SHELF LIFE STUDY OF HOMEMADE NOODLES INCORPORATED WITH BELL PEPPER (*Capsicum* sp.)

By

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ABSTRACT

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Noodles serve as staple food in Asia and is gaining popularity worldwide due to its convenience and low cost. Therefore, noodle production increases for past few years to fulfill consumers' demand. In this study, red bell pepper (*capsicum annum* L.) was incorporated into homemade noodles to study its shelf life. Different concentrations of red bell pepper (0, 5, 10 and 20 %) were incorporated into noodles. For microbiological determination, total plate count (TPC) and yeast and mold test (YM) were performed. TPC showed that all samples including control started to have bacterial growth at day 4 with the highest log cfu/g (>4.06). For YM, noodles with 20 % bell pepper had highest count (7.04 log cfu/g) among all samples at day 8. Water activity of all noodles showed average water activity of 0.978 ± 0.00, which were not significantly different from each other ($p>0.05$). Proximate analysis indicated that ash and fiber content were significantly different ($p<0.05$) among samples but not significantly different in moisture, protein and fat content ($p>0.05$). Total phenolic test revealed that noodles with 20 % bell pepper had highest phenolic content (0.36 ± 0.05 mg/g), having significant difference among samples

(p<0.05). Out of five sensory attributes (appearance, aroma, taste, texture and overall acceptance), only taste attribute has significant difference among all samples including control (p<0.05) where noodles with 10 % of bell pepper had highest mean score value (53.33 ± 5.32). In conclusion, red bell pepper is less effective in prolonging shelf life of noodles although noodles with 20 % bell pepper has the highest phenolic content, this may be due to the loss of capsaicin of bell pepper during preparation, reducing the initially low capsaicin content in bell pepper to a negligible level and further caused the low efficiency of microbial inhibitory effect of capsaicin.

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DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

_____

TAN YEE QING

APPROVAL SHEET

This project report entitled **"SHELF LIFE STUDY OF HOMEMADE NOODLES INCORPORATED WITH BELL PEPPER (*Capsicum* sp.)"** was prepared by TAN YEE QING and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **TAN YEE QING** (ID No: **13ADB04005**) has completed this final year project entitled "**SHELF LIFE STUDY OF HOMEMADE NOODLES INCORPORATED WITH BELL PEPPER (*Capsicum* sp.)**" under the supervision of Dr. Tan Yen Nee from the Department of Agricultural and Food Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in PDF format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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TAN YEE QING

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
A_w	Water activity
Cfu/g	Colony forming unit per gram
$CuSO_4$	Copper (II) sulfate
FC	Folin-Ciocalteau
GA	Gallic acid
HCl	Hydrochloric acid
H_2SO_4	Sulfuric acid
N	Normality
NaOH	Sodium hydroxide
K_2SO_4	Potassium sulfate
RDA	Recommended dietary allowance
ROS	Reactive oxygen species
SPSS	Statistical package for the social sciences
TPC	Total plate count
YM	Yeast and Mold

CHAPTER 1

INTRODUCTION

Noodles are categorized under cereal foods where most commonly, they are made from wheat. The basic raw materials to prepare noodles are flour, salt and water. The nutritional value of noodles mainly come from flour as flour is the main raw material in producing noodles where noodles may contain protein, carbohydrates and trace amount of fat and mineral. Therefore, noodles can be consumed as a complete meal (Ojure and Quadri, 2012; Zhang and Ma, 2016). However, Ojure and Quadri (2012) stated that there are little study and evidence on the nutrient content of noodles and the process, wheat flour refinement that is carried out during production of noodles may cause the lost in some of the nutrients such as B vitamins, certain minerals and dietary fiber.

Cereal foods can be considered as the most economical energy food because they are served as the main energy contributor to human. Therefore, noodles are widely recognized as staple food for many Asian countries (Zhang and Ma, 2016). Notably, China shows the most significant consumption rate of noodles because as suggested by the study of Zhang and Ma (2016), China had sold a total of 8.6 billion yuan to 20.26 billion yuan of noodles, showing amazing sales, consumption and production rate. In other word, China is the largest consumer of noodles as of 2016 (Zhang and Ma, 2016). Nevertheless, noodles is gaining popularity and acceptability worldwide especially in Western countries, such as Australia due to their convenience

(versatility – can be applied with different cooking methods and fast cooking characteristics; simplicity – simple and little time needed for preparation), organoleptic appeal (palatability), low cost and satiety (Bui & Small, 2007; Ojure and Quadri, 2012; Omeire, Umeji and Obasi, 2014).

Fresh noodles are easily perishable due to high water content and nutrient compounds, causing it to be susceptible to microorganism growth and enzymatic activities. To prolong shelf life and maintain freshness of noodles, the most commonly used chemical preservatives are calcium propionate and potassium sorbate (Li et al., 2012). It is not advisable to consume too much preservative-containing food products because adverse effect may be arise. In the case of potassium sorbate that is used to inhibit microbial growth in noodles, there were reports stating that contact dermatitis and urticaria can be triggered by sorbates (Sharma, 2015).

Nowadays, the public are becoming more and more health conscious as a trend of "green" consumerism can be identified. They would prefer natural "green" foods (foods that have less synthetic food products included) and concern about the health consequences that may be caused by food additives that are commonly used currently. Therefore, in order to extend the shelf life and in the same time maintaining freshness of perishable foods, natural preservatives are more preferable over synthetic preservatives due to consumer behavior nowadays where consumers desire safe and natural products of late (Del Nobile et al., 2009). Apart from that,

the natural food compound incorporated should exhibit antimicrobial properties in order to inhibit microbial growth to prevent food spoilage. Synthetic compounds may act as a more effective barrier against food spoilage but they do not specifically attack the targeted microorganisms but also exhibit effects on non-target. This may harm human body because synthetic compounds may kill some beneficial microorganisms found in the gut (probiotics). Therefore, natural food compounds with antimicrobial action are more preferable due to their biodegradable properties and they would not cause toxic and allergic reactions (Ksibi et al., 2015).

As the name suggests, *capsicum* sp. belongs to the genus *capsicum* and is a member of Solanaceae or Nightshade family. Vegetables that are categorized under this genus are bell peppers, chilies, tomatoes, eggplants and so on. The botanical name for bell pepper is *Capsicum annuum* L. *Capsicum annuum* L. is available in many color, ranging from green, yellow, orange and red. Bell peppers are rich in vitamin A, C and antioxidant vitamin E. Furthermore, they also contain high concentration of antioxidants, such as flavonoids (luteolin, quercetin and hesperidin), carotenoids (alpha- and beta-carotene, lutein and zeaxanthin) and capsaicinoids (Chávez-Mendoza et al., 2015). Capsaicin enable *capsicum* sp. to preclude bacterial growth, such as *Escherichia coli* as it can act as antibacterial agent. This property leads to the possibility of *capsicum* sp. to prolong the shelf life of food products if incorporated into a respective product (Othman et al., 2011).

As mentioned earlier, people nowadays prefer natural preservative as they are more aware about their health. Bell pepper (*Capsicum annum* L.) as a vegetable may act as natural preservative by inhibiting microbial activity due to its antibacterial properties (due to capsaicin). Hence, bell pepper was incorporated into homemade noodles to determine whether or not it is effective in prolonging the shelf life of noodles. Thus, the objectives of this project were:

1. To prepare newly formulated homemade noodles incorporated with bell pepper, then further examine and evaluate shelf life of noodles using microbial and water activity tests.
2. To determine the nutritional value of homemade noodles incorporated with different concentration (0, 5, 10 & 20 %) of bell peppers by proximate analysis.
3. To determine antioxidant activities of homemade noodles incorporated with bell peppers at different concentrations by total phenolic test.
4. To determine acceptance level of consumers towards homemade noodle incorporated with bell peppers at different concentrations by carrying out sensory evaluation.

CHAPTER 2

LITERATURE REVIEW

2.1 Bell Pepper (*Capsicum annum* L.)

2.1.1 Sensory Attributes

Bell pepper (*Capsicum annum* L.) is a type of *capsicum* sp. as it is included in the *capsicum* genus and is a member of the Solanaceae family. The climate of North and South America is often dry and warm, providing a favorable condition for bell pepper to grow (Othman et al., 2011). Consumer acceptance towards bell peppers is high as it is one of the most common vegetable found in the culinary world (eg. Christmas ornaments) due to its attractive characteristics sensory properties (color, texture and flavor), especially its intense color and strong flavor as well as highly nutritious in the same time (Gomez et al., 2014). Bell pepper is famous for its variation in many color, ranging from green, yellow, orange to red, depending on its ripeness (Nadeem et al., 2011). Most commonly, green bell peppers serve as the unripen/immature form while red bell peppers are the mature form of peppers. However, some green bell peppers are also mature and ripen, depending on the location on where they are breed. While bell peppers undergo maturation process, chlorophyll (pigments that is responsible for the green color of green bell peppers) started to degrade, and in the same time synthesizing capsanthin (3,3'dihydroxy-β,κ-carotene-6'-one) and capsorubin (3,3'dihydroxy-κ,κ-carotene-6,6'dione) that caused the red color of the red bell pepper, changing color of bell peppers from green to red (Gomes et al., 2014). In some rare cases, brown and white bell peppers

can also be harvested. Other than having attractive appearance due to its distinctive color, bell pepper is well known for its characteristics bell shape where standard bell pepper features either 3 or 4 lobes with crunchy and thick fleshy texture, differing it from other *capsicum* sp. (Chávez-Mendoza et al., 2015). Flavor wise, the pungency of *capsicum* sp. is contributed by capsaicinoids, which act as one of its characteristic attribute (Othman et al., 2011). However, according to a research by Soetarno S. (1997), bell pepper is not as pungent and spicy as chili pepper, so it is also known as sweet pepper.

2.1.2 Health Benefits

Bell pepper is well known for its high nutritional value as it contains high concentration in phytonutrients and also exhibit antioxidant activities. Phytonutrients, such as vitamin C (ascorbic acid), vitamin A, vitamin B6, β-carotene, phenolic compounds (natural antioxidants), minerals (eg: folate, manganese, molybdenum, potassium, thiamine, zinc, copper, chromium, cadmium, iron, lead and cobalt), folic acid and other biotic compounds can be found in bell peppers (Kothari et al., 2010; Bello, Boboye and Akinyosoye, 2015). A remarkably high content of ascorbic acid at which by consuming 100 g serving of fresh bell pepper, this can fulfill the criteria of RDA (60 mg per day) for daily intake of ascorbic acid (Nadeem et al., 2011). In the case of red bell pepper, its lycopene content is higher than green and yellow pepper which is useful in preventing cancer (Nadeem et al., 2011). Bell peppers exhibit therapeutic activity due to presence of capsaicin (a type of active ingredient in *capsicum* sp.) and their inherent

nutraceutical compounds, enabling them to be used as herbal medicine, not only in Africa but also other countries as they can successfully prevent, diagnose or treat certain illness (Chávez-Mendoza et al., 2015). According to Othman et al. (2011), Gomez et al. (2014) and Bello, Boboye and Akinyosoye (2015), examples of medicinal functions of bell peppers due to their considerable amount of capsaicin, phenolic compounds (carotenoids such as lutein) and minerals are:

1. Anti-carcinogenic as it can fight against cancer (eg: progress of adult T-cell of leukemia cells can be inhibited by capsaicin),
2. Hinder posterior sub-capsular cataracts due to its high carotenoids content,
3. Effective against neurogenic inflammation,
4. Prevent ulcer at intestinal lining by triggering the release of mucus due to presence of minerals,
5. Useful against arthritis pain, inflammation and some peripheral painful states (for instance, rheumatoid arthritis) due to its analgesic property,
6. Able to reduce high cholesterol level and avoid obesity and
7. Exhibit beneficial physiologically and psychologically effect on gastrointestinal tract, respiratory, cardiovascular, thermoregulation and sensory system.

Chávez-Mendoza et al. (2015) suggested that there were epidemiological studies proved that the decline in number of death cases caused by cancer, heart diseases and degenerative disorders (for instance, aging and macular degeneration in women) is directly proportional to the amount of consumption of fruits and vegetables. Therefore, it is believed that we can be healthier and stay away from diseases by

consuming more fruits and vegetables, such as bell pepper with such nutritious property and its ability to defend our body from diseases. Incorporation of the nourishing nutraceutical compounds from bell pepper into functional foods is encouraged to increase their bioavailability in functional foods than from their natural sources (Gomes et al., 2014). Further studies can be carried out to determine whether or not bioavailability of bell pepper can be increase if incorporated into non-functional foods.

2.1.3 Antimicrobial Properties

2.1.3.1 Capsaicin

Capsaicinoids are a group of bioactive compound that accounts for the pungency attribute of the members in genus *capsicum* where the pungency trait is regulated by *Pun1* gene. They belong to the alkaloid group as they contain at least one nitrogen atom. There are many different member in capsaicinoids group, the most well-known will be capsaicin. The structure that different types of capsaicinoids share in common is that they contain vanillylamide in branched fatty acid with 9 to 11 carbons while the difference is the number of double bond found at the fatty acid side chain (Cisneros-Pineda et al., 2007). The synthesis site of capsaicinoids in *capsicum* sp. is at the placenta. Therefore, capsaicinoids are most abundantly found at placenta, the whitish inner part with attached seeds (Peña-Alvarez, Ramírez-Maya and Alvarado-Suárez, 2009). Other than placenta, seeds and pericarp also contain capsaicinoids as after capsaicinoids are finished synthesizing, they are packaged into vacuoles by epidermal specialized cells and slowly distributed to

seeds and internal pericarp surface (Cisneros-Pineda et al., 2007). As mentioned earlier, pungency of the *capsicum* sp. are mainly caused by capsaicin (8-methyl-*N*-vanillyl-trans-6-nonenamide) where capsaicin is the major constituent (71 %) in capsaicinoids, followed by dihydrocapsaicin at which capsaicin and dihydrocapsaicin are the most abundantly found (90 %) capsaicinoids as compared to others (Othman et al., 2011). The difference between capsaicin and dihydrocapsaicin is the number of double bond found at the fatty acid side chain at the 9th carbon (Cisneros-Pineda et al., 2007). Figure 2.1 showed the structure of capsaicin:

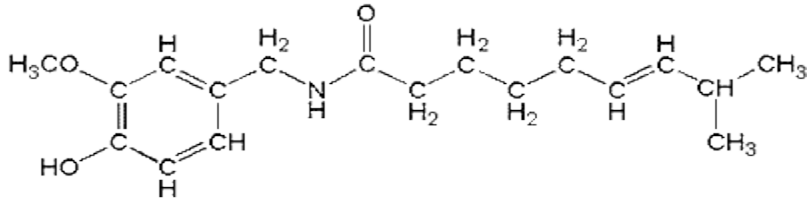

Figure 2.1: Structure of capsaicin

2.1.3.2 Antimicrobial Activities of Capsaicin

As mentioned in 2.1.2, despite therapeutic activity, the presence of capsaicin enable *capsicum* sp. to act as antimicrobial agent by inhibiting the growth of microorganisms, including bacteria and fungi (Soetarno et al., 1997). Guil-Guerrero et al. (2016) and Bello, Boboye and Akinyosoye (2015) suggested that antimicrobial effect can be exhibited by capsaicin where capsaicin is able to retard the proliferation of *Escherichia coli* and *Psedomonas solanacearum* and in the same time, it can inhibit *Bacillus subtilis* growth. By referring to Othman et al. (2011), a total concentration of 2.5 mg/g of capsaicin can be found in red bell

pepper. Therefore, red bell pepper is expected to display antimicrobial property and if it is incorporated into food products, it should be able to prolong the shelf life as it can hinder microbial growth, leading to prevention of food spoilage.

2.1.3.3 Microbiological Risk and Assessment for Determination of Shelf Life of Noodle

Microbiological risk of noodles are considered as high due to its high moisture content and sufficient nutrients where these two factors provide favorable growing condition that "attract" the invasion of microorganisms. As stated by Akigbemidu, Musa and Kuforiji (2015), microbial quality is the acceptability of a food compound with a tolerable number of microorganisms in a food compound. In order to achieve the safety and stability of noodles, their microbial quality needs to be taken into consideration to ensure noodles are shielded from potential microbial contamination and spoilage (Akigbemidu, Musa and Kuforiji, 2015). In the case of noodles, by referring to a study by Akigbemidu, Musa and Kuforiji (2015), the possible bacteria genera that may grow on noodles were *Pseudomonas*, *Aeromonas*, *Bacillus*, *Streptococcus* and *Staphylococcus sp.* while fungi genera that could be isolated from spoiled noodles were *Candida, Aspergillus, Rhodotorula, Penicillium* and *Mucor*. In this project, microbial assessment were carried out to check whether incorporation of bell pepper was useful in extending shelf life of noodle by delaying or reducing microbial growth. In order to identify whether aerobic bacteria (for instance, Pseudomonas) is able to grow on noodle at mesophilic temperature (37 ℃), total plate count (TPC) or also known as standard plate count or aerobic plate

count was performed by using plate count agar (PCA) as the medium (Del nobile et al., 2009). Phattra and Maweang (2009) also suggested that acidified potato dextrose agar (PDA) could be used as agar medium to determine when yeast and mold start to grow on the noodles incorporated with bell pepper. Only number of colonies that fall in the range of 25 – 250 colonies are included in the calculation of cfu/mL.

2.1.4 Antioxidant Properties

Phenolic compounds are also known as antioxidants as they are the compounds that contribute to the antioxidative properties of a compounds. Due to inability of human body to synthesize them and they must be obtained through diet, they are essential to human body (Materska and Perucka, 2005). As the name suggests, antioxidants are meant to inhibit or delay the occurrence of oxidation by eliminating free radicals and ROS. This can be done by:

i. preventing their formation,
ii. scavenging any free radicals and ROS found and
iii. serving as their substrate.

This is because the stress caused by oxidation will result in free radicals and ROS to be formed, damaging the commodity with free radicals by inducing lipid peroxidation of body cells. Lipid peroxidation may be associated with the rancidity off-odor of fat products where the off odor is resulted by the volatile aldehydes, ketones, alcohols, furans, hydrocarbons or acids. These are the products of chemical reaction between fat molecules with oxygen (Sainsburry et al., 2016).

There are two types of antioxidants functional in human body. Fat soluble and water soluble antioxidants are required to adapt to the unique environment of human body. Hence, it is said to have two lines of antioxidant defense with the presence of fat and water soluble antioxidants. In order to prevent oxidation in cell cellular membrane which consists of hydrophobic phospholipids, fat soluble antioxidants, vitamin E (most potent, carries out chain breaking mechanism within cell phospholipid membrane), β-carotene and co-enzymes are vital. The second line of defense is constituted by water soluble antioxidants, vitamin C, catalase, glutathione peroxidase and superoxide dismutase inside the cell as they need to dissolve in the water-based cell interior to scavenge free radicals and ROS (Nadeem et al., 2011).

Antioxidant properties may also related to antimicrobial properties. For example, flavonoids, categorized under phenolic compounds with antioxidant properties are able to bind to cell wall of bacteria as well as soluble and extracellular proteins to form complexes (eg: tannins can form complex with wall proteins), and hence causing the death of the microorganism by destabilizing and breaking the microbial cell wall (Bello, Boboye and Akinyosoye, 2015).

Basically, in the food industry, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are the most widely used synthetic additives used to prevent oxidation in food products. However, natural antioxidants gain greater preference from consumers due to the carcinogenic properties of synthetic antioxidants.

Natural antioxidants are more preferable as effective health promoting agent as well (Medina-Juárez et al., 2012).

According to Medina-Juárez et al. (2012), phytonutrients need to be extensively studied as the composition and levels of phytonutrients with antioxidant properties found in plants does not correspond to the plant's total antioxidant capacity. This is because antioxidant capacity depends on type and concentration of phytonutrients, synergistic and the inhibitory interaction among molecules present in the compound (Medina-Juárez et al., 2012). Therefore, understanding on the antioxidant properties of a compound is essential for the food manufacturers so they can fully utilize the therapeutic and nutritional properties of dietary phenolic antioxidants in vegetal plants (phytonutrients) to develop functional foods (Conventional food with improved health benefits) (Materska and Perucka, 2005).

2.1.4.1 Mechanism of Action of Antioxidant

As the name suggests, antioxidants (mainly phenolic compounds) are able to prevent oxidation from occurring due to their tendency in neutralizing free radicals by rapidly donating their electron to a highly reactive free radicals to get paired. This is to ensure the initially unstable free radical with a lone electron is able to achieve its stable state after accepting an electron from antioxidant, leading to the pairing of radical's lone electron with the antioxidant electron, which neutralizes and stabilizes the free radical. A stabilized radical would not damage body cell as the chain reaction of oxidation has been disrupted. This prevents the induction of

diseases such as cancer and aging which is caused by the "snatching" of electron by radical from body cells to pair up with the respective radical's lone electron if no electron is provided by antioxidant, oxidizing the cells and triggering diseases like aging (Lobo et al., 2010; Nadeem et al., 2011). This mechanism of action is known as chain breaking mechanism (Lobo et al., 2010). Similarly in the case of foods, foods may be damaged by free radicals, especially high fat foods because oxidation may cause rancidity of high fat foods. Therefore, antioxidants incorporated in food products can disrupt the oxidation chain by chain breaking mechanism as well. The pathway of chain breaking mechanism is shown in Figure 2.2:

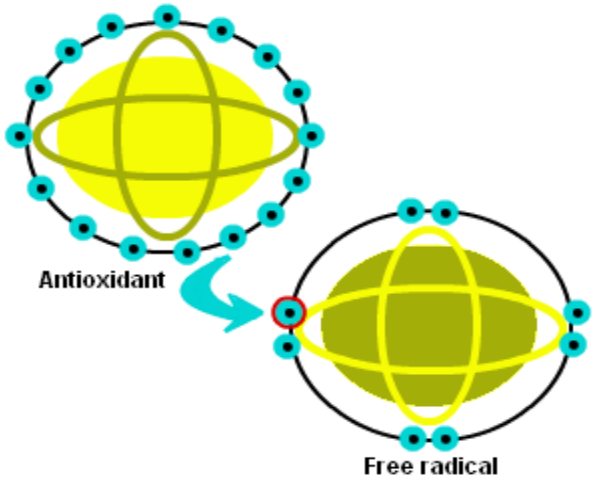

Figure 2.2: Mechanism of action of antioxidant

Another type of mechanism that can be carried out by antioxidant is by quenching chain initiating catalyst to remove ROS or other secondary antioxidants (reactive nitrogen species initiators). Other different patterns of actions of antioxidants such as by electron donating, metal ion chelating, establishing co-antioxidants and

regulating gene expression can be utilized by antioxidants to perform their antioxidant properties on biological systems (Lobo et al., 2010).

2.1.4.2 Phenolic Compounds in Bell Pepper and Their Functions

Every phenolic compound contains at least one aromatic ring with at least one hydroxyl (OH) group attached to it. To enhance better understanding of the phenolic structure, Figure 2.3 displayed the basic structure of phenolic compound:

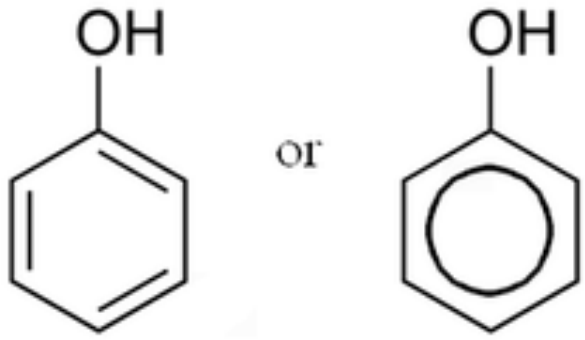

Figure 2.3: Basic structure of phenolic compound

Each phenolic compound in bell pepper plays their own role in preventing oxidative damage in human body. The function of the phenolic compounds were important in promoting human health, sometimes acting as a barrier against diseases, such as cancer and degenerative disorders. The phenolic compounds that present in bell pepper and their functions are presented in the table below (Chávez-Mendoza et al., 2015; Ksibi et al., 2015 and Lobo et al., 2010):

Table 2.1: Phenolic Compounds in Bell Pepper

Phenolic Compounds	Examples	Functions
Flavonoids	-Quercetin (Flavonol)	- Prevent oxidation and hence, significantly reduces oxidative damages to body cells.
	-Luteolin (Flavone)	- Reduces the risk of degenerative diseases, for instance cancer, cataracts, diabetes, cardiovascular diseases, Parkinson's and Alzheimer's disorders.
	-Hesperidin (Flavanone glycoside)	- Enhance optimal functioning of brain by preventing the oxidation of essential fats found in brain cells, preventing damages to brain cells, further causing brain disorder, for examples, Parkinson's and Alzheimer's disorders.
Carotenoid	-β-carotene	<u>Can act as anti-carcinogenic agent:</u> - Able to revise the liver metabolism effects of carcinogens.
		- Protect body against cancer due to its immuno-enhancement property.

Table 2.1 (continued): Phenolic Compounds in Bell Pepper

Phenolic Compounds	Examples	Functions
Carotenoid	-β-carotene	<u>Can act as anti-carcinogenic agent:</u> - Exhibit pro-vitamin A activity, hence it is useful in inhibiting ultraviolet light induced carcinogenesis.
		- Prevent genetic damage from products caused by oxidation.
Oxygenated Carotenoids	-Capsantine -Capsorubin -Cryptocapsin	- Effective in quenching and neutralizing free radicals.
	-Lutein (Xantophyll)	- Helps in the prevention of cataracts and macular degeneration.
Capsaicinoi-nds	-Capsaicin -Dihydrocapsaicin	- Antimicrobial agent by inhibiting microbial growth.

By referring to Table 2.1, the most abundant phenolic compounds that can be found in bell pepper is flavonoids. Theoretically, carotenoid is categorized under terpenoid group but after it is oxygenated (after accepting oxygen), it proclaimed the ability to scavenge free radicals present in human body. Therefore, in this case, oxygenated carotenoids (eg:capsantine, capsorubin and cryptocapsin) can be known as phenolic compounds due to their antioxidant properties. On the other hand, bell pepper is also well known for its high concentration of carotenoids, for

instance β-carotene and zeaxanthin as well as α-carotene, luteolin and cryptoxanthin in lower concentration (Chávez-Mendoza et al., 2015). Some carotenoids are antioxidant and some are not as they do not contain aromatic ring with OH group attached to it.

2.1.5 Comparison between Different Colors of Bell Peppers

In some cases, green bell pepper is considered the immature form of bell pepper as it had not been through maturity stage at which it was harvested from mother plant before it ripe (premature harvesting). In this case, red bell pepper serves as the mature (ripened) form (Shotorbani, Jamei and Heidari, 2012). As it goes through maturity stage, its chlorophyll layer starts to degrade, eventually synthesizing capsanthin and capsorubin, which contributes to the red color of red bell pepper (Gomes et al., 2014). Colon carcinogenesis can be prevented by the increasingly capsanthin (which made up half of total carotenoids in red bell pepper) and capsorubin content in red bell pepper as it slowly getting mature (Gomes et al., 2014). Besides, lycopene content in red bell pepper increases as well where this exhibits beneficial effect on attacking certain types of cancer (Nadeem et al., 2011). Maturation process changes the phytonutrient content, such as phenolic content of bell pepper (Shotorbani, Jamei and Heidari, 2012). This was proven by a research by Sun et al. (2007) where total phenolic test, FC method was performed to differentiate the phenolic content between different colored bell peppers. As determined by FC test, red bell pepper contains highest total phenolic content (4.2 µmol catechin equivalent/g) as compared to green (2.4 µg/g), yellow (3.3 µg/g) and

orange pepper (3.4 µg/g). On the other hand, β-carotene, capsanthin, quercetin and luteolin content of red bell pepper was recorded as highest among green, yellow and orange peppers with value of 5.4, 8.0, 34.0 and 11.0 µg/g respectively (Sun et al., 2007). As proven by another study by Shotorbani, Jamei and Heidari (2012), red bell pepper contains higher β-carotene as compared to green and yellow peppers (9 times more than the green verison). Besides the difference in antioxidant content, the ascorbic acid and capsaicinoids in red bell peppers are higher than green and yellow bell peppers (Haejin et al., 2014). The ascorbic acid content increases (double up compared to green bell peppers) may be due to more ascorbic acid is synthesized to withstand and prevent tissues from oxidative stress induced by the higher light intensities as the season goes by. Apart from that, the reason why more capsaicinoids present in mature peppers is may be the capsaicin synthase (biosynthetic enzyme) reaches its optimum temperature to produce capsaicin as the temperature increases from 23.3 – 25.0 ℃ (immature stage) to 34.4 – 36.1 ℃ (mature stage) (Haejin et al., 2014). Furthermore, the taste of peppers is also taken into consideration where due to green bell peppers are prematurely harvested, so they have more bitter taste compared to red bell peppers. The sweet and fruity taste of red bell peppers is assumed to gain more interest from consumers than green peppers. Hence, incorporation of red bell pepper is said to be more nutritious than green and yellow pepper as suggested by previous studies because it contains higher nutrients as well as phenolic compounds.

2.2 Water Activity (A_w)

The degradation and deterioration of food quality and safety are in positive correlation with the a_w of the food products. This is because a_w represent the state of the water, reflecting the amount of availability of water for microbial growth and metabolic processes. Once the growth microorganisms reaches a certain level, the particular food is declared spoiled. A food with higher a_w is more susceptible to microbial spoilage because if the amount of free water (unbound water) is higher, the mobility of molecules in the system is higher and hence there are more free water can be utilized by microorganisms for growth, fastening the food spoilage. This is because water is the main criteria for microbial proliferation (Agoda-Tandjawa, Dieudé-Fauvel and Baudez, 2016; Medvedová, Valík and Studeničová, 2009; Secchi et al., 2017). A study by Li et al. (2011) stated that they obtained a a_w value of 0.979 for noodles, indicating that noodles are high in a_w and belong to high moisture food, causing them to be susceptible to microbial spoilage. Song and Kang (2016) reported that lower a_w can decrease the D value of microorganism. Therefore, there are several food preservation method, for example salting and dehydration that can decrease the a_w to an acceptable level in order to prevent early spoilage. Foods will be safer with lower a_w.

2.3 Proximate Analysis

Proximate analysis, includes analysis of moisture, ash, protein, fat, carbohydrate and fiber content. These tests enable food manufacturers to identify the proximate composition and nutritional value of a food compound. Noodles belong to high moisture food as Li et al. (2016) stated that water content of noodles made up of approximately 30 %, resulting in its susceptibility to microbial growth. This is because previous studies by Li et al. (2011) showed that a water content of 23 – 24 % is sufficient for microbial growth. Red bell peppers are also high in minerals, for instance, potassium, phosphorus and manganese (Kothari et al., 2010; Bello, Boboye and Akinyosoye, 2015), hence ash content for red bell pepper is expected to be increasing if more red bell peppers are incorporated. Due to red bell peppers are a type of vegetables, so they definitely will contribute fiber to the food products, hence noodles incorporated with red bell peppers should have higher fiber content than control noodles and increasing from noodles with lower concentration of red bell peppers to noodles with higher concentration of red bell peppers. There was limited research on the fat and protein content of red bell peppers but as stated by Ojure and Quadri (2012), the noodles itself may contribute to protein and little amount of saturated fatty acids. A study by Nadeem et al. (2011) proposed that a green bell pepper contains 0.33 g of fat, 0.99 g of protein, 10.63 g of carbohydrate, 2.73 g of dietary fiber, 46.79 Cal of calorie and 195.58 kJ of energy. However, there was limited studies on the proximate composition of red bell pepper.

2.4 Other Studies Regarding Methods to Prolong the Shelf Life of Food Products

2.4.1 Chemical Methods (Incorporation of Other Bioactive Compounds)

There are a few studies regarding the addition of bioactive compounds to noodles in order to lengthen its shelf life. The details about those studies were presented in Table 2.2:

Table 2.2: Studies Regarding Incorporation of Other Bioactive Compounds into Foods to Inhibit Microbial Growth

Compounds	Inhibitory Action
1. Flaxseed	- Showed positive result in inhibiting mold spoilage on noodle incorporated with: 6 % flaxseed flour: Inhibit the growth of *Fusarium graminearum* and *Aspergillus flavus* (Xu et al., 2008) 9 % flaxseed flour: Reduce the growth count of *Penicillium chrysogenum* and *Penicillium sp.* (Xu et al., 2008).
2. Chitosan	- Able to inhibit growth of total coliform, mesophilic and psychrophilic bacteria and *Staphylococcus sp*. on homemade fresh pasta (Del Nobile et al., 2009).
3. Grapefruit seed extract (GFSE)	- Effective against total coliform, mesophilic and psychrophilic bacteria and *Staphylococcus sp.* on homemade fresh pasta (Del Nobile et al., 2009).

Table 2.2 (continued): Studies Regarding Incorporation of Other Bioactive Compounds into Foods to Inhibit Microbial Growth

Compounds	Inhibitory Action
4. Thymol	- Antibacterial agent to reduce growth count of mesophilic and psychrophilic bacteria and *Staphylococcus sp.* on homemade fresh pasta (Del Nobile et al., 2009).

2.4.2 Physical Methods (Packaging)

2.4.2.1 Modified Atmosphere Packaging (MAP)

Despite prolonging shelf life of food products by chemical methods (adding bioactive compounds) as well as improving nutritional value or sensory properties of foods, efforts should also be put on the physical methods, such as packaging of the food products as packaging shields the products from the outer environment and directly come in contact with the products, hence the types and quality of packaging certainly exhibit effects on the products. For instance, modified atmosphere packaging (MAP) can be implied on food products to prolong their shelf life. As the name suggests, the atmospheric environment of the internal packaging of food products are modified to a level that is not suitable for microbial growth. Low oxygen (O_2), high carbon dioxide (CO_2) and suitable amount of nitrogen (N_2) are desired in MAP. This is because if CO_2 presence at high level, it is able to kill bacteria and fungi due to its bacteriostatic and fungistatic properties. O_2 level is preferable to be low because a lot of microbes are aerobic (requires O_2

to survive). N_2 is inert and will not react with any other molecules, it can maintain the packaging shape by preventing it from collapsing (Sanguinetti et al., 2016).

2.4.2.2 Studies that Implied MAP on Food Products

Sanguinetti et al. (2016) conducted a study that they attempt to maintain safety and prolong the shelf life of gluten-free fresh filled pasta by MAP. Their attempt was successful because when the pasta was air-packed (normal packaging), their shelf life was 14 days as after this certain period of time, visible mold was observed on the surface of pasta. Molds only started to grow on the surface of pasta in MAP starting from 42 days. Therefore, this study proved that the application of MAP is an effective technique in extending the shelf life of pasta. In this research, if the microbial colonies formed were too few to count (TFTC), the symbol "<" was included in front of the cfu/g value. This symbol was convenient as whenever this symbol was observed, it can be understand that pasta spoilage have not occur yet as the colonies formed were TFTC, where the amount of microorganisms grew was not sufficient to cause spoilage yet.

Apart from that, Secchi et al. (2017) also utilized MAP on whey cheesecakes to extend their shelf life. In this case, MAP modifies the internal environment of the packaging to an oxygen concentration of less than 1 % and carbon dioxide concentration of more than 20 %, contributing to antimicrobial properties (Secchi et al., 2017). In the research by Secchi et al. (2017), by modifying the internal packaging environment to a ratio of 70:30 for nitrogen to carbon dioxide ratio, they

successfully extended the shelf life of whey cheesecakes to 45 days where control cheesecakes can only stand for 11 days. A portion of the microbial counts for the samples of whey cheesecakes were showed in the table below:

Table 2.3: Microbial Counts of Whey Cheesecakes Samples (cfu/g) after Combining MAP and OVP Techniques in the Study of Secchi et al. (2017)

Microbial Media	Experimental Batches	Storage Time (Days)				
		0	10	20	40	60
PCA*	Control	<1.0 x 10^1	2.5 x 10^3	-**	-	-
	MAP	7.3 x 10^2	1.2 x 10^3	2.8 x 10^6	2.7 x 10^6	-

*: PCA = Plate Count Agar.
** Sampling and Calculation on cfu/g was stopped due to visible mold growth on cheesecakes.

As observed in Table 2.3, the cfu/g for control on day 0-10 was recorded as less than (<) 1.0 x 10^1 because the colonies formed was too few to count and was negligible. It was viewed as slight microbial contamination. Visible mold started to grow on control plates starting from day 21 while cheesecakes with MAP managed to stay mold-free until day 45. According to the results, MAP is able to retard microbial growth and prevent early food spoilage, prolonging the shelf life.

CHAPTER 3

MATERIALS AND METHODS

3.1 Noodles Preparation

The basic ingredients for homemade noodles, wheat flour, water and salt as well as the ingredient of interest of this project, red bell pepper (*Capsicum annum* L.) were purchased from local supermarket, Tesco, Kampar. The basic ingredients needed to prepare the dough for the control noodles were 100 g of flour, 46 mL of water and 2 g of salt (pre-dissolve in water). These three ingredients were measured and mixed together thoroughly until a consistent dough was formed. Then, the dough was left to rest for 20 min. After 20 min, a domestic noodle making machine was used to obtain dough sheets by gradually reducing the thickness of dough. It was then sliced into noodle strands using the same machine. The noodle strands were subjected to cooking for 6 min, drained for 10 min and packed into individual plastic bag and lastly, they were stored at 4 ℃ refrigerator. Noodles incorporated with 5, 10 and 20 % of red bell pepper were prepared. Fresh red bell pepper was prepared and blended where the amount used was in accordance to the concentration required (5, 10 or 20 %). Then, blended red bell pepper was mixed with an appropriate amount of flour, water and salt as shown in Table 3.1 so that the noodle with required concentration of red bell pepper can be prepared. The dough sheeting, cooking, draining, packaging and storing procedures were similar to preparation of control (Li et al., 2012).

Table 3.1: The ingredients (flour, salt, water and bell pepper) and their amount required to be incorporated into control and noodle samples incorporated with 5, 10 and 20 % of bell pepper

	Flour (g)	Salt (g)	Water (g)	Bell Pepper (g)	Total weight of dough (g)
0 % Bell Pepper	67	2	31	0	100
5 % Bell Pepper	64	2	29	5	100
10 % Bell Pepper	60	2	28	10	100
20 % Bell Pepper	53	2	25	20	100

3.2 Shelf Life Determination

3.2.1 Microbial Analysis

3.2.1.1 Total Plate Count (TPC)

The procedures for TPC was performed according to as stated by Li et al. (2016) with slight modification. Twenty five grams of sample were sampled from the cooked control noodles and noodle incorporated with 5 %, 10 % and 20 % bell pepper which were stored in the refrigerator after preparing. Two hundred and twenty five milliliters (mL) of sterile saline solution (0.85 % NaCl) was mixed with each sample. A stomacher was used to homogenize the samples in a Stomacher bag for 2 min. Sterile saline solution was serial diluted into several dilutions and this

was carried out by initially pipetting 1 mL of homogenized sample into a sterile tube and then subsequently adding 1 mL of solution into the next solution, forming dilution from 10^{-1} to 10^{-6}. For microbial assessments, all agar and apparatus (such as test tubes) were autoclaved at 121 ˚C for around 2 h before subjected to any microbial tests. Sterile / autoclaved plate count agar (PCA) was poured into empty sterile Petri plates and allowed for solidification. After solidification of agar, 0.1 mL of each dilution were pipetted into respective plates and then the inoculum was spread plated evenly on the agar surface by glass spreader. Then, the plates were labelled accordingly. For each dilution, two plates were spread-plated with 0.1 mL inoculum of the same dilution (duplicate). Lastly, each PCA plates were incubated at 37 ℃ for 48 h to detect if there is any growth of aerobic mesophilic bacteria (AMB). This can be done by observing whether colonies are formed on the surface of PCA plates for each plates. Only 25 to 250 colonies were taken into consideration in calculating the cfu/mL and log cfu/g of the bacterial colonies grown on PCA plates. Plates with less than 25 colonies were recorded as too few to count (TFTC) while too numerous to count (TNTC) was recorded for plates with more than 250 colonies.

3.2.2.2 Yeast and Mold (YM)

Procedures of YM test were similar to TPC. The difference was the agar used for YM test was acidified potato dextrose agar (PDA) instead of PCA. After spread plating each dilution into respective plates, incubation condition of the plates were set at 26 ℃ for 72 h. To detect if any YM present, whether or not colonies were formed on the plates was observed (King et al., 1986; Phattra and Maweang, 2015). Similar to TPC, TFTC was labelled if colonies were less than 25 colonies and if more than 250 colonies, TNTC was labelled as the range of fungal colonies that was included in the calculation was 25 to 250 colonies.

3.2.2 Water Activity (A_w) Test

Water activity of every noodle samples, including control were determined by LabSwift-aw water activity meter (Brand name: Novasina) as mentioned by Xu et al. (2008) with slight modification. The containers that were designed specifically to be inserted into the water activity meter were filled up completely by control noodles. Then, the container that contained the control sample was inserted into the meter and the process of measuring the water activity of the noodles started when "start" button was pressed. The final water activity value displayed on the screen was recorded when "beeping" sound was heard. These steps were repeated by replacing control with noodles incorporated with 5, 10 and 20 % of bell pepper.

3.3 Proximate Analysis

3.3.1 Moisture Content

The four cooked noodle (control, 5, 10 and 20 % bell pepper) were subjected to moisture content analysis following Association of Official Analytical Chemist (AOAC) 934.06 official method. Twelve empty aluminum pans were oven-dried for 3 h at 105 ℃ until they reached a constant weight and transferred to desiccator to cool. The pans were weighed. Approximately 6 g of the four samples were weighed and added to respective aluminum pans. The samples were cut into smaller pieces before adding to the pans and they were distributed evenly on the pans using spatula. The sample-containing pans were dried in the oven for at least 7 h at 105 ℃. They were cooled in desiccator after drying. Lastly, pans and their dried samples were reweighed. Moisture content analysis for each sample was carried out in triplicate. Moisture content (in percentage) of the samples can be determined using equation below (Olivera and Salvadori, 2009; Kumoro, Johnny and Alfilovita, 2016):

$$M\ (\%) = \frac{W_i - W_f}{W_i} \times 100$$

Where,

M (%) = Percentage of the moisture content of samples (%)

W_i = Initial weight of noodle samples before drying (g)

W_f = Final weight of noodle samples after drying (g)

3.3.2 Ash Content

AOAC 923.03 method was applied to measure the total ash content of samples. Incineration of 12 crucibles and their lids were carried out in a muffle furnace at 550 ℃ for 8 h or overnight to burn off impurities on the surface of the crucibles. The crucibles were allowed to cool in a desiccator for 30 min and before weighing. Each samples were used and approximately 1.5 g form each dried samples were weighed into the respective crucibles. Hot plates were used to burn the crucibles containing samples until no more fumes can be observed releasing from the samples. The crucibles were then incinerated in muffle furnace. The furnace was gradually increased to a temperature of 550 ℃ for at least 8 h until no black carbon particle present to obtain permanent weight. The crucibles that contained the ash were cooled in desiccator. The crucibles together with ash were weighed. The samples should be subjected to further ashing in the furnace if the samples do not turn grey. Ash analysis for each sample was carried out in triplicate. The ash content (in percentage) can be determined using formula below (Ahmed, Qazi and Jamal, 2015; Kumoro, Johnny and Alfilovita, 2016):

$$\text{A (\%)} = \frac{(W_a + W_p) - W_p}{W_s} \text{ x } 100$$

Where,

A (%) = Percentage of ash content of samples (%)

W_a = Weight of ash (g)

W_p = Weight of crucibles (g)

W_s = Weight of samples (g)

3.3.3 Protein Content

The determination of crude protein content in each samples followed AOAC 920.87 official method. Kjedahl method was carried out. Around 0.5 to 1.0 g of sample was crushed into smaller pieces and wrapped in filter paper, followed by insertion into digestion flask. Similarly, dried samples were required. Half spatula of Kjedahl catalyst ($CuSO_4$ and K_2SO_4) which were prepared beforehand at ratio of 1:10 and 200 mL of concentrated sulphuric acid (H_2SO_4) were added subsequently. The flasks were placed in holder and subjected to digestion using digestion unit. They were heated for 1 – 2 h and boiled briskly until the solution turned clear green. The digestion process ended, and the digests were set to undergo distillation. The solutions were cooled and were installed in the distillation unit. Twenty five milliliters of 4 % boric acid was added into conical flask and mixed with 3 drops of indicator (mixture of methyl red and bromocresol green), forming pink solution. This is to provide visual color changes when it reaches its end point. The conical flask was connected to the distillation unit and the distillation process was allowed to begin where appropriate amount of distilled water and 32 % NaOH were added subsequently. Changing of color from pink to blue color indicates distillation was completed. After distillation, 0.2 N HCl was used in the titration process to get titrated by the boric acid mixture. Titration was said to reach completion when the blue color of the boric acid mixture changed from blue to pink. The crude protein content (in percentage) was calculated using formula below (Ahmed, Qazi and Jamal, 2015; Kumoro, Johnny and Alfilovita, 2016):

$$P\ (\%) = \frac{(V\ x\ N) x\ 1.4007 \frac{g}{mol}\ x\ 100\ x\ 6.25}{W}$$

Where,

P (%) = Percentage protein content of samples (%)

V = Volume of 0.2 N HCl used in sample titration (mL)

N = Normality of HCl (N)

W = Weight of samples (g)

14.007 = Molecular mass of nitrogen

6.25 = Conversation factor of protein-nitrogen

3.3.4 Fat Content

Standard method of AOAC to determine crude fat in cooked noodle was followed. Extraction beakers and boiling stones were placed in an oven at 105 ℃ for 1 h to make sure the weight of all the beakers are stable. The weight of extraction beakers together with boiling stones were recorded as M_1. Three grams of the four dried samples were weighed (M_0). Filter papers were used to wrap the four dried samples. The samples were inserted into respective extraction thimble and inserted into respective beaker. Appropriate amount of solvent (petroleum ether) was added to each beaker to make sure all the sample were fully immersed in the solvent. A piece of cotton wool was inserted on top of the sample before connecting the beakers to Soxtherm for fat analysis. Extraction of fat was carried out for approximately 2 h. After extraction, the beakers containing boiling stones and extracted fat (if any) were dried in oven at 105 ℃ as there may be remaining solvent that were not dry off yet. They were cooled down in desiccator for 1 h. The thimble and boiling stones

were removed. The extraction beakers were reweighed (M_2). Crude fat content (in percentage) can be determined using formula below (Ahmed, Qazi and Jamal, 2015):

$$F\ (\%) = \frac{(M_2 - M_1)}{M_0} \times 100$$

Where,

F (%) = Percentage of fat content of samples (%)

M_0 = Weight of samples (g)

M_1 = Weight of extraction beaker and boiling stones before extracting fat (g)

M_2 = Weight of extraction beaker and extracted fat (if any) (g)

3.3.5 Fiber Content

Crucibles were pre-ashed for 30 min in muffle furnace at 600 ℃. The crucibles were cooled in 105 ℃ oven for 30 min first then transferred to desiccator for 30 min, ready to be inserted with digested sample. RF fiber bags were dried in oven for 1 h at 105 ℃ and then cooled for 30 min in desiccator. The weight of fiber bags for samples were measured and recorded as M_1 whereas fiber bag for blank was recorded as B_1. Glass spacer was inserted into fiber bag before adding dried samples (M_2) into the fiber bag. Fiber bags together with the glass spacers were loaded into carousel and inserted into a beaker. Then, the dried samples were subjected to digestion process using Gerhardt Fiberbag system. To digest fiber, a beaker that contained the carousel was added with 360 mL of 0.13 mol/L H_2SO_4. The carousel was rotated for about 1 min to mix the sample with H_2SO_4. The hot plate of the Gerhardt Fiberbag system was preheat for 5 min before inserting the beaker. After

preheating, the beaker was placed on the hot plate and heated to boil. After the solution start to boil, the hot plate setting was reduced to allow gentle simmering for about 30 min. The carousel was removed from the beaker, the solution and soluble within the beaker was discarded. The beaker was added with 360 mL of 0.313 mol/L NaOH before inserting carousels with fiber bags into the beaker. Similarly, it was allowed for gentle simmering for 30 min after it boiled. Fiber bags with glass spacers were unloaded from carousel and glass spacers and inserted into the pre-ashed crucibles. They were dried for 4 hours or overnight in an oven at 105 ℃ and then cooled in desiccator for 30 min. The weight of fiber bags, dried digested samples and pre-ashed crucibles were recorded as M_3 while empty fiber bags and pre-ashed crucible were recorded as B_3. Then, they were incinerated in muffle furnace at 600 ℃ for 4 h or overnight. They were cooled in 105 ℃ oven for 30 min and then in desiccator for 30 min. Weight of crucibles, fiber bag ash and sample ash (M_4) and weight of crucible and empty fiber bag ash (B_4) were obtained. The percentage of fiber of samples were obtained by using formula below (Kumoro, Johnny and Alfilovita, 2016):

$$F\ (\%) = \frac{(M_3 - M_1 - M_4) - [(B_3 - B_1 - B_4)\ x\ 100]}{M_2}$$

Where,

F (%) = The percentage of fiber

M_1 = Weight of fiber bag for samples (g)

B_1 = Weight of empty fiber bag as blank (without sample) (g)

M_2 = Weight of sample (g)

M_3 = Weight of fiber bags, dried digested samples and pre-ashed crucibles (g)

B_3 = Weight of empty fiber bags and pre-ashed crucibles (g)

M_4 = Weight of crucibles, fiber bag ash and sample ash (g)

B_4 = Weight of crucible and empty fiber bag ash (g)

3.4 Total Phenolic Test

To quantify the total phenolic compound in each sample, FC's reagent assay was performed where the phenolic content were expressed as GA equivalents (mg/g). Standard curve was generated by using the standard GA solution between the concentrations of 0.05 to 0.35 mg/mL. A 0.5 mg/mL of standard GA solution was used as the stock solution. In order to prepare the stock solution, distilled water was used to slowly dissolved the 0.5 g of GA, then the overall volume of the solution was topped up to 10 mL using 10 mL volumetric flask. Then, this stock solution was further diluted into lower GA concentration, with lowest concentration (0.05 mg/mL) to highest concentration (0.35 mg/mL). Next, 0.1 mL of each concentration (0.05 – 0.35 mg/mL) was pipetted using micropipette into a universal bottle and mixed with 2 mL sodium bicarbonate (Na_2CO_3). Then, it was left undisturbed for 2 min. After the incubation period, 0.3 mL of 80 % ethanol was added before adding 0.1 mL of 1 N FC. The mixture was incubated for 30 min before obtaining its absorbance at 720 nm using spectrophotometer. After obtaining all the absorbance reading, a standard curve was plotted ($y = 2.7299x$ and $r^2 = 0.9831$; where y and x stand for absorbance and Gallic acid concentration respectively while r^2 represents the correlation coefficient). For the samples, the phenolic compounds from each sample (noodle incorporated with 5 %, 10 % and 20 % bell pepper) were extracted

by triturating 5 g of each sample with 20 mL of 80 % ethanol for 2 min, forming extract with the concentration 250 µM. The procedure in adding respective chemicals (Na_2CO_3, ethanol and FC), incubation time and measuring absorbance of the samples were similar to the preparation of standard curve, the only difference was GA used in preparation of standard curve was replaced with noodle samples. The total phenolic content of each sample were calculated using the standard curve obtained earlier. The unit of the total phenolic content obtained from the standard curve would be mg/mL, it needs to be converted to mg/g before subjecting to data analysis (Khare, Biswas and Sahoo, 2014; Materska and Perucka, 2005).

3.5 Sensory Evaluation

Hedonic test (9 point rating scale) was carried out to measure the acceptability of the cooked noodle incorporated with 5, 10 and 20 % of bell pepper. Control was also included in this sensory evaluation. An untrained panel of 50 consumers were recruited. Appropriate amount of plastic containers were coded randomly according to Table of Random Number. A master sheet was constructed. The cooked noodles were placed in the coded plastic container accordingly and served randomly according to the master sheet. Panelists were asked to rate the appearance, aroma taste, texture and overall appearance of each of the samples based on 9-point rating scale, with ‘Dislike Extremely’ (1) and ‘Like Extremely’ (9) at either end with a middle point of ‘Neither Like nor Dislike’ (5). Water was provided so that panelists can rinse their mouth between samples before rating. This was carried out in isolated booths to avoid interference (Olivera and Salvadori, 2009).

3.6 Statistical Analysis

Statistical analysis of the data obtained from each test was similar to as stated by Xu et al. (2008) with slight modification. SPSS software (Version 20) was used instead of SAS software to conduct ANOVA to analyze the data obtained from each tests. The means of the data were separated using Tukey test at a 95 % confidence interval ($p<0.05$) where means were significantly different from each other if $p<0.05$ but not significantly different if $p>0.05$.

CHAPTER 4

RESULTS

4.1 Shelf Life Determination

4.1.1 Microbial Analysis

4.1.1.1 Total Plate Count (TPC)

The cfu/g and log cfu/g for each sample (control and noodles incorporated with 5, 10 and 20 % of bell pepper) were calculated at 2 days interval starting from the day the noodles were prepared (day 0) to day 8. Therefore, in TPC test, two factors, which were days and noodle samples were involved. After conducting ANOVA, it was showed that there were significant difference ($p<0.05$) among the log cfu/g for all the samples, including control. Furthermore, the microbial count for the noodle samples were also significantly different ($p<0.05$) from each other at different days. Hence, there were significant difference ($p<0.05$) for both of the factors. This can be observed more clearly by the indication of superscript next to the mean value of the respective log cfu/g at which the mean were compared and separated by Tukey post hoc test to identify which mean was significantly different ($p<0.05$) from the others. According to Table 4.1, TPC results showed that all the plates of control and noodles incorporated with 5, 10 and 20 % of bell pepper started to have bacterial growth at day 4. Starting from day 4, log cfu/g for control were recorded as more than 4.06. Noodles incorporated with 5 and 20 % of bell pepper shared similar trend in their log cfu/g where both of them increased from day 4 to 8. However, there was an exception with noodles incorporated with 10 % of bell

pepper as it displayed different trend. Its microbial count decreased at day 6 (less than 3.06 log cfu/g) compared to day 4 (less than 3.36 log cfu/g) then increased again at day 8 (3.84 log cfu/g). By referring to Australian Standard (1993) and Food and Drug Administration Philippines (2013), noodles are declared spoiled when its bacterial count exceeded 10^5 cfu/g. Therefore, noodles incorporated with 5 and 20 % bell pepper spoiled at day 8 and day 6 respectively as their cfu/g exceeded 10^5 cfu/g at this point. Control was considered spoiled since day 4 because the cfu/g value was more than 1.14×10^4. Although it did not has an exact value, but it was considered spoiled because the colonies formed on all of the plates were too numerous to count. Overall, the log cfu/g for noodles incorporated with 20 % of bell pepper was the highest at day 8, indicating it spoiled the most compared to others. This showed that capsaicin in bell peppers exhibit low effectiveness of antimicrobial inhibitory effect. This is because although the log cfu/g of noodles incorporated with 5 and 10 % of bell pepper were lower than control (more than 4.06 log cfu/g) at day 4 and 6, but the log cfu/g of noodles incorporated with 20 % of bell pepper was higher than control at day 6 (5.25 log cfu/g) and was the highest (6.23 log cfu/g) at day 8.

Table 4.1: Microbial count of control and noodle samples (with 5, 10 and 20 % of bell pepper incorporation) using TPC method at day 0, 2, 4, 6 and 8

	Day 0	Day 2	Day 4	Day 6	Day 8
Noodles incorporated with 0 % of Bell Pepper					
Cfu/g	-	-	$>1.14 \times 10^4$	$>1.14 \times 10^4$	$>1.14 \times 10^4$
Log cfu/g	-	-	$>4.06^{aA}$	$>4.06^{aB}$	$>4.06^{aC}$
Noodles incorporated with 5 % of Bell Pepper					
Cfu/g	-	-	$<1.14 \times 10^3$	2.35×10^3	1.18×10^6
Log cfu/g	-	-	$<3.06^{cD}$	3.37^{bC}	6.07^{aB}
Noodles incorporated with 10 % of Bell Pepper					
Cfu/g	-	-	$<2.27 \times 10^3$	$<1.14 \times 10^3$	6.90×10^3
Log cfu/g	-	-	$<3.36^{bC}$	$<3.06^{cD}$	3.84^{aD}
Noodles incorporated with 20 % of Bell Pepper					
Cfu/g	-	-	4.15×10^3	1.77×10^5	1.71×10^6
Log cfu/g	-	-	3.62^{cB}	5.25^{bA}	6.23^{aA}

$^{A-D}$: Data within the same column with same superscript are not significantly different ($p>0.05$) while data with different superscript are significantly different ($p<0.05$).

$^{a-c}$: Data within the same row with same superscript are not significantly different ($p>0.05$) while data with different superscript are significantly different ($p<0.05$).

4.1.1.2 Yeast and Mold (YM)

Similar to TPC test, fungal count for YM was also conducted at 2 days interval for all the samples, including control. There were significant difference (p<0.05) among samples and days as indicated by the superscript next to each mean value (Table 4.2). Superscripts were determined by Tukey test which separated the means of each sample. According to Table 4.2, all plates except plates for noodles incorporated with 10 % of bell pepper started to have fungal growth at day 4. In this case, uncommon results were obtained for control and noodles incorporated with 10 % of bell pepper because for noodles incorporated with 10 % of bell pepper, there was no fungal growth at day 4 but instead, its fungal count started at day 6. However, the count at day 8 (5.54 log cfu/g) was even lower than count on day 6 (6.38 log cfu/g). Fungal growth for control was unusual as the log cfu/g was higher at day 4 (less than 3.36 log cfu/g) compared to day 6 (less than 3.08 log cfu/g) and day 8 (less than 3.06 log cfu/g) where day 8 had the lowest count. According to the results, noodles with 10 % of bell pepper was considered as spoiled as its yeast and mold count (2.38 x 10^6 cfu/g) exceeded the cut-off between spoiled and unspoiled noodles (10^5 cfu/g) (Australian Standard, 1993; Food and Drug Administration Philippines, 2013) at day 6. Even although its count decreased on day 8 and became lower than day 6, but its count still exceeded 10^5 cfu/g at day 8.

Noodles with 5 and 20 % of bell pepper showed similar trend as in TPC where their count gradually increased from 3.57 and 4.38 log cfu/g to 6.39 and 7.04 log cfu/g respectively from day 4 to day 8. These two types of noodles were declared as

spoiled at day 8 and 6 respectively as they exceeded the cut-off point (10^5 cfu/g) (Australian Standard, 1993; Food and Drug Administration Philippines, 2013). Similar to TPC, noodles with 20 % of bell pepper had highest log cfu/g (7.04 log cfu/g) and considered spoiled the most among others at day 8.

Table 4.2: Fungal count of control and noodle samples (with 5, 10 and 20 % of bell pepper incorporation) using YM method at day 0, 2, 4, 6 and 8

	Day 0	Day 2	Day 4	Day 6	Day 8
Noodles incorporated with 0 % of Bell Pepper					
Cfu/g	-	-	$<2.27 \times 10^3$	$<1.19 \times 10^3$	$<1.14 \times 10^3$
Log cfu/g	-	-	$<3.36^{aC}$	$<3.08^{bD}$	$<3.06^{cD}$
Noodles incorporated with 5 % of Bell Pepper					
Cfu/g	-	-	3.75×10^3	1.26×10^4	2.43×10^6
Log cfu/g	-	-	3.57^{cB}	4.10^{bC}	6.39^{aB}
Noodles incorporated with 10 % of Bell Pepper					
Cfu/g	-	-	-	2.38×10^6	3.43×10^5
Log cfu/g	-	-	-	6.38^{aA}	5.54^{bC}
Noodles incorporated with 20 % of Bell Pepper					
Cfu/g	-	-	2.40×10^4	1.01×10^6	1.09×10^7
Log cfu/g	-	-	4.38^{cA}	6.00^{bB}	7.04^{aA}

$^{A-D}$: Data within the same column with same superscript are not significantly different ($p>0.05$) while data with different superscript are significantly different ($p<0.05$).
$^{a-c}$: Data within the same row with same superscript are not significantly different ($p>0.05$) while data with different superscript are significantly different ($p<0.05$).

4.1.2 Water Activity (A_w) Test

Two factors were included in water activity test, which were the different samples and days. Results showed that there were no significant difference (p>0.05) among the samples as well as days. According to Table 4.3, mean water activity value for each sample on different days showed no significant difference (p>0.05). Noodle samples shared a mean value of 0.978 ± 0.00 at which this value was determined by obtaining the overall mean value of the mean value of samples and days.

Table 4.3: Water activity of control and noodle samples (with 5, 10 and 20 % of bell pepper incorporation) at day 0, 2, 4, 6 and 8

	Day 0	Day 2	Day 4	Day 6	Day 8	Mean
Noodles incorporated with 0 % of Bell Pepper						
Average A_w	0.978^{aA} ± 0.00	0.977^{aA} ± 0.00	0.978^{aA} ± 0.00	0.978^{aA} ± 0.00	0.978^{aA} ± 0.00	0.977^{aA} ± 0.00
Noodles incorporated with 5 % of Bell Pepper						
Average A_w	0.978^{aA} ± 0.00	0.977^{aA} ± 0.00	0.978^{aA} ± 0.00	0.980^{aA} ± 0.00	0.979^{aA} ± 0.00	0.978^{aA} ± 0.00
Noodles incorporated with 10 % of Bell Pepper						
Average A_w	0.977^{aA} ± 0.00	0.977^{aA} ± 0.00	0.977^{aA} ± 0.00	0.978^{aA} ± 0.00	0.978^{aA} ± 0.00	0.977^{aA} ± 0.00

[A]: Data within the same column with same superscript are not significantly different (p>0.05).
[a]: Data within the same row with same superscript are not significantly different (p>0.05).

Table 4.3 (continued): Water activity of control and noodle samples (with 5, 10 and 20 % of bell pepper incorporation) at day 0, 2, 4, 6 and 8

	Day 0	Day 2	Day 4	Day 6	Day 8	Mean
Noodles incorporated with 20 % of Bell Pepper						
Average A_w	0.979^{aA} ± 0.00	0.978^{aA} ± 0.00	0.977^{aA} ± 0.00	0.979^{aA} ± 0.00	0.978^{aA} ± 0.00	0.978^{aA} ± 0.00
Mean	0.978^{aA} ± 0.00	0.977^{aA} ± 0.00	0.977^{aA} ± 0.00	0.979^{aA} ± 0.00	0.978^{aA} ± 0.00	0.978 ± 0.00

[A]: Data within the same column with same superscript are not significantly different (p>0.05).
[a]: Data within the same row with same superscript are not significantly different (p>0.05).

4.2 Proximate Analysis

For proximate analysis, as stated in Table 4.4, only ash and fiber content showed significant difference (p<0.05) among samples while the samples were not significantly different (p>0.05) from each other in moisture, fat and fiber content.

Table 4.4: Moisture, ash, protein, fat and fiber content of control and noodle samples (with 5, 10 and 20 % of bell pepper incorporation)

	Content (%)				
	Moisture	Ash	Protein	Fat	Fiber
0 % Bell Pepper	71.33^{a} ± 7.25	1.01^{c} ± 0.09	29.07^{a} ± 0.36	0.00^{a} ± 0.00	0.14^{c} ± 0.01
5 % Bell Pepper	69.73^{a} ± 0.80	1.13^{c} ± 0.01	29.13^{a} ± 0.11	0.00^{a} ± 0.00	0.29^{b} ± 0.02

[a-c]: Data within the same column with same superscript are not significantly different (p>0.05) while data with different superscript are significantly different (p<0.05).

Table 4.4 (continued): Moisture, ash, protein, fat and fiber content of control and noodle samples (with 5, 10 and 20 % of bell pepper incorporation)

	Content (%)				
	Moisture	Ash	Protein	Fat	Fiber
10 % Bell Pepper	68.02[a] ± 1.57	1.26[b] ± 0.03	29.19[a] ± 0.31	0.00[a] ± 0.00	0.40[b] ± 0.04
20 % Bell Pepper	67.46[a] ± 1.14	1.47[a] ± 0.01	29.28[a] ± 0.44	0.00[a] ± 0.00	0.58[a] ± 0.04

[a-c]: Data within the same column with same superscript are not significantly different (p>0.05) while data with different superscript are significantly different (p<0.05).

4.2.1 Moisture Content

Moisture content slightly decreased from control (71.33 ± 7.25 %) to noodles incorporated with 20 % of bell pepper (67.46 ± 1.14 %) but there were no significant differences (p>0.05) noticed among all samples, including control.

4.2.2 Ash Content

Control and noodles incorporated with 5, 10 and 20 % of bell pepper were incinerated until grey pinkish ash were obtained for each sample. According to Table 4.4, ash content showed significant increase (p<0.05) from control (1.01 ± 0.09 %) to noodles incorporated with 20 % of bell pepper (1.47 ± 0.01 %). The ash content of control was significantly different (p<0.05) from noodle incorporated

with 10 % (1.26 ± 0.03 %) and 20 % (1.47 ± 0.01 %) of bell pepper but showed no significant difference (p>0.05) with the noodles incorporated with 5 % of bell pepper (1.13 ± 0.01 %). The ash content of each samples were showed in Table 4.5 and 4.6.

Table 4.5: Images of ash content for control and noodles with 5 % of bell pepper

Control	Noodles incorporated with 5 % of bell pepper
	
Figure 4.1: Ash of control	Figure 4.2: Ash of noodles with 5 % of bell pepper

Table 4.6: Images of ash content for noodles with 10 and 20 % of bell pepper

Noodles incorporated with 10 % of bell pepper	Noodles incorporated with 20 % of bell pepper
	
Figure 4.3: Ash of noodles with 10 % of bell pepper	Figure 4.4: Ash of noodles with 20 % of bell pepper

4.2.3 Protein Content

There was a slight increase in protein content from the lowest protein content of control noodles (29.07 ± 0.36 %) to the noodles with 20 % of bell pepper that contained the highest protein content (29.28 ± 0.44 %). However, the increase in protein content was not significant (p>0.05). Figure 4.5 showed the clear green solution of each sample after digestion process.

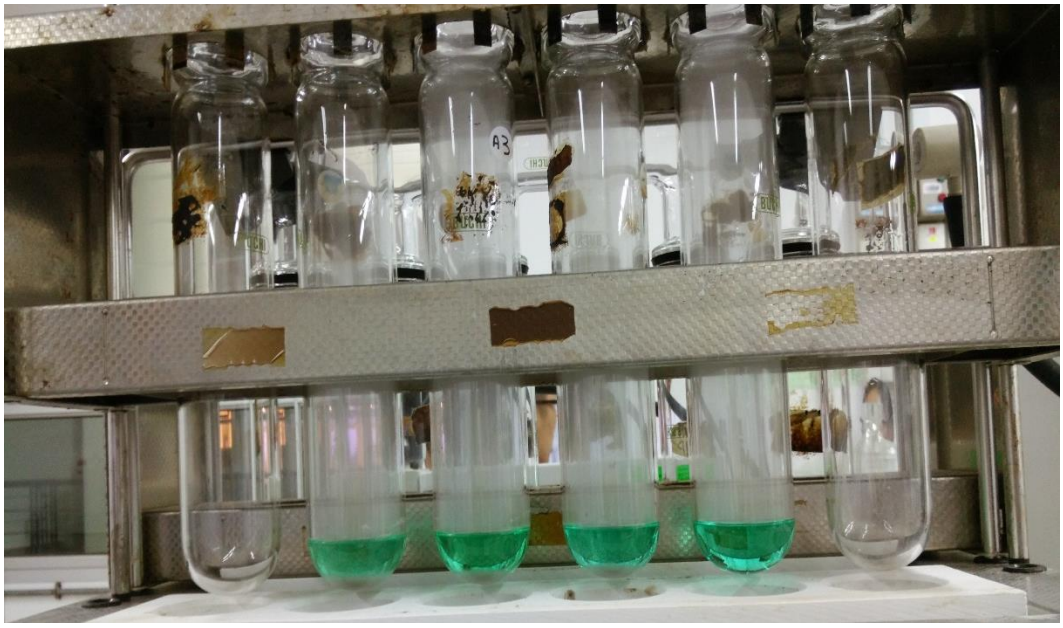

Figure 4.5: Color of the solution of control noodles and noodle samples incorporated with 5, 10 and 20 % of bell pepper in digestion tube after digestion process. (From left to right: Control, noodles with 5, 10 and 20 % of bell pepper)

4.2.4 Fat Content

Fat for each sample were extracted using Soxtherm analyzer as shown in Figure 4.6. However, no fat was detected for each of the sample, hence recorded as 0.00 ± 0.00 %. Hence, the fat content of each sample were not significantly different from each other (p>0.05) as no results were obtained.

Figure 4.6: Images of fat extraction using Soxtherm analyzer

4.2.5 Fiber Content

The fiber content for control was the lowest (0.14 ± 0.01 %). Fiber content increased as higher concentration of bell peppers were incorporated into the noodles with the highest fiber content in noodles incorporated with 20 % of bell pepper (0.58 ± 0.04 %), showing a significant increase (p<0.05) in fiber content. Noodles with 5 and 10 % of bell pepper did not differ from each other but they differed from control and noodles with 20 % of bell pepper. The fiber content of control and noodles with 20 % of bell pepper were significantly different (p<0.05) from each other as well.

4.3 Total Phenolic Test

4.3.1 Standard Curve

The initially colorless GA turned to blue color where the intensity of the blue color was in correspondence with the GA concentration (Figure 4.8). The higher GA concentration, the higher intensity of blue color of the solution. Different GA concentration, ranging from 0.05 to 0.35 mg/mL were subjected to FC assay, obtaining absorbance that correspond to the respective GA concentration. By plotting the absorbance obtained against their corresponsive GA concentration, standard curve as shown in Figure 4.7 was obtained.

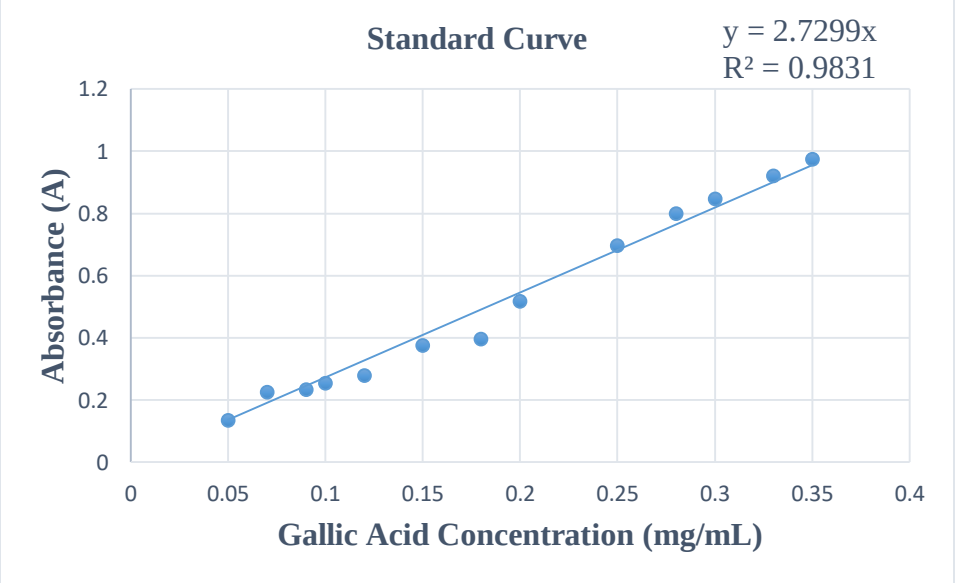

Figure 4.7: Standard curve of absorbance against Gallic acid concentration

Figure 4.8: Color of different concentration of GA after incubating with Na_2CO_3, ethanol and FC before subjecting to spectrophotometer

4.3.2 Phenolic Content of Control and Noodle Samples Incorporated with 5, 10 and 20 % of Bell Pepper

Total phenolic content of control and noodles incorporated with 5, 10 and 20 % bell pepper were obtained by using the standard curve as shown in Figure 4.7. Significant difference in total phenolic content (p<0.05) was observed among all the samples, including control. By referring to Table 4.7, total phenolic content was the highest for noodles incorporated with 20 % of bell pepper (0.36 ± 0.05 mg/g). There was a significant increase (p<0.05) in the total phenolic content (mg/g) from control to noodles incorporated with 20 % bell pepper where it increased from 0.13 ± 0.01 to 0.36 ± 0.05. Total phenolic content of control and noodles incorporated with 5 % of bell pepper were not significantly different (p>0.05) from each other whereas noodles incorporated with 5 and 10 % of bell pepper showed no significant difference (p>0.05) as well. Noodles incorporated with 20 % bell pepper was significantly different (p<0.05) from the others.

Table 4.7: Absorbance and total phenolic content of control and noodle samples incorporated with 5, 10 and 20 % of bell pepper

	Total Phenolic Content (mg/g)
0 % Bell Pepper	$0.13^{c} \pm 0.01$
5 % Bell Pepper	$0.16^{bc} \pm 0.01$
10 % Bell Pepper	$0.26^{b} \pm 0.00$
20 % Bell Pepper	$0.36^{a} \pm 0.05$

[a-c]: Data within the same column with same superscript are not significantly different (p>0.05) while data with different superscript are significantly different (p<0.05).

Figure 4.9: Color of sample extract after incubating with Na_2CO_3, ethanol and FC before subjecting to spectrophotometer

4.4 Sensory Evaluation

According to Table 4.8, out of five sensory attributes (appearance, aroma, taste, texture and overall acceptance), only consumer acceptance for the taste attribute showed significant difference ($p<0.05$) whereas consumer acceptability for the appearance, aroma, texture and overall acceptance were not significantly different ($p>0.05$). Consumer panelists tend to like and prefer noodles incorporated with 10 % of bell pepper more than others with the highest acceptability of 53.33 ± 5.32 mean score value. The taste attribute of noodles with 10 % of bell pepper was significantly different ($p<0.05$) from control and noodles incorporated with 5 % of bell pepper but not significantly different ($p>0.05$) from noodles incorporated with 20 % of bell pepper. In overall, the results showed that consumer panelists prefer control noodles as the overall consumer acceptability for control was the highest with a mean score value of 55.50 ± 6.89.

Table 4.8: Mean score value of sensory evaluation regarding appearance, aroma, taste, texture and overall acceptance of control and noodle samples incorporated with 5, 10 and 20 % of bell pepper

	Appearance	Aroma	Taste	Texture	Overall Acceptance
0 % Bell Pepper	57.00[a] ± 6.57	47.17[a] ± 5.91	44.50[b] ± 4.14	52.33[a] ± 8.29	55.50[a] ± 6.89
5 % Bell Pepper	50.17[a] ± 6.52	47.00[a] ± 4.47	45.67[b] ± 4.68	50.83[a] ± 5.27	50.67[a] ± 6.68
10 % Bell Pepper	51.33[a] ± 6.25	49.67[a] ± 5.75	53.33[a] ± 5.32	50.17[a] ± 3.76	50.50[a] ± 5.09
20 % Bell Pepper	47.83[a] ± 5.74	48.83[a] ± 4.54	48.33[ab] ± 4.41	48.17[a] ± 7.20	46.50[a] ± 6.25

[a-b]: Data within the same column with same superscript are not significantly different (p>0.05) while data with different superscript are significantly different (p<0.05).

CHAPTER 5

DISCUSSION

5.1 Shelf Life Determination

5.1.1 Microbial Analysis

5.1.1.1 Total Plate Count (TPC)

TPC results showed that control noodles and noodles incorporated with 5 and 20 % of bell pepper started to spoil at day 4, 8 and 6 respectively. Microbial count of noodles with 10 % bell pepper did not exceed 10^5 cfu/g (Australian Standard, 1993; Food and Drug Administration Philippines, 2013), so it was not considered spoiled yet. According to Table 4.1, starting from day 4 to day 8, the colonies formed on the plates of control were too numerous to count, no actual plates were available for colonies counting, hence their log cfu/g was recorded as more than 4.06. There are limited studies on the fluctuation of microbial count as indicated by noodles incorporated with 10 % of bell pepper, it may be due to the environment condition during preparation, cooking or storing process. Furthermore, it may be due to technical error while pipetting. Pipetting error may occur if an inaccurate amount of inoculum is pipetted and placed on the plates prior to spread plating where not exactly 0.1 mL of inoculum was pipetted. Thus, microbial count on the plates may be less accurate and inconsistent as the amount of inoculum pipetted was not accurate and consistent. If lesser amount of inoculum containing the potential bacteria was pipetted, there may be lower microbial count at day 6. Apart from that, improper way of sterilization of glass spreader prior to spread plating may also be

a factor in decreasing microbial count at day 6. The glass spreader was flamed using Bunsen burner to sterilize it before spread plating. After sterilizing, the glass spreader may not be cool enough and when the still-hot glass spreader may kill off some bacteria when it came in contact with inoculum to spread them evenly on the surface of the plates, leading to lower microbial count due to the death of some bacteria. Besides, the bacteria grow on this types of noodles at day 6 may be psychrophilic bacteria (bacteria that are able to grow under cold condition) as they were stored at a refrigerator at 4 °C. Therefore, incubation temperature of TPC (37 °C) might not be suitable to recover this type of bacteria. However, this argument needs to be further studied as there was limited research on this.

Supposedly, noodles incorporated with bell pepper should be able to inhibit microbial growth and has lower microbial count than control due to the presence of capsaicin. However, the results showed that the log cfu/g of noodles incorporated with 20 % of bell pepper was the highest at day 8. Researches performed by Li et al. (2013) and Li et al. (2014), they stated that large amount (approximately 61 %) of the capsaicin would be lost during preparation if directly incorporated into noodles. Boiling or cooking process may cause a moderate decrease in the capsaicinoids content (Loizzo et al., 2015). Othman et al. (2011) conducted a research to determine the amount of capsaicin and dihydrocapsaicin in *capsicum* sp. Their results revealed that the capsaicin amount (mg/g) in red bell pepper is negligible, reflecting the low concentration of capsaicin in bell pepper. This was strengthen by another statement by Bello, Boboye and Akinyosoye (2015) where

they stated that the concentration of capsaicin is negligible in sweet bell pepper but hot chili or jalapeno peppers contains much higher capsaicin content. As a consequence, the initially low concentration of capsaicin was reduced even more after preparation and cooking process as indicated by the studies of Li et al. (2013), Li et al. (2014) and Loizzo et al. (2015), leading to the inefficiency of bell pepper in inhibiting microbial growth due to level of capsaicin is too low to perform its antibacterial function. Furthermore, the moisture content of red bell pepper itself is around 90 % (90.1 g in 100 g) (Rufián-Henares, J., Guerra-Hernández, E. and García-Villanova, B., 2013), which is very high, hence after its incorporation into noodle, the moisture content may induce more microbial growth, leading to the higher log cfu/g of noodles incorporated with higher concentration of bell pepper. In addition to the high moisture content, bell pepper is also rich in nutrients, such as potassium and phosphorus (Jyoti, Syed and Pritee, 2014), providing a favorable condition for microbial growth and catabolic activity as a study by Tahseen et al. (2016) showed that a high amount of potassium and phosphorus were utilized by bacteria for their proliferation, leading to the higher microbial count of noodles with higher amount of bell pepper. In order to declare noodles are spoiled, the cfu/g needs to be higher than 10^5 cfu/g (Australian Standard, 1993; Food and Drug Administration Philippines, 2013) where in this case, noodles with 20 % bell pepper perished the most at day 8 (1.71×10^6 cfu/g). In addition, there may be some interaction between the basic ingredients of the noodles (wheat flour, salt and water) and composition of bell pepper that inhibit the bioactivity of the capsaicin in

constraining microbial growth. However, there was limited studies on the inhibitory effect of noodles ingredients on the antimicrobial property of capsaicin.

5.1.1.2 Yeast and Mold Test (YM)

Noodles incorporated with 5 and 20 % of bell pepper perished at day 8 and 6 respectively while control's fungal count did not the cut-off point (10^5 cfu/g) (Australian Standard, 1993; Food and Drug Administration Philippines, 2013) and noodles incorporated with 10 % of bell pepper started to perish at day 6 because the samples exceeded 10^5 cfu/g at that respective day. The accuracy of results of YM for the control and noodles incorporated with 10 % of bell pepper were less convincing. YM results for control was considered less accurate and it should follow the trend of TPC where the colonies formed should be too numerous to be count as well. According to Table 4.2, the log cfu/g of control in YM were decreasing from day 4 to day 8, which was absolutely not logic because as the day progressed, the microbial growth should be increasing, there should be increment in the log cfu/g of control (3.36) at day 4 and not reducing to less than log 3.06 cfu/g at day 8. This condition was again observed in the case of noodles incorporated with 10 % of bell pepper where there was also a decline in the log cfu/g (from 6.38 to 5.54 log cfu/g). All types of samples started to have fungi growing on the plates at day 4, including the noodles with the highest percentage (20 %) of bell pepper so there should be fungal count for noodles incorporated with 10 % of bell pepper starting at day 4. This condition and the decreasing of log cfu/g might occur due to pipetting error when pipetting the inoculum onto acidified

potato dextrose agar (PDA) plates. Hence, lesser amount of inoculum that contained the fungi may be cultured on the plates, leading to lesser fungal growth on acidified PDA plates. Moreover, while sterilizing the glass spreader during spread plating, the glass spreader may be still hot and not cool enough, hence killing off all or a portion of the yeast and mold when the hot glass spreader came in contact with the inoculum.

The reason why noodles incorporated with bell pepper had higher log cfu/g than the control may be similar to TPC. The noodle cooking process may cause the loss of capsaicinoids, causing reduction of the initially low concentration of capsaicin to even lower level, and hence losing its effectiveness in inhibiting microbial growth (Li et al., 2013; Li et al., 2014; Loizzo et al., 2015; Othman et al., 2011). Furthermore, bell pepper now with low or without efficiency in antimicrobial activities due to low level or even negligible capsaicin, the high moisture (90 %) and nutrient content (contains potassium and phosphorus that are required for microbial growth) may cause it to be more susceptible to fungal growth, hence having higher log cfu/g than control (Jyoti, Syed and Pritee, 2014; Rufián-Henares, J., Guerra-Hernández, E. and García-Villanova, B., 2013; Tahseen et al., 2016). Control might not be as rich moisture and nutrient content as the bell pepper, so there was lesser invasion of yeast and mold. Similar to TPC, there might be inhibitory interaction between noodles ingredients and microbial activity of capsaicin which results in the inefficiency of antimicrobial function of bell pepper. However, this needs to be further studied.

5.1.2 Water Activity (A_w) Test

There was no significant difference in the a_w of all samples including control (p>0.05) where they all shared a mean value of 0.978 (± 0.001) according to Table 4.3. This is because noodles belong to high moisture food (Li et al., 2012). This may be due to the cooking process of noodles, leading to its high moisture content after absorbing the boiling water to get cooked. Then, the cooked noodles were drained for 10 min to remove most of the water instead of drying in oven prior to storing in a 4 °C refrigerator, leading to the high availability of water in noodles. This is because this project was meant for homemade noodles, drying noodles in oven is inconvenient for most people as normally, they will just prepare, cook and store the noodles if there are any leftover. Unless, if he or she is interested to store the noodles for a certain period of time, then he or she may try to dry the noodles. However, this condition is a seldom case. A_w (0.978) of all the noodles was in conformity with a research performed by Li et al. (2011) where this research obtained a_w of 0.979 for their control noodle. This enhance the accuracy of the a_w obtained in this project. A_w of 0.978 is high enough for microbial growth because in order to inhibit the growth of most of the spoilage microorganisms, the a_w needs to be lower than 0.90 (Thomas et al., 2014). Incorporation of bell pepper did not cause any difference in the a_w of noodles, hence the noodles were still susceptible to microorganisms and enzymatic activities. A_w was a factor that contribute to the spoilage of all noodles samples including control because the higher the a_w, the more free water available for usage by microorganisms for their growth and metabolic processes, the higher the microbial count and thus, the faster the food

spoilage. Therefore, a_w of a food should be reduced to a level that microorganisms could not multiply to avoid early food spoilage (Arslan and Özcan, 2011).

5.2 Proximate Analysis

The data for proximate analysis, including determination of moisture, ash, protein, fat and fiber content were recorded in Table 4.4. By referring to Table 4.4, only ash and fiber content showed significant difference among all noodles samples (p<0.05), including the control. As shown in Figure 4.1, 4.2, 4.3 and 4.4, pinkish grey ash were observed for each sample. Normally, ash appeared as whitish grey. In this case, the pinkish grey color of the samples' ash may be due to the red coloration of bell pepper, contributed by capsanthin and capsorubin (Gomes et al., 2014), hence the leftover minerals (ash) may appear as pinkish grey color. Bell pepper is a good source of minerals, hence resulting in the increasingly ash content from the noodles incorporated with lower to higher concentration of bell pepper (5 to 20 %) where it significantly increased (p<0.05) from control (1.01 ± 0.09 %) to noodles with 20 % bell pepper (1.47 ± 0.01 %) (Bello, Boboye and Akinyosoye, 2015). Therefore, noodles incorporated with 20 % bell pepper has the highest ash content because it contained the highest percentage of bell pepper. This is because the minerals come from bell pepper, the more the bell pepper incorporated, the higher the ash content.

For fiber analysis, noodles incorporated with 20 % of bell pepper contained the highest percentage of fiber (0.58 ± 0.04 %) compared to other samples, including control.

However, the moisture, protein and fat content were not significantly different among all samples (p>0.05). For protein analysis, the samples were said to be completely digested when clear green solution were formed for each samples as shown in Figure 4.5 where nitrogen in the samples bind to the sulphate ion (SO_4^{2-}) contributed by the concentrated H_2SO_4 used for digestion of samples, forming ammonium sulphate ($(NH_4)_2SO_4$). The amount of nitrogen content was determined by quantifying the volume of 0.2 N HCL needed to change the color of blue solution to pink solution when end point was reached during titration process. By applying the calculation in Chapter 3 to determine protein content, it can be observed that the protein content slightly increased because there are traces of protein in bell pepper (Nadeem et al., 2011).

There was a slight decline in moisture content from control to noodles incorporated with 20 % bell pepper from (71.33 ± 7.25 %) to (67.46 ± 1.14 %). This may be due to technical error while draining the noodles for 10 min after cooking and prior to storage. Lastly, no fat was extracted from control and each sample so the value recorded was zero for all of the noodle samples, including control. Supposedly, there are little amount of saturated fatty acids in noodles (Ojure and Quadri, 2012), it might be too little to be detected.

5.3 Total Phenolic Test

Folin-Ciocalteau assay was carried out to determine the total phenolic content of control and noodles incorporated with 5, 10 and 20 % of bell pepper. Their total phenolic content were quantified as Gallic acid equivalences (mg/g). It can be observed in Table 4.7 that total phenolic content increased gradually from control to noodles incorporated with 20 % of bell pepper, indicating a significant difference (p<0.05) among them with the highest phenolic content was recorded as 0.36 ± 0.05 mg/g for noodles incorporated with 20 % of bell pepper, corresponding to their high antioxidant activity. The phenolic content of the noodles was in accordance to its antioxidant activity. Bell pepper is high in high phenolic compounds such as flavonoids (eg: quercetin and luteolin) as well as carotenoids (β-carotene) and oxygenated carotenoids (eg: capsorubin) (Chávez-Mendoza et al., 2015). Therefore, the higher the concentration of bell pepper incorporated, the higher the total phenolic content. This could be reflected by the results in Table 4.7. Blue color solution was observed as shown in Figure 4.9 due to reduction of FC agent into phosphotungstate and phosphomolybdenum, forming a blue chromophore (Blainski, Lopes and de Mello, 2013). The higher the intensity of the blue complex, the higher the total phenolic content as more phenolic compounds were reduced by FC reagent, hence developing more blue complexes. Spectrophotometer is used to measure the absorbance of the blue chromophores, hence the absorbance reflects the total phenolic content. The wavelength that needs to be set to read the absorbance varies among different product. In this case, the suitable wavelength to measure absorbance in noodles incorporated with bell pepper was 720 nm (Khare,

Biswas and Sahoo, 2014). The possible reason why wavelength was set at 720 nm is at this wavelength, the wavelength can be absorbed best by the phenolic compounds of bell pepper and showed the most accurate absorbance value, reflecting the total phenolic content of the noodle samples, including control.

However, by comparing to the results of a research on the total phenolic content of red bell pepper conducted by Shotorbani, Jamei and Heidari (2013), the phenolic content extracted from fresh red bell pepper was around 3.25 mg/g which was much more higher than the noodles with the highest phenolic content compared to others in this project, which was the noodles incorporated with 20 % of red bell pepper (0.36 ± 0.05 mg/g). In the research of Shotorbani, Jamei and Heidari (2013), the phenolic content was directly extracted from red bell pepper without incorporating into food matrix and cooking. This is because volatilization and thermal decomposition may be induced by the heat exerted during cooking process (Silva et al., 2013), leading to the loss of phenolic content. This argument is also supported by another study where it stated that significant losses of phenolic content would occur due to cooking because the cooking heat exerted on the food incorporated with phenolic compounds would dehydrate and reduce the stability of the food matrix when the food is completely in contact with the boiling water, leading to leakage of phenolic compounds from the food (Loizzo et al., 2015). According to Materska and Perucka (2005), low temperature would also exhibit negative effect on total phenolic content of the food. The ice crystals formed at low temperature may induce loss of phenolic compounds from food as the membrane of matrix is

damaged (Materska and Perucka, 2005). In this case, the phenolic content in bell pepper was first blended and incorporated into noodles. During this process, the oxidation of the phenolic content may occur, reducing the phenolic content. In addition to this, the noodles with bell pepper were subjected to cooking, leading to the loss of more phenolic content as this was proven by previous studies. This could be the reason that leads to the low efficiency of microbial inhibitory effect as discussed in TPC and YM. This is because phenolic compounds with antioxidant properties are able to cause the death of microorganisms by binding to the microbial cell wall, soluble and extracellular proteins to form complexes, destructing the cell wall and hence, killing the microorganisms (Bello, Boboye and Akinyosoye, 2015). As a result, the total phenolic content directly extracted from red bell pepper without any treatment (eg: cooking) has higher phenolic content than those subjected to treatment.

5.4 Sensory Evaluation

A total of 50 panelists participated in the sensory evaluation of control and noodles incorporated with 5, 10 and 20 % of bell pepper to evaluate their sensory attributes, which were appearance, aroma, taste, texture and overall appearance by using 9-scale hedonic test. Most of the panelists (35 of them) were female where there was only 15 male panelists who took part in this session. Due to the sensory evaluation was conducted at food science laboratory, majority of the panelists (40 of them) were students while 9 lecturers were invited to test the control and samples and there was 1 panelist who work as a microbiologist. Table 4.8 showed that out of all

five sensory attributes, only taste attribute was significantly different among control and samples (p<0.05). Panelists have highest acceptance for the noodle incorporated with 10 % bell pepper with mean score value of 53.33 ± 5.32. This may be due to the characteristic taste contributed by the bell pepper. However, they do not prefer noodle incorporated with 5 and 20 % bell pepper because they may think that taste contributed by 5 % bell pepper was too little to be traced while taste of 20 % bell pepper was too intense. As shown in Table 4.8, the mean value of appearance, aroma, texture and overall appearance for the control and each sample shared the same superscript, indicating they were not significantly different from each other (p>0.05). Panelists may find it hard to differentiate these four attributes because they were untrained. For some panelists, this was their first time taking part in a sensory evaluation. Due to lack of practice and unsure on how to evaluate and score the intensities of sensory attributes, majority of the panelists tend to score "5" (Neither like nor dislike) for each attribute. Although there was no significant difference (p<0.05) in the overall acceptance, but it can be observed that panelists preferred control more than others in overall with a mean score value of 55.50 ± 6.89, this may be because panelists are not ready to accept new flavored noodle as the mean score value of appearance (57.00 ± 6.57) and texture (52.33 ± 8.29) were also higher for control compared to other samples as well. Consumers may not be able to accept the reddish color of noodles contributed by the capsanthin and capsorubin of red bell pepper as well as the texture of noodles after incorporation of bell pepper.

5.5 Future Study

A research by Othman et al. (2011) showed that there was negligible amount of capsaicin in bell pepper, contributing to the condition that bell pepper is less effective in inhibiting microbial growth. Therefore, for future study, bell pepper can be replaced by other *capsicum* sp. which has higher capsaicin content, so it can do a better job in prolonging shelf life of noodles due to its better microbial inhibitory effect. For instance, hot chili or jalapeno peppers which contain higher concentration of capsaicin (Bello, Boboye and Akinyosoye, 2015) may act as an alternative to bell pepper as natural preservative in noodles or other kind of food with lower moisture content.

Due to higher concentration of capsaicin incorporated, to prevent the capsaicin from causing too much spiciness in the mouth, retention of the capsaicin should be carried out. In order to achieve this, layered noodles can be prepared by having capsaicin-enriched dough as the middle layer, getting sandwiched by two layers of gastro-resistant wheat flour dough. As a result, this suits consumers who cannot withstand the intense spiciness caused by the high capsaicin content in the mouth while chewing because capsaicin-enriched layered noodles enables capsaicin to be released only when it reached stomach and small intestine (Li et al., 2011).

Three layers of noodles may be too tough for certain consumers. Therefore, soy protein isolate and microbial transglutaminase can be added to soften the texture of the noodles to a desirable level that can accepted by the consumers (Li et al., 2011).

CHAPTER 6

CONCLUSION

In conclusion, incorporation of red bell pepper (*capsicum annum* L.) into homemade noodles was found out to be less effective in prolonging the noodles' shelf life, indicating the low efficiency of microbial inhibitory effect of capsaicin due to loss of capsaicin during noodles preparation. TPC and YM assays showed that the newly formulated noodles incorporated with 5, 10 and 20 % of bell pepper started to have microbial count at day 4, showed no difference compared to control which also had microbial growing at the same day but control had higher microbial count. The microbial growth for noodles with the highest concentration of bell pepper (20 %) was highest at day 8 where its count for bacterial and fungal growth as determined by TPC and YM assay were recorded as 6.23 and 7.04 log cfu/g respectively. Water activity for all the samples, including control shared mean value of 0.978 ± 0.00, indicating the susceptibility of noodles to microbial spoilage.

Nutritional value in term of ash and fiber content of noodles increases along with incorporation of bell pepper into noodles. Ash and fiber content showed significant increase ($p<0.05$) in noodles as more bell pepper was incorporated. Noodles with 20 % bell pepper contained highest ash (1.47 ± 0.01 %) and fiber content (0.58 ± 0.04 %) compared to the ash (1.01 ± 0.09 %) and fiber content (0.14 ± 0.01 %) of control. However, all samples did not showed significant difference ($p>0.05$) in moisture, protein and fat content.

Noodles with 20 % of bell pepper contained highest total phenolic content (0.36 ± 0.05 mg/g) as bell pepper is rich in phenolic compounds (eg: flavonoids), showing highest antioxidant activities. Antioxidant activities can be related to antimicrobial activities as binding of antioxidants to microbial cell wall can disrupt the cell wall, killing the microorganism and can prolong the shelf life of food product.

For sensory evaluation, although results showed that consumers prefer the taste of noodles with 10 % of bell pepper (53.33 ± 5.32 mean score value) with a significant difference ($p<0.05$) from control and noodles with 5 % of bell pepper, consumers may not be ready to accept noodles with bell pepper because the overall acceptance was the highest for control (55.50 ± 6.89 mean score value). This may be due to they could not accept the appearance and texture after incorporating noodles with bell pepper as for control, these two attributes had highest mean score value of (57.00 ± 6.57 mean score value) and (52.33 ± 8.29 mean score value) respectively.

Thus, the outcome of this project suggested that red bell pepper is not an effective natural preservative in prolonging shelf life of homemade noodles because the initially low capsaicin content in red bell pepper is reduced to a negligible level after preparation and cooking process, hence reducing its efficiency in microbial inhibitory effect. Although noodles with highest concentration of bell pepper (20 %) had highest total phenolic content, the amount may not be significant enough to inhibit microbial growth. There may be some loss of phenolic compounds through cooking, oxidation and low temperature during storage.

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APPENDIX

QUESIONNAIRE OF SENSORY EVALUATION

Panel No. : Date :

Gender :

Age :

Occupation :

Product: Homemade Noodles

Instruction: You are given four coded samples. Kindly evaluate these samples in terms of appearance, aroma, taste, texture and overall acceptance. Please rate how much you LIKE or DISLIKE each other according to the scale given below for the respective sensory attribute.

9 Like Extremely

8 Like Very Much

7 Like Moderately

6 Like Slightly

5 Neither Like Nor Dislike

4 Dislike Slightly

3 Dislike Moderately

2 Dislike Very Much

1 Dislike Extremely

Please rinse your mouth with water before tasting each sample. Evaluate the samples starting from the left.

Sensory Attribute	**Sample Code**			
Appearance				
Aroma				
Taste				
Texture				
Overall Acceptance				

Comments: _____

Thank You.