colour coordinate b* for ‘sambal pijat’ in air tight container at three different temperature.

Packaging	Sambal pijat- Air tight container														
Temperature	5°C					8°C					26°C				
Readings/ b*	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	30.03	30.08	30.01	30.04	0.0361	30.03	30.08	30.01	30.04	0.0361	30.03	30.08	30.01	30.04	0.0361
1st week	25.93	25.30	25.36	25.53	0.3477	25.61	26.66	26.52	26.26	0.5701	24.15	24.29	24.29	24.24	0.0808
2nd week	25.71	25.94	25.54	25.73	0.2007	25.67	25.64	25.88	25.73	0.1308	20.00	20.98	20.03	20.34	0.5573
3rd week	24.65	24.80	25.52	24.99	0.4651	22.67	22.55	22.53	22.58	0.0757	19.16	19.15	19.14	19.15	0.0100

4th week	17.36	17.18	17.18	17.24	0.1039	19.40	19.47	19.49	19.45	0.0473	16.11	16.19	16.19	16.16	0.0462
5th week	14.24	14.24	14.17	14.22	0.0404	14.29	13.58	14.25	14.04	0.3989	13.82	13.78	13.36	13.65	0.2548
6th week	13.82	13.93	13.54	13.76	0.2011	14.49	14.14	14.31	14.31	0.1750	13.44	13.00	13.47	13.30	0.2631
7th week	13.30	13.64	13.71	13.55	0.2193	14.85	14.76	14.68	14.76	0.0850	9.85	9.73	9.54	9.71	0.1563
8th week	13.22	13.72	13.21	13.38	0.2916	13.63	13.09	13.66	13.46	0.3208	9.48	9.76	9.89	9.71	0.2095

C.13 Triplicate measurement of moisture content (%) for control at three different temperatures.

Moisture content(%)															
Control															
Temperature	5°C					8°C					26°C				
Readings	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	76.3300	75.6700	77.3300	76.4433	0.8358	76.3300	75.6700	77.3300	76.4433	0.8358	76.3300	75.6700	77.3300	76.4433	0.8358
1st week	76.7700	75.5700	76.4700	76.2700	0.6245	73.0500	74.1100	74.6200	73.9267	0.8009	76.2000	76.6300	76.5810	76.4703	0.2354
2nd week	75.2500	75.0800	74.4200	74.9167	0.4384	72.6400	72.7900	73.7200	73.0500	0.5851	75.4400	75.0000	75.6700	75.3700	0.3404
3rd week	75.4400	72.3700	73.1600	73.6567	1.5941	72.1500	74.4200	72.2800	72.9500	1.2747	74.6700	74.3300	73.6700	74.2233	0.5085
4th week	76.1100	71.4800	73.1600	73.5833	2.3439	73.0900	72.7900	72.7900	72.8900	0.1732	75.6900	73.2600	72.0000	73.6500	1.8757
5th week	70.1000	71.7600	72.8800	71.5800	1.3987	72.7300	71.7500	71.9000	72.1267	0.5279	72.2300	74.5800	72.2000	73.0033	1.3655
6th week	71.0200	71.8600	71.8500	71.5767	0.4821	72.1700	70.4150	71.2890	71.2913	0.8775	73.8500	72.0000	73.0800	72.9767	0.9293
7th week	70.3900	72.0800	70.7900	71.0867	0.8832	69.8700	71.9000	71.1500	70.9733	1.0265	73.2600	72.2900	71.8500	72.4667	0.7214
8th week	71.3200	71.0900	70.1300	70.8467	0.6312	69.8300	70.8500	69.2300	69.9700	0.8190	72.3480	72.2100	72.2440	72.2673	0.0719

C.14 Triplicate measurement of moisture content (%) for ‘sambal pijat’ in vacuum packaging at three different temperatures.

Packaging	Sambal pijat- Vacuum packaging														
Temperature	5°C					8°C					26°C				
Readings/ Moisture	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	66.6700	67.0000	69.0000	67.5567	1.2608	66.6700	67.0000	69.0000	67.5567	1.2608	66.6700	67.0000	69.0000	67.5567	1.2608
1st week	67.7500	68.9800	67.0000	67.9100	0.9996	67.5100	67.7100	67.5400	67.5867	0.1079	69.5100	69.4100	66.3400	68.4200	1.8020
2nd week	67.7500	68.0800	68.3000	68.0433	0.2768	67.3300	68.0900	67.6100	67.6767	0.3844	68.9800	68.9300	69.0000	68.9700	0.0361
3rd week	67.7600	68.2000	69.0800	68.3467	0.6721	68.0800	68.0100	68.5100	68.2000	0.2707	69.0800	69.0000	69.3600	69.1467	0.1890
4th week	68.4200	69.3100	67.3100	68.3467	1.0020	66.6700	68.7700	69.8400	68.4267	1.6126	68.7700	70.1000	69.9300	69.6000	0.7238
5th week	67.7600	68.6500	69.0000	68.4700	0.6393	68.4700	68.7600	68.0500	68.4267	0.3570	69.6100	69.6400	69.7400	69.6633	0.0681
6th week	69.6200	68.3300	68.0100	68.6533	0.8523	67.1890	68.8420	69.5280	68.5197	1.2024	69.3100	69.9600	69.7300	69.6667	0.3296
7th week	68.7020	69.0120	68.8050	68.8397	0.1579	68.7700	69.0800	68.4600	68.7700	0.3100	69.6950	69.4230	70.1000	69.7393	0.3407
8th week	69.2500	69.1800	68.7500	69.0600	0.2707	69.6100	68.2700	69.4100	69.0967	0.7229	69.7700	69.5800	71.0100	70.1200	0.7766

C.15 Triplicate measurement of moisture content (%) for ‘sambal pijat’ in aluminium foil pouch at three different temperatures.

Packaging	Sambal pijat- Aluminium foil pouch														
Temperature	5°C					8°C					26°C				
Readings/ Moisture	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	66.6700	67.0000	69.0000	67.5567	1.2608	66.6700	67.0000	69.0000	67.5567	1.2608	66.6700	67.0000	69.0000	67.5567	1.2608
1st week	68.5200	68.1100	67.4300	68.0200	0.5505	67.8800	67.0000	67.2200	67.3667	0.4580	67.4000	67.9600	68.3600	67.9067	0.4822
2nd week	68.9300	69.4600	68.4900	68.9600	0.4857	68.3000	68.4200	66.8900	67.8700	0.8508	69.0000	69.8000	69.6500	69.4833	0.4252
3rd week	68.2400	69.4400	69.8100	69.1633	0.8208	68.1610	68.5640	67.7270	68.1507	0.4186	69.6400	70.6300	71.3400	70.5367	0.8538
4th week	68.5200	68.0000	69.4100	68.6433	0.7130	67.7500	67.7300	69.1800	68.2200	0.8314	71.0700	72.6900	68.1800	70.6467	2.2846
5th week	69.5400	69.7700	68.0100	69.1067	0.9567	68.8100	68.1300	68.7100	68.5500	0.3672	70.3300	71.1500	70.9800	70.8200	0.4328
6th week	68.8700	68.7500	68.4200	68.6800	0.2330	68.2100	68.6500	69.1800	68.6800	0.4857	71.4500	71.5500	71.5600	71.5200	0.0608
7th week	67.9900	68.5600	67.9600	68.1700	0.3381	69.6100	68.2700	69.4100	69.0967	0.7229	71.7600	71.8760	71.8650	71.8337	0.0640
8th week	70.6320	68.3400	68.8360	69.2693	1.2059	68.6400	69.4300	69.3600	69.1433	0.4373	71.9870	71.9760	71.9890	71.9840	0.0070

C.16 Triplicate measurement of moisture content (%) for ‘sambal pijat’ in air tight container at three different temperatures.

Packaging	Sambal pijat- Air tight container														
Temperature	5°C					8°C					26°C				
Readings/ Moisture	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	66.6700	67.0000	69.0000	67.5567	1.2608	66.6700	67.0000	69.0000	67.5567	1.2608	66.6700	67.0000	69.0000	67.5567	1.2608
1st week	68.4200	68.1000	68.9800	68.5000	0.4454	67.5500	67.7600	67.4400	67.5833	0.1626	67.2100	68.5200	67.4300	67.7200	0.7015
2nd week	67.7300	69.6800	68.7200	68.7100	0.9750	67.2450	67.8750	68.7460	67.9553	0.7537	68.7300	69.2800	67.8300	68.6133	0.7320
3rd week	69.0660	69.5756	67.6500	68.7639	0.9977	68.2100	68.8700	67.4200	68.1667	0.7260	69.0200	69.6500	69.7400	69.4700	0.3923
4th week	68.2000	68.2000	70.4900	68.9633	1.3221	67.7500	67.7300	69.1800	68.2200	0.8314	70.4900	70.2300	69.4800	70.0667	0.5244
5th week	68.4200	69.6400	68.8700	68.9767	0.6170	66.1000	69.6400	69.0800	68.2733	1.9029	70.1540	70.2560	70.1890	70.1997	0.0518
6th week	69.7100	68.6300	68.6500	68.9967	0.6178	68.3500	68.7700	68.6500	68.5900	0.2163	70.7890	70.9860	70.8790	70.8847	0.0986
7th week	69.0600	69.7000	69.6400	69.4667	0.3535	68.5400	70.2000	67.6600	68.8000	1.2898	71.0420	71.2350	71.0120	71.0963	0.1210
8th week	69.4800	70.2700	69.0800	69.6100	0.4944	67.5700	69.2100	69.6500	68.8100	1.0962	71.2340	71.3420	71.3450	71.3070	0.0632

C.17 Triplicate measurement of pH value for control at three different temperatures.

pH value															
Control															
Temperature	5°C					8°C					26°C				
Readings/ pH	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	4.34	4.34	4.35	4.34	0.0058										
1st week	4.53	4.55	4.55	4.54	0.0115	4.32	4.32	4.31	4.32	0.0058	4.39	4.38	4.39	4.3867	0.0058
2nd week	4.57	4.57	4.58	4.57	0.0058	4.45	4.47	4.46	4.46	0.0100	4.54	4.53	4.5	4.5233	0.0208
3rd week	4.64	4.65	4.65	4.65	0.0058	4.48	4.48	4.47	4.48	0.0058	4.61	4.62	4.60	4.6100	0.0100
4th week	4.68	4.71	4.70	4.70	0.0153	4.55	4.54	4.55	4.55	0.0058	4.63	4.62	4.61	4.6200	0.0100
5th week	4.71	4.71	4.70	4.71	0.0058	4.73	4.72	4.72	4.72	0.0058	4.63	4.63	4.64	4.6333	0.0058
6th week	4.74	4.75	4.74	4.74	0.0058	4.73	4.74	4.75	4.74	0.0100	4.66	4.65	4.65	4.6533	0.0058
7th week	4.86	4.88	4.89	4.88	0.0153	4.80	4.82	4.81	4.81	0.0100	4.72	4.71	4.71	4.7133	0.0058
8th week	5.05	5.03	5.05	5.04	0.0115	4.88	4.88	4.87	4.88	0.0058	4.97	4.98	4.99	4.9800	0.0100

C.18 Triplicate measurement of pH value for ‘sambal pijat in vacuum packaging at three different temperatures.

Packaging	Sambal pijat- Vacuum packaging														
Temperature	5°C					8°C					26°C				
Readings/ pH	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	3.96	3.95	3.95	3.95	0.0058	3.96	3.95	3.95	3.95	0.0058	3.96	3.95	3.95	3.95	0.0058
1st week	4.03	4.05	4.04	4.04	0.0100	4.06	4.04	4.03	4.04	0.0153	4.03	4.05	4.04	4.04	0.0100
2nd week	4.05	4.04	4.05	4.05	0.0058	4.07	4.09	4.08	4.08	0.0100	4.05	4.04	4.05	4.05	0.0058
3rd week	4.07	4.06	4.05	4.06	0.0100	4.09	4.08	4.07	4.08	0.0100	4.07	4.06	4.05	4.06	0.0100
4th week	4.15	4.15	4.14	4.15	0.0058	4.14	4.13	4.14	4.14	0.0058	4.15	4.15	4.14	4.15	0.0058
5th week	4.20	4.19	4.2	4.20	0.0058	4.18	4.17	4.19	4.18	0.0100	4.20	4.19	4.2	4.20	0.0058
6th week	4.22	4.22	4.23	4.22	0.0058	4.23	4.24	4.24	4.24	0.0058	4.22	4.22	4.23	4.22	0.0058
7th week	4.23	4.24	4.22	4.23	0.0100	4.25	4.26	4.24	4.25	0.0100	4.23	4.24	4.22	4.23	0.0100
8th week	4.26	4.25	4.25	4.25	0.0058	4.28	4.29	4.27	4.28	0.0100	4.26	4.25	4.25	4.25	0.0058

C.19 Triplicate measurement of pH value for ‘sambal pijat in aluminium foil pouch at three different temperature

Packaging	Sambal pijat- Aluminium foil pouch														
Temperature	5°C					8°C					26°C				
Readings/ pH	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	3.96	3.95	3.95	3.95	0.0058	3.96	3.95	3.95	3.95	0.0058	3.96	3.95	3.95	3.95	0.0058
1st week	4.04	4.04	4.02	4.03	0.0115	4.01	4.01	3.98	4.00	0.0173	4.41	4.44	4.45	4.43	0.0208
2nd week	4.07	4.08	4.08	4.08	0.0058	4.07	4.07	4.06	4.07	0.0058	5.05	5.05	5.06	5.05	0.0058
3rd week	4.09	4.11	4.1	4.10	0.0100	4.09	4.11	4.11	4.10	0.0115	5.56	5.55	5.54	5.55	0.0100
4th week	4.12	4.11	4.1	4.11	0.0100	4.15	4.14	4.15	4.15	0.0058	5.58	5.57	5.59	5.58	0.0100
5th week	4.14	4.14	4.15	4.14	0.0058	4.17	4.18	4.16	4.17	0.0100	5.62	5.61	5.6	5.61	0.0100
6th week	4.17	4.18	4.19	4.18	0.0100	4.20	4.22	4.21	4.21	0.0100	5.65	5.66	5.67	5.66	0.0100
7th week	4.21	4.2	4.20	4.20	0.0058	4.23	4.24	4.25	4.24	0.0100	5.71	5.70	5.72	5.71	0.0100
8th week	4.34	4.34	4.33	4.34	0.0058	4.31	4.32	4.33	4.32	0.0100	5.74	5.75	5.76	5.75	0.0100

C.20 Triplicate measurement of pH value for ‘sambal pijat in air tight container at three different temperatures.

Packaging	Sambal pijat- Air tight container														
Temperature	5°C					8°C					26°C				
Readings/ pH	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD	Reading 1	Reading 2	Reading 3	Average	SD
0 week	3.96	3.95	3.95	3.95	0.0058	3.96	3.95	3.95	3.95	0.0058	3.96	3.95	3.95	3.95	0.0058
1st week	4.06	4.04	4.05	4.05	0.0100	4.03	4.05	4.04	4.04	0.0100	4.21	4.22	4.2	4.21	0.0100
2nd week	4.09	4.08	4.08	4.08	0.0058	4.07	4.06	4.06	4.06	0.0058	5.10	5.11	5.10	5.10	0.0058
3rd week	4.12	4.11	4.13	4.12	0.0100	4.13	4.12	4.13	4.13	0.0058	5.21	5.20	5.21	5.21	0.0058
4th week	4.15	4.16	4.17	4.16	0.0100	4.17	4.16	4.17	4.17	0.0058	5.83	5.80	5.80	5.81	0.0173
5th week	4.18	4.19	4.20	4.19	0.0100	4.19	4.19	4.20	4.19	0.0058	5.94	5.95	5.96	5.95	0.0100
6th week	4.22	4.21	4.22	4.22	0.0058	4.22	4.23	4.22	4.22	0.0058	6.10	6.11	6.11	6.11	0.0058
7th week	4.24	4.23	4.24	4.24	0.0058	4.26	4.27	4.25	4.26	0.0100	6.17	6.16	6.18	6.17	0.0100
8th week	4.28	4.29	4.30	4.29	0.0100	4.30	4.32	4.32	4.31	0.0115	6.34	6.33	6.32	6.33	0.0100