COMPARISON BETWEEN PRIMARY METHOD ANALYSIS AND NIR ANALYSIS IN DETERMINATION OF NUTRIENT CONTENT IN TEMPEH

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JUDUL **COMPARISON BETWEEN PRIMARY METHOD ANALYSIS & NIR ANALYSIS IN DETERMINATION OF NUTRIENT CONTENT IN TEMPEH**

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COMPARISON BETWEEN PRIMARY METHOD ANALYSIS & NIR ANALYSIS IN DETERMINATION OF NUTRIENT CONTENT IN TEMPEH

NUR HAZWANI BINTI ROSLI

A thesis submitted in partial fulfillment of the
requirements for the award of the degree of
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APRIL, 2010

I declare that this thesis entitled "*Comparison between primary method analysis & NIR analysis in determination of nutrient content in tempeh*"is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special dedication to my beloved
father and mother

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ABSTRACT

The objective of this study is to compare the analysis of nutrient content in tempeh by using primary method analysis and Near Infrared (NIR) analysis and the nutrient content utilized from using different types of packaging also been compared. The nutrient content that is analyzed is protein content, fat content, fiber and also ash content. Random samples from Kuantan area were taken to be analyzed with primary method analysis and NIR analysis. Tempeh samples with different types of packaging also been analyzed on the nutrient content. From here the nutrient utilized in each types of packaging were compared. From the results, it shows that there are similarities on the analysis between conventional method and NIR analysis. The percentage differences are about 2 to 20% difference depending on the errors during the analysis. The results also show that the plastic packaging utilizes lower protein content than paper packaging but preserved longer by using plastic packaging.

ABSTRAK

Objektif kajian ini dijalankan adalah untuk mengkaji kandungan nutrisi yg terkandung didalam tempe dengan menggunakan analisa kaedah utama dan juga Near Infrared (NIR). Objektif kedua kajian ini adalah untuk mengkaji perbezaan kandungan nutrisi yang akan terhasil jika tempe dibungkus dengan menggunakan 2 jenis pembungkusan yang berbeza iaitu pembungkusan dengan menggunakan kertas dan juga menggunakan plastik. Kandungan nutrisi yang dikaji didalam kajian ini adalah protein, lemak, fiber dan juga abu. Sampel yang dikaji diambil secara rawak di sekitar kawasan Kuantan. Analisa untuk sampel tempe yang berlainan jenis pembungkusan kemudiannya akan dibandingkan. Daripada keputusan kajian yang dilakukan, terdapar persamaan nilai kandungan nutrisi diantara analisa kaedah utama dan juga kaedah NIR. Peratus perbezaan antara kedua jenis analisa adalah didalam lingkungan 2 hingga 20%. Kajian juga menunjukkan bahawa pembungkusan kertas dapat menaikkan kandungan nutrisi didalam tempe, namun begitu, untuk memastikan kandungan nutrisi ini dapat bertahan lebih lama, pembungkusan secara plastik adalah lebih baik.

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LIST OF SYMBOLS/ABBREVIATIONS

NIR	-	Near-Infrared
mL	-	Milliliter
g	-	Gram
mg	-	Milligram
µg	-	Microgram
%	-	Percentage
ºC	-	Degree celcius
M	-	Molar
kg	-	Kilogram
nm	-	Nanometer
NaOH	-	Sodium Hydroxide
H_2SO_4	-	Sulfuric acid
MARDI	-	Malaysian Agricultural Research and Development Institute

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Tempeh is a traditional fermented soybean food. It is a fermented food made by the controlled fermentation of cooked soybeans with a Rhizopus mould as the tempeh starter. The tempeh fermentation by the Rhizopus mould binds the soybeans into compact white cake (Tempeh 2009,25 June). It is made by cooking and dehulling of soybeans and inoculation with different strains of Rhizopus (*R. oligosporus, R. oryzae, and R. stolonifer*) which will lead to solid substrate fermentation (Steinkraus K.H., 1983). Fermentation also influences the content of desirable constituents such as vitamins, protein and fatty acids (Baumann U., 1995).

Tempeh provides the staple food for a large population in Indonesia and Malaysia. Like tofu, tempeh is made from soybeans, but tempeh is a whole soybean product with different nutritional characteristics and also different textural qualities. Tempeh's fermentation process and its retention of the whole bean give it higher content of protein, dietary fiber and vitamins compared to tofu, as well as firmer texture and also with stronger flavor. Because of its nutritional value, tempeh is used worldwide in vegetarian cuisine. Tempeh is a low cost nutritious food and can be consumed by all socio-economic groups.

Figure 1.1 : A piece of uncooked tempe

Table 1.1: Nutritional table for 100g of Tempe

Water	54.9	g
Energy	199	kcal
Energy	833	kJ
Protein	19.0	g
Fat	7.7	g
Sat. fatty acids	1.11	g
Mono-unsat. fatty acids	1.7	g
Poly-unsat. fatty acids	4.3	g
Carbohydrates	17.0	g
Fiber	4.8	g
Ash	1.4	g
Isoflavones	53	mg
Calcium, Ca	93.0	mg
Iron, Fe	2.3	mg
Magnesium, Mg	70.0	mg
Phosphorus, P	206	mg

Potassium, K	367	mg
Sodium, Na	6.0	mg
Zinc, Zn	1.81	mg
Copper, Cu	0.67	mg
Manganese, Mn	1.43	mg
Selenium, Se	8.8	µg
Vitamin C	0.0	mg
Thiamine (B1)	0.131	mg
Riboflavin (B2)	0.111	mg
Niacin (B3)	4.63	mg
Panthotenic acid (B5)	0.355	mg
Vitamin B6	0.299	mg
Folic acid	52.0	µg
Vitamin B12	1.0	µg
Vitamin A	69	µg
[Source: USDA Nutrient Database for Standard Reference]		

From Table 1.1, we can see that tempeh contains a lot of nutrient content that can give a lot of benefits to human body. The most noticeable nutrient content is the amount of protein in the tempeh. With the large amount of protein in tempeh, it makes tempeh popular with the health conscious consumer.

With the numerous and the richness of nutrition in tempeh, it rapidly becomes popular. This is because market nowadays demands for the quality of the food itself

which refers back to the nutrient content of the food. Customers nowadays are very well educated and also knowledgeable. However, in Malaysia the production of tempeh is mainly from the small scale industries with traditional way of making. Therefore, the specific facts and also figures of the nutrient content in the traditionally made tempeh are still uncertain due to some reason such as contamination.

1.2 Problem Statement

A lot of products in the market nowadays have their own nutritional labelling attach to their packaging. This gives the customers choices on how to choose their food preferences. However, as in the previous part, major production of tempeh in Malaysia is made traditionally. Therefore, there is no nutritional labelling that is attached to the packaging. This is because; there are no proper scientific data of the nutritional facts on tempeh from the entrepreneurs (small scale industrialist) themselves.

Nowadays tempeh has been gaining lots of attention from either local or overseas researchers on its nutrient content. There are a lot of research planned or been done on tempeh. The main reason for this attention is because of its high nutrient content. There has been research done on the nutrient content of tempeh. However, the research was done under the perfect condition of tempeh and under controlled condition. For this study, the tempeh that will be studied are made from the small scale producer from Kuantan area.

The perfect condition for tempeh is about 36-48 hours after fermentation. That is where the nutrient content of tempeh is at its peak condition. However, in this study also, the nutrient content of aged tempeh also will be studied. Since the utilization of the nutrient content in tempeh is actually from the fermentation process,

this study will also check on the effect of fermentation time to the nutrient content in tempeh.

For the determination of nutrient content in tempeh, the method use is by using physical method which is by using Near-Infrared (NIR). However, before the analysis by using NIR can be done, the tempeh samples must first be analyzed by using primary method to set a standard analysis in NIR. However, after setting the standard analysis and analyzing the tempeh samples by s using NIR, there are slight difference on the result between NIR analysis and primary method analysis. In this study, the difference of the analysis will be analyzed.

1.3 Objectives

The objective of this study is to determine the nutrient content of tempeh and also to compare between NIR analysis and primary method analysis. The comparison of nutrient content of tempeh with different packaging such as plastic packaging and paper packaging is also to be studied.

1.4 Scope of Study

To achieve the objectives of this study, these scopes have been identified. The scopes are

i) Focus on the small scale traditional producer
ii) Focus on Kuantan area tempeh producer
iii) Analyzing by using Near-Infrared and primary method
iv) Analyzing between plastic packaging and paper packaging on the nutrient content of tempeh

v) 1 week of fermentation time for analyzing on the effect on the nutrient content

vi) Nutrient element to be studied

a. Protein
b. Fat
c. Fiber
d. Ash

1.5 Rational and Significance

Although the study on the nutritional content of tempeh have been done worldwide, and tempeh has been gaining its popularity among the health conscious customers, in Malaysia, as been told in the previous part major production of tempeh is made traditionally by the small scale industries. Therefore there is not much information that is given to the industrialist about the nutritional facts. They also usually use their own instinct and experience to determine the amount of tempeh starter or vinegar that should be put for the soaking or fermentation process.

Near Infrared (NIR) also has been gaining attention from the industries as the equipment is actually a faster route to do the analysis of nutrient content of samples. However, the analysis using NIR is not yet being approved as one of the reliable analysis to determine the chemical content in the samples. By doing this study, the difference between the primary method analysis and NIR analysis can be studied and analyzed.

By doing this study also, it can helps the small scale industrialist. This is because by using the results from the study, the exact amount of nutrient content in the tempeh produce by these small scale industrialists can actually be determined. From here, the nutritional facts of tempeh produced can be slowly formed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Consumers nowadays usually demand for a wide variety of product which is in high quality, nutritious and also will offer a good value to them. The consumers also very concerned on the safety of the food product that they are buying which we can see nowadays they are testing of food for allergies, pesticides residues and also products from genetic modification of food materials. Many consumers are very interested in the relationship between diet and health. That is why the nutritional facts are very important for every product because these consumers will utilize the nutrient content and health claim information from the food labels to make purchase choices (Kirchner E. M., 1997).

Nutrition labelling regulations differ in countries around the world. In United States, the Food and Drug Administration have made regulations in 1973 where the food must be labelled with regard to their nutritional value. The nutrition label included the following: serving size, number of servings per container, calories per serving, grams of protein, carbohydrate, fat per serving and also percentage of U.S. Recommended Dietary Allowance (USDRA) per serving of protein, vitamins, thiamine, riboflavin, niacin, calcium and iron (Schultz H. W., 1981).

In Malaysia, there are Malaysian Food Act 1983 and Food Regulations 1985, which protect the public against hazard and fraud in preparation, sale and use of

food. Currently, the Food Regulations does not require mandatory nutrition labelling for food products, except for special purpose foods such as infant formula and cereal based foods and food that have been enriched or fortified (Kementerian Kesihatan Malaysia 2009, 23 August). Even though in Malaysia there are no specific regulations on the nutritional labelling, the labelling is very important so that the product can create a competitive advantage in the market. Tempeh's product that is produced by the small scale industrialist did not have the nutritional labelling which makes the consumers hesitate whether to buy the product that have the nutritional labelling or not. They are also less competitive because of this lack of information.

2.2 Processing steps of tempeh

From the website on the tempeh production, it stated that there are five processing steps of making tempeh (Tempeh 2009,25 June). The steps are:

1. Cracking the soybeans
2. Soaking and dehulling the soybeans
3. Cooking the soybeans
4. Inoculating the soybeans with tempeh starter
5. Incubating the beans (fermentation process)

2.2.1 Cracking the soybeans

Before the soybeans can be used to make tempeh, the soybeans have to be cracked in half. This will make the process of dehulling easier. To crack the soybeans, usually a loosely set of grain mill is used. There are also stores that sell dehulled soybeans.

2.2.2 Soaking and dehulling soybeans

After cracking the soybeans, the soybeans then soaked in water for 6 to 18 hours or overnight. During the soaking period, the hulls of the soybean will rise at the surface of the soaking water when the soybeans were squeeze by hands with a kneading motion. The water then poured off with the hulls. Then fresh water is added and the step is repeated.

During the soaking process, the first stage of fermentation process also happens. The spontaneous and uncontrolled fermentation of soybeans occurs during the soaking stage where it results because of the fungal fermentation. Usually the fermentation results in an acidification of the beans. The fermentative acidification during soaking inhibits the multiplication of spoilage. However the acidification during the soaking process can be controlled by recycling part of the soak water from the previous batch as inoculums which will result in the soak water pH to be 4.1-4.9 depending on the soaking temperature and recycling rate (M. J. R. Nout, 1987).

2.2.3 Cooking the soybeans

The beans are put in a cooking pot and also water to cover the soybeans. Add the vinegar before cooking the beans and after that the beans were cooked for about 30 minutes. After that the water were drained off and dried the soybeans by continue heating them in the pot on medium heat for a few minutes and until the beans are dry. The soybeans then allowed to cool down to below 35°C.

2.2.4 Inoculating the soybeans with tempeh starter

The soybeans then sprinkled with tempeh starter. The tempeh starter and the beans then mix with a clean utensil to distribute the tempeh starter evenly. To reduce the risk of spoilage and make the fermentation faster, the tempeh starter must be mix very well with the beans.

Figure 2.1: Tempeh starter

Tempeh starter, also called powdered tempeh starter (PTS), is a dried mixture of live Rhizopus spores with substrate, which can be soybeans or rice. Tempeh starter will push the process into the desired direction. In tempeh fermentation, to produce good quality tempeh, tempeh starter with a very high count of desirable Rhizopus molds is needed. Tempeh can be produced by two Rhizopus strains: Rhizopus oryzae or Rhizopus oligosporus, both of which can be isolated from fresh Indonesian tempeh (Tempeh 2009,25 June).

Tempeh starter can be divided into two types which is Indonesian style (traditional) and Western style (modern). In Indonesia, where tempeh originated and is still produced in small tempeh shops, the tempeh master always uses dried tempeh starter. They make it by placing a handful of cooked and inoculated soybeans between two hibiscus leaves, allowing them to incubate for a few days until the soybeans are covered with black spores and finally drying them in the sun. They use this starter by rubbing the hibiscus leaves above the soybeans to be inoculated. As you can understand, this type of tempeh starter can easily be contaminated with other molds or bacteria. However, the climatic conditions in Indonesia are so ideal for tempeh fermentation that this type of contamination is not known to cause problems.

In Western countries, where tempeh production is rather new, tempeh factories always use pure cultures to make sure that the quality of the finished tempeh is consistent and to minimize the risk of failed batches. There are no specific legal standards for tempeh starter, but good quality tempeh starter should contain millions of Rhizopus spores, contain no contaminating, coliform or pathogenic bacteria. Tempeh starter is often extended with sterile rice flower or starch to standardize the spore count.

2.2.4.1 Rhizopus Moulds

Tempeh contains natural antibiotics. This is because the Rhizopus moulds in the tempeh starter produce natural, heat-stable antibiotic agents against some disease-causing organisms. The process of fermentation makes the soybeans softer, since enzymes produced by the mould predigests a large portion of the basic nutrients. The Rhizopus moulds produce an enzyme phytase which breaks down phytates, thereby increasing the absorption of minerals such as zinc, iron and calcium (Tempeh 2009,25 June).

2.2.5 Incubating the beans (fermentation process)

The well mixed soybeans and also tempeh starter then put into plastic bags. The plastic bags have been perforate with holes at a distance of about 1 cm. This is done to make sure that the mould could breathe. The packed beans then placed in an incubator at 30°C or at a warm place for about 36 to 48 hours. During this time, tempeh fermentation will takes place. Then the container should be filled completely with white mycelium and the entire contents can be lifted out as a whole piece.

Processing of soybeans into tempeh (fermentation process) brought about favorable nutritional changes including reduction in the level of phytic acid, starch and the flatulence-causing oligosaccharides stachyose and raffinose; whereas thiamine concentrations were reduced, riboflavin and nicotinic acid contents increased during fermentation (W. B. Van der Piet, 1987).

2.3 Nutrient Content of tempeh

Even though tempeh can be categorized as one of the cheap, basic foodstuff in Indonesia and also in Malaysia, it contains a lot of nutrient that is very good for human health. The fermentation process that is used to make tempeh influences the nutrient content. The increased content of some vitamins of the B-group, especially riboflavin, niacin, vitamin B6 and vitamin B12 is due to the fungal and bacterial activities during the fermentation process. Even though the finished tempeh contains high nutrient content, it stated in the research on the changes of the content of fat during tempeh fermentation that there are some nutrient loses from the finished tempeh due to the preparatory treatment of soybeans before the fermentation process (J. Denter, 1998).

2.3.1 Protein

Protein is one of the main components in soybean. From Table 1.1 in chapter 1, we can see that there are slight differences in the protein content in raw soybeans and also in tempeh. In raw soybeans, there are about 36.49g of protein in it while in tempeh the amount is reduced to 19g. This happens because of the process happens in between before the soybeans become tempeh. Proteins are an abundant component in all cells, and almost all except storage proteins are important for biological functions and cell structure. Food proteins are very complex. They are

composed of elements including hydrogen, carbon, nitrogen, oxygen and sulfur. (S. Suzanne Nelson, 2003). The protein in tempeh is excellent for diabetic patients, who tend to have problem with animal sources of protein. The protein and fiber in tempeh can also prevent high blood sugar levels and helps in keeping blood sugar level under control.

Protein serve as the building material of muscles and other animal tissues and in plants, they play crucial metabolic roles as enzymes and enzyme inhibitors, participate in the transport and binding of oxygen and metal ions and perform immunological functions. During their development, cereal grain and legume seeds deposits large quantities of storage proteins in granules known also as protein bodies. In soybeans, these proteins constitute 60-70% of the total protein content and the granules in 80% are made of proteins (Zdzislaw E. Sikorski, 2002).

In a research paper wrote by Sparringa and Owens, the research paper mainly aims on to identify until to what extent the proteins were utilized during the fermentation process of tempeh. The experiment was done by fermenting bacteria free tempeh which is prepared with acidified soybeans cotyledons and *Rhizopus oligosporus* at 30 degree C. From here, the protein oxidation which is estimated from the ammonia production, was 5g at 24hours, 10g at 46 hours. The total amount of soy protein hydrolyzed, including the one that is incorporated into mould biomass, was estimated to be 80g at 28 hours incubation, 95 g at 46 h, and 100 g at 72 h. The hydrolyzed protein at 46 h represented 25% of the initial protein. Of this hydrolyzed protein, it is suggested that approximately 65% remained in the tempeh as amino acids and peptides, 25% was assimilated into mould biomass, and 10% was oxidized (Sparringa R. A., 1999).

2.3.2 Ash

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. Ash content represents the total mineral content in foods. The ash content of most fresh foods rarely is greater than 5%. Pure oils and fats generally contain little or no ash, products such as cured bacon may contain 6% ash, and dried beef may be as high as 11.6%. Fats, Oils and shortenings vary from 0.0 to 4.09% ash, while dairy products vary from 0.5 to 5.1%. It would be expected that grain and grain products with bran would tend to be higher in ash content than such products without bran. Nuts and nut products contain 0.8-3.4% ash, while meat. Poultry and seafood contains 0.7-1.3%. In finished tempeh there is about 1.4g of ash (S. Suzanne Nelson, 2003).

2.3.3 Moisture

In soybeans : Water 8.54 g

In tempeh :Water 54.9 g

(Source : USDA Nutrient Database)

As we can see from the nutrient table of both raw soybeans and also tempeh, we can see that the water content greatly increases from before the fermentation process and after the fermentation process to become tempeh. Because of high water content in tempeh makes it easily to cause spoilage. This is because the microbial growth has always linked to the water activity. This is why when preparing the soybean to be fermented, it is very crucial to make sure that there are no contamination happens to avoid spoilage.

2.3.4 Fat

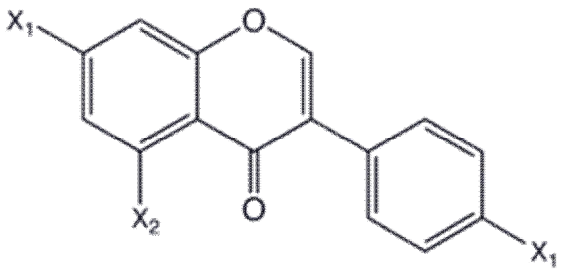
Fats consist of a wide group of compounds that are generally soluble in organic solvents and largely insoluble in water. Chemically, fats are generally triesters of glycerol and fatty acids. Fats may be either solid or liquid at normal room temperature, depending on their structure and composition. Although the words "oils", "fats", and "lipids" are all used to refer to fats, "oils" is usually used to refer to fats that are liquids at normal room temperature, while "fats" is usually used to refer to fats that are solids at normal room temperature. "Lipids" is used to refer to both liquid and solid fats, along with other related substances (Maton Anthea, 1993). Fats generally refer to the lipids that are in solid at room temperature.

Foods contain many types of lipids, but those which tend to be the greatest importance are the triacylglycerols and the phospholipids. Liquid triacylglycerols at room temperature are referred to as oils, such as soybean oil and olive oil and generally are from plant origin. Solid triacylglycerols at room temperature are termed as fats (S. Suzanne Nelson, 2003). Fats impart physical properties to foods and thereby affect the sensory, nutritional, safety and storage characteristics (Richard O. A., 2005).

2.3.5 Fiber

Tempeh contains high fiber content. One serving of tempeh (100g) contains more fiber than most people’s consume in one day. Fiber is essential for a healthy digestive tract as well as preventing many chronic diseases.

2.3.6 Isoflavones



Isoflavone (X_1 and X_2 = H),

genistein (X_1 and X_2 = OH) and

daidzein (X_1 = OH, X_2 = H).

Figure 2.2 : Structure of Isoflavones

Isoflavones are phytochemicals, which are compounds found only in plants. They are also a type of phytoestrogen that resembles human estrogen in chemical structure yet are weaker. By mimicking human estrogen at certain sites in the body, isoflavones provide many health benefits that help you to avoid disease. Isoflavones are found in soybeans, chick peas and other legumes. However, soybeans are unique because they have the highest concentration of these powerful compounds.

Table 2.1 : Isoflavones levels in some foods (per 100g):

Tempeh	**43.52 mg**
Peas bean	2.42 mg
Peanuts	0.26 mg
Navy bean	0.20 mg
Chickpeas	0.10 mg
Lentils	0.10 mg
Bread	0.02 mg
Black bean	0.00 mg

(Source:USDA-Iowa State University Database on the Isoflavone, Rel. 1.3 - 2002)

From the table above we can see that tempeh (fermented soybeans) contains the highest isoflavones. Some of the health benefit of isoflavones are they can lower the cancer risk, improved bone health, relieves menopause symptoms and also lowers cholesterol.

Soy contains many isoflavones, but the most beneficial are Daidzein (Da) and Genistein (Ge). When the tempeh is fried, the Da and Ge contents significantly decreased as much as 21% and 58%, respectively, compared to raw tempeh. Heat applied during tempeh frying caused the decarboxylation of the compounds (Hasnah Haron, 2009).

2.3.7 Carbohydrates

Carbohydrates are important in foods as major source of energy, as imparters of crucial textural properties, and as dietary fiber which influences physiological processes. Digestible carbohydrates, which are converted into monosaccharides, which are absorbed, provide metabolic energy. Worldwide, carbohydrates account for more than 70% of the caloric value of the human diet. It is recommended that all person should limit calories from fat(the other significant source) to not more than 30% and that most of the carbohydrate calories should come from starch (S. Suzanne Nelson). Therefore, from the amount of carbohydrates contained in tempeh and also how these carbohydrates came from natural sources and starch, tempeh could and should be one of the choices that people will choose to make a healthy diet.

2.4 Near Infrared (NIR)

Near infrared (NIR) spectroscopy extracts usable information from the absorption spectral characteristic of a sample irradiated by light in the NIR region (G. Dotzlaw, 1993). The NIR region (780 – 2500 nm) is dominated by overtone and combination bands of fundamental vibrations occurring in the mid infrared.

It has been recognized that NIR reflectance is sensitive to particles size, shape and distribution of powders of granular samples (W. W. Wendtlandt, 1966). A sensor that has sensitivity to two measureable quantities is said to exhibit cross-sensitivity. For these sensors, calibration involves maximising the wanted and minimising the unwanted signal. For primary NIR reflectance applications, i.e. determining the chemical compounds in granular or powdered samples, particles size effects on the spectra are considered as the unwanted signal or noise. Therefore, to ensure adequate precision in quantitative chemical analysis, the particles size effects

are reduced by grinding the sample finely to a near-uniform size followed by proper sampling (E. W. Ciurcza(1986)k, P. C. Williams(1987)).

The cross sensitivity of NIR can be exploited for particle size analysis. Due to its proven reliability and speed in multi-constituent monitoring and control (A. Robertson, 1989), and the availability of fiber optic probes, NIR reflectance is receiving renewed interest as a potential online sensor for particles size. Because only one sensor is needed to monitor both chemical constituents and particles size (J. L. Ilari, 1988), NIR reflectance has advantages in powder analysis over other methods.

2.4.1 Principle of NIR

In NIR diffuse reflectance spectroscopy, the requirements of classical absorption spectroscopy are not completely satisfied because the sample is non-homogenous and scattering (W. F. McClure, 1994). The theory of NIR reflectance spectroscopy is not fully defined, but empirical results show that Beer-Lambert's law holds, at least in principle (W. F. McClure1994).

An NIR beam incident on a powdery or granular material of a weakly-absorbing medium, thick enough to prevent transmission, will penetrate the layer and its direction will be changed each time a particle boundary is encountered. The changes in direction are a result of reflection, refraction and random diffraction at the surface of various particles. The combination of these effects is called light scattering. As scattered light encounters more boundaries of particles, further scattering occurs in all directions and part of it is absorbed. Scattering and absorption occurs simultaneously in the layer until finally the remaining attenuated light re-emerges from the entry surface. This light is called diffuse reflectance (G. S. Birth, 1987).

Each time the radiation interacts with a sample particle, the chemical constituents in the sample can absorb a portion of the radiation. Therefore the diffusely reflected radiations contain information about the chemical composition of the sample, as indicated by the amount of energy absorbed at specific wavelengths (S. Suzanne Nelson, 2003).

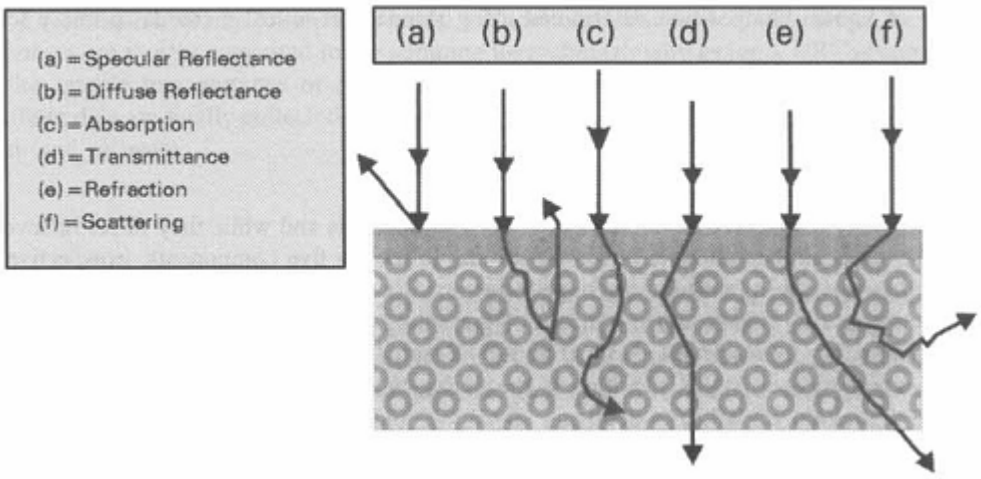


Figure 2.3: Interaction of Near infrared radiation with solid particle in a sample

2.4.2 Application of NIR to Food Analysis

Theory and applications of NIR spectroscopy to food analysis has found its biggest use in the grain, cereal product and oilseed processing industries. NIR techniques using reflectance measurements from ground or powdered samples have been adopted as approved methods of analysis by the American Association of cereal chemist (AACC, 2000.). NIR also can be used for other food products to measure moisture, protein, lactose, monitoring sugar content (S. Suzanne Nelson, 2003).

CHAPTER 3

METHODOLOGY

3.1 Introduction

The overall methodology that is used in this study is:

i) Preparation of tempeh samples.
ii) Standard analysis for tempeh samples.
    a. Protein
    b. Fiber
    c. Fat
    d. Ash
iii) Analysis of tempeh samples using NIR.

The methods used were given by Malaysian Agricultural Research and Development Institute (MARDI)

3.2 Preparation of tempeh samples

In order to do the analysis for the study, the samples are prepared for two purposes:

i) Standard analysis
ii) Analysis using NIR

For the standard analysis by using primary method, the samples were taken from a few kiosks from Kuantan area. These samples then will be duplicate to make sure that the analysis is as accurate as possible. These samples will be analyzed by using the method chosen according to the suitability and availability of materials. A few of the methods are suggested by MARDI. From the results taken from the primary analysis, the results will then be used to do the analysis using NIR where the primary analysis results are used as the standard in the NIR.

For the NIR analysis, the samples were taken from random producers or kiosk from Kuantan area. These samples were taken randomly as for the determination of nutrient content in the tempeh samples that is produced by the small scale producers around Kuantan area.

3.3 Standard analysis for tempeh samples

In this study there are four (4) types of nutrient content that will be studied. The nutrient contents are :

a. Protein
b. Fiber
c. Fat
d. Ash

3.3.1 Standard analysis for Protein

This method is called Protein Determination using Kjeldahl Method. This method can be applied to few type of food samples such as from animal origin, grains and cereals and also legumes.

Reagents:

a. Sulphuric acid (98%)
b. Kjeldahl tablet (5 g each)
c. Sodium Hydroxide
d. pH indicator (0.5 g Bromothymol Blue in 500 mL ethanol (95%) and 500 mL distilled water)
e. Activated charcoal (granular)
f. Boric Acid (4%)

Material and equipment:

a. Kjeldahl digestion and distillation apparatus
b. 500 ml Kjeldahl flasks
c. volumetric flask

Method:

Digestion

Parameter	< 0.5 g sample	1 g sample	< 5 g sample
Sulphuric Acid (98%)	10 mL	20 mL	30 mL
Kjeldahl Tablet (5 g each)	1	2	3
Warm up time	30 min	30 min	30 min
Digestion time	90 min	90 min	90-120 min

Table 3.1: Amount for digestion process

Scrubber

1. To prepare 5 L of sodium hydroxide (8%) (NaOH) : Dissolve 400 g NaOH in 5 L distilled water
2. Add pH indicator
3. Add activated charcoal (granular)

Distillation

1. To prepare 1 L of boric acid (4%) : Dissolve 40 g boric acid in 800 mL distilled water. Adjust to pH 4.65 using NaOH (10 %). Fill up to 1 L with distilled water
2. Tp prepare 1 L sulphuric acid (0.25 M) : Add 13.3 mL sulphuric acid (98%) into volumetric flask and make up to 1 L with distilled water
3. Tp prepare 500 mL NaOH (10 %) : Dissolve 50 g NaOH in 500 mL distilled water

Water	50 mL
NaOH (32%)	90 mL
Reaction time	5 s
Distillation time	240 s
Steam power	100%
Stirrer speed	5^5

Table 3.2: Standard setting on the equipment

Titration

1. Titrate distillate sample from KjelFlex with sulphuric acid (0.25 M) and stop once it reaches slight purple or pH 4.65
2. Record the volume of titrant used

Calculation of % Nitrogen

For 0.25 M sulphuric acid or 0.5 M hydrochloric acid :

$$\% N = \frac{(V_{sample} - V_{blank})\ mL \times 0.05 \times 14.0067}{\text{Weight of sample in g}} \quad \text{(eq 3.1)}$$

Calculation of % Protein

% Protein = % Nitrogen x empirical protein factor (eq 3.2)

Food	**Factor**
General	**6.25**
Animal origin	
Eggs and eggs product	6.25
Gelatine	5.55
Meat and meat products	6.25
Fish, sea animals	6.25
Milk, milk products, cheese, whey	6.38
Grains & Cereals	
Barley, oats, rye	5.83
Corn	6.25
Rize	5.95
Wheat	5.70
Full grain products	5.83
Bran	6.31
Fruits	
Fruits and fruits products	6.25
Legumes	
Vegetables and products made of vegetables (except soy)	6.25
Beans	6.25
Soy and soy products	5.71
Nuts	
Nuts (treenut, coconut, chestnut)	5.30
Peanuts	5.46
Almonds	5.18
Seeds	
Oilseed (except of peanuts)	5.30

Table 3.3: Empirical protein factors for the Kjeldahl method

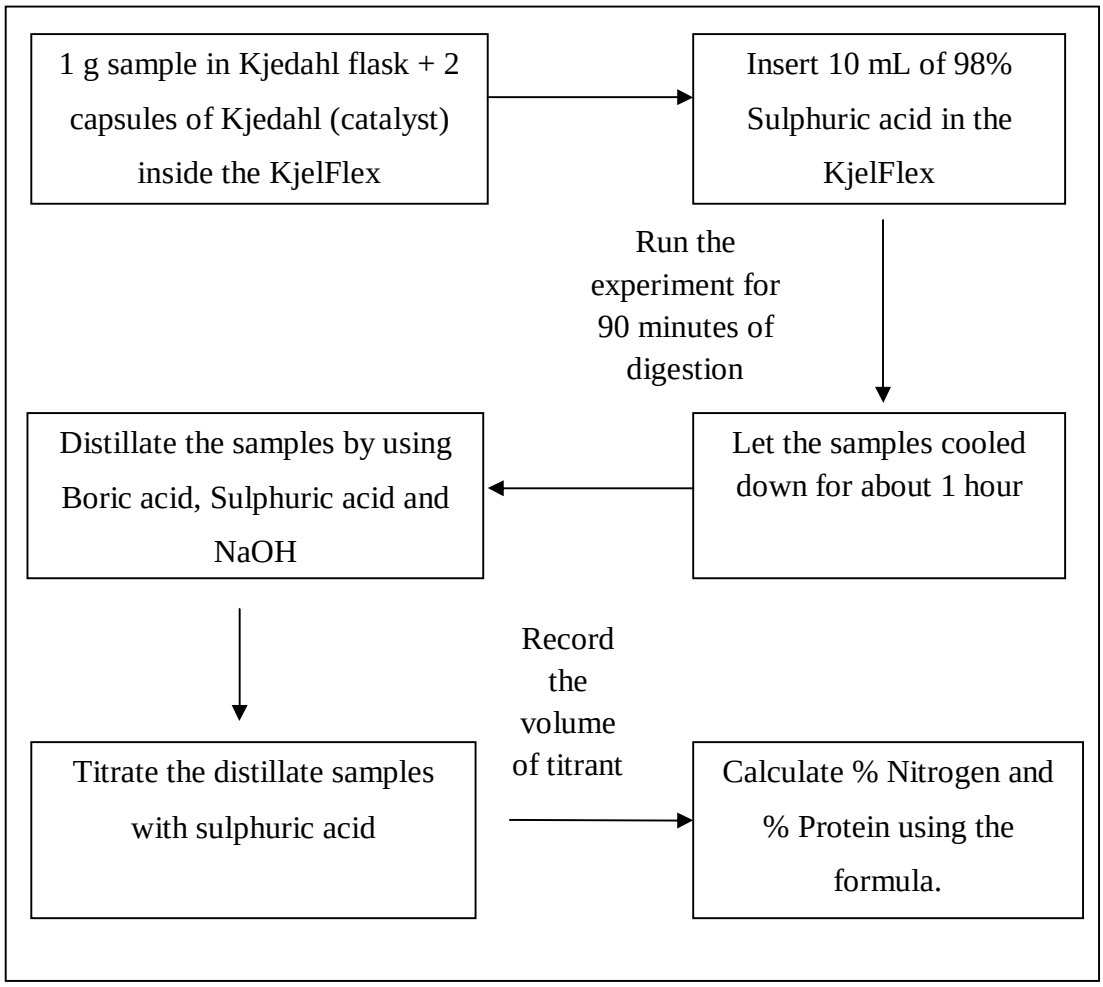


Figure 3.1: Determination of protein by Kjeldahl method

3.3.2 Standard analysis for Fiber (Filter Bag Technique, ANKOM ^{2000})

This method determine crude fiber which is the organic residue remaining after digesting with 0.255N H_2SO_4 and 0.313N NaOH. The compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignin. This method is applicable for all feed materials such as grains, meals, pet food, mixed feeds, forages and the following oilseeds: corn and soybeans

Reagents:

a. Sulphuric acid solution 0.255N
b. Sodium hydroxide solution 0.313N

Materials and equipments:

a. Analytical balance
b. Oven
c. Electric muffle furnace
d. Filter bag
e. Heat sealer
f. Desiccators pouch
g. Digestion instrument
h. Crucible and cap
i. Marking pen (solvent and acid resistant)

Method:

1. Use a solvent and acid resistant marker to label the filter bag. Weight filter bag (W1) and zero balance.
2. Weight about 1 g of sample (W2) and put inside the filter bag. Using a heat sealer, completely seal the upper edge of the filter bag.

3. Weight one blank bag include in run to determine blank bag filter bag correction.(C1)
4. Extract fat from sample by placing all bags into a 250 ml container. Add enough petroleum ether to cover bags and soak for 10 minutes. Pour off solvent and allow bags to air-dry.
5. Place a maximum of 24 filter bag into the Bag Suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessels and place the Bag Suspender weight on top of the empty 9^{th} tray to keep it submerged.
6. Set the temperature until 70°C and start the machine.
7. When the crude fiber extraction and rinsing process is complete, open the lid and remove the samples. Gently press out excess water from bags. Place bags in 250 ml beaker and add enough acetone to cover bags and soak for 3-5 minutes.
8. Remove the bags from acetone and dry it. Completely dry in oven at 102°C within 2-4 hours.
9. Remove the bags from the oven and cool it in desiccators for a while
10. Ash the entire bags in crucible for 2 hours at 600°C, cool it in desiccators and calculate the crude fiber by using the formula.

Calculation of % Crude Fiber:

$$\% \text{ Crude Fiber} = \frac{(W_3 - (W_1 \times C_1))}{W_2} \times 100 \qquad \text{(eq 3.3)}$$

W_1 = Bag tare weight

W_2 = Sample of weight

W_3 = Weight of organic matter

C_1 = Ash corrected blank bag factor

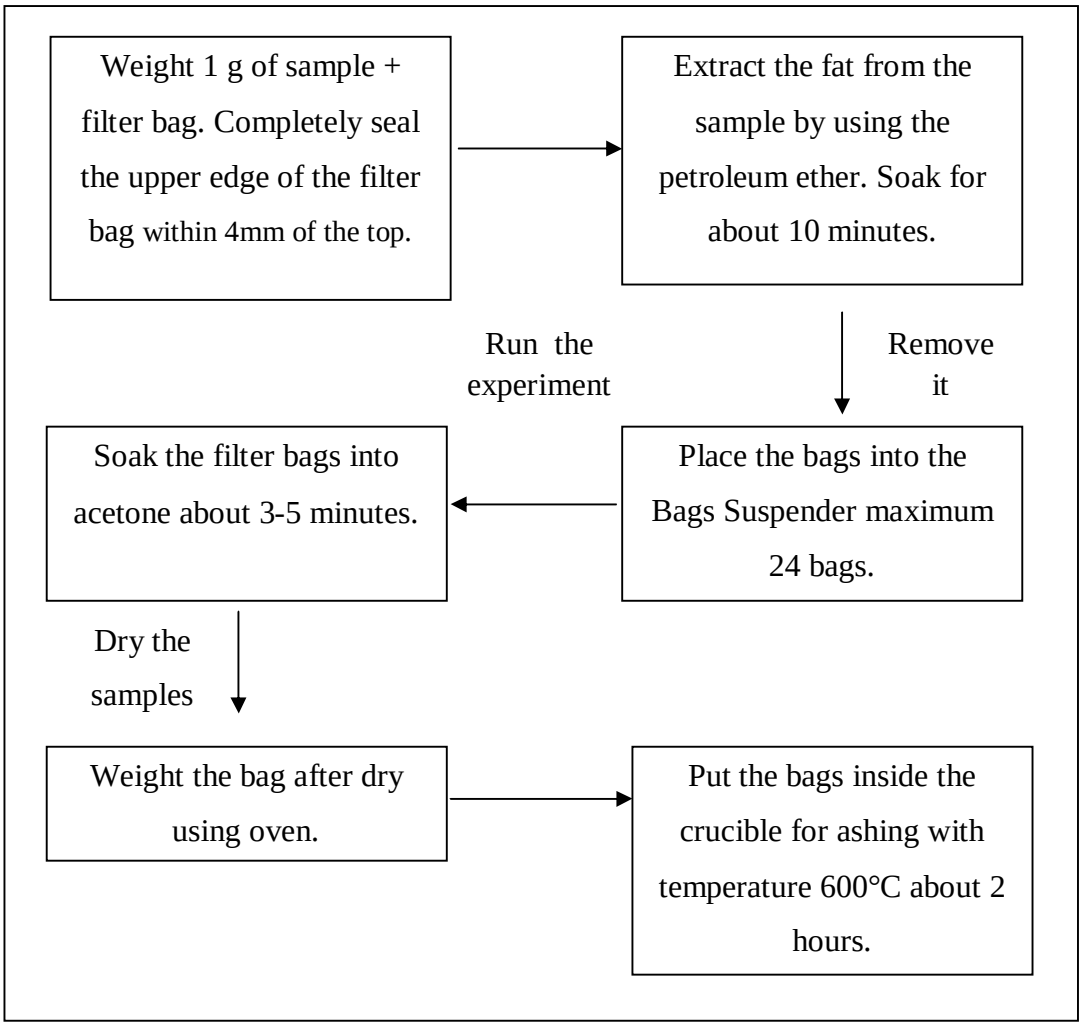


Figure 3.2: Determination of crude fiber

3.3.3 Standard analysis for Crude Fat (Soxhlet Method)

In this method, the fats are extracted from the sample with petroleum ether and evaluated as a percentage of the weight before the solvent is evaporated.

Reagents:

a. Petroleum ether
b. Cotton wool free of fat
c. Anti bumping granules

Materials and equipment:

a. Petroleum ether, boiling point 60–80°C.
b. Soxhlet extraction apparatus.
c. Heating mantle
d. Dryer
e. Extraction thimbles
f. Round bottom flask 150 ml

Method:

1. Weight 2 g sample (S) into the timble and place cotton wool in the top of the timble.
2. Insert the timble in a soxhlet extractor.
3. Accurately weight a clean, dry 150 ml round bottom flask with antibumping (W1) and put about 90 ml of petroleum ether into the flask.
4. Assemble the extraction unit over the heating mantle.
5. Heat the solvent in the flask until it boils. Adjust the heat source so that solvent drips from the condenser into the sample chamber at the rate of about 6 drops per seconds.
6. Continue the extraction for 8 hours.

7. Remove the extraction unit from the heat source and detach the extractor and condenser. Replace the flask on the heat source and evaporate off the solvent.
8. Place the flask in an oven at 120°C and dry the content until a constant weight is reached about 1-2 hours.
9. Cool the flask in a desiccators and weight the flask and content (W2)

Calculations for % Crude Fat:

Crude fat content (%) = $\frac{(W2-W1)}{S} X\ 100\%$ (eq 3.4)

Where:

W1 = weight of round bottom flask with antibumping

W2 = weight of round bottom flask after dry

S = Weight of sample

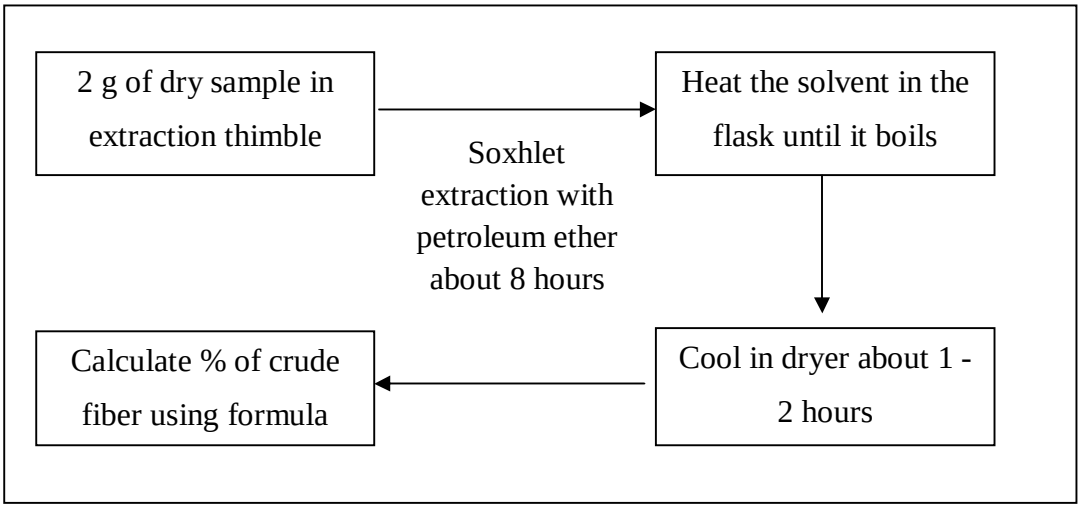


Figure 3.3: Determination of lipids by Soxhlet's method

3.3.4: Standard analysis for Ash

This method is used to determine ash content in feedstuffs by calcination. Ash is considered as the total mineral or inorganic content of the sample.

Materials and equipment:

a. Porcelain crucibles
b. Crucible furnace
c. Dryer
d. Desiccators

Method:

1. Place 2.5 to 5 g of dry sample in a crucible previously calcined and brought to constant weight.
2. Place the crucible in a furnace and heat at 130°C for 1 hours; leave to cool and transfer to a dryer.
3. Carefully weigh the crucible again with the ash.

Calculations for Ash:

$$\% \text{ Ash} = \frac{A-B}{C} \times 100\ \% \qquad \text{(eq 3.5)}$$

Where:

A = weight of crucible with sample (g)
B = weight of crucible with ash (g)
C = weight of sample (g)

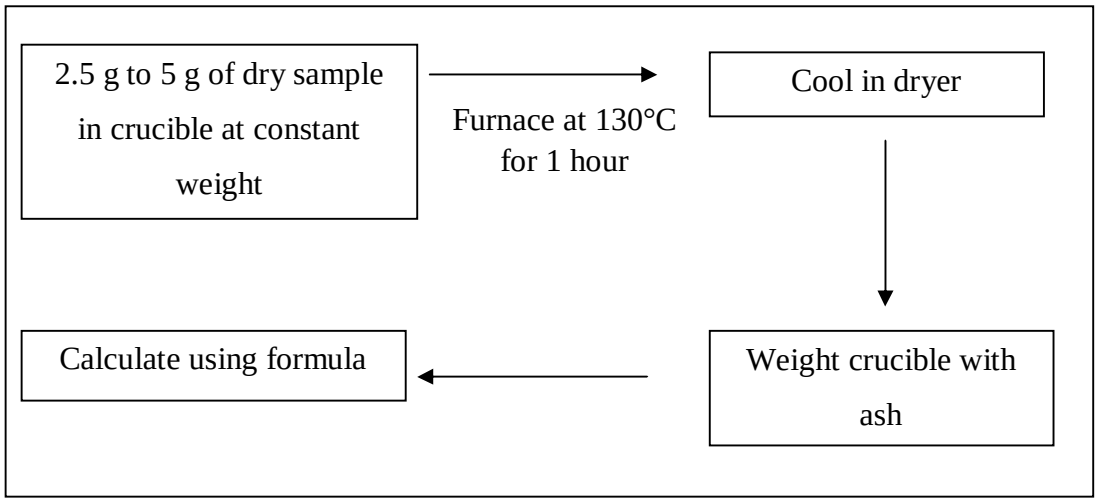


Figure 3.4: Determination of ash content in feed ingredients

3.3.5 Standard analysis for moisture content

This method determine the moisture content in solid food samples. This method can be applied to soy products samples.

Materials and equipment:

a. Air oven
b. Porcelain crucibles
c. Analytical balance
d. Desiccators

Method:

1. Weight 2 g of test portion in porcelain crucibles
2. Uncover the test portion and dry dish from unnecessary moist
3. Cover the samples and content in oven provided with opening for ventilation
4. Maintain 130 ± 3°C (1 hour of drying starts when the oven temperature reach 130°C)
5. Cover the samples while still in oven, then transfer to the desiccators
6. Weight soon after reaching room temperature

Calculation for % Moisture content:

$$\% \text{ Moisture content} = \frac{\text{wet sample (g)} - \text{dry sample (g)}}{\text{Wet sample (g)}} \times 100 \qquad \text{(eg 3.6)}$$

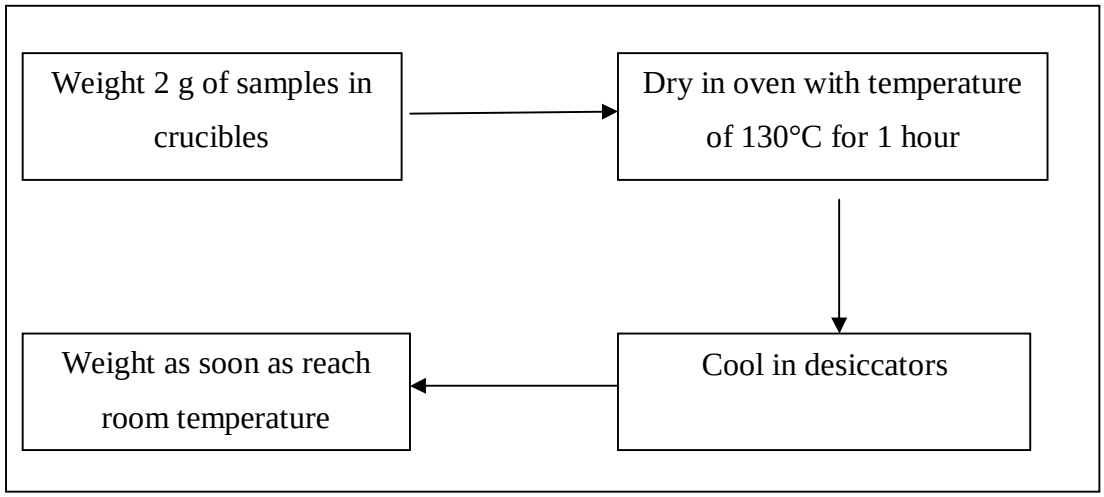


Figure 3.5: Determination of moisture content

3.4 Analysis of tempeh samples by using Near infrared (NIR)

After doing the standard analysis by using the primary methods listed above, the results from the analysis were then being used as the standard in the NIR instrument. From here, the samples used in analyzing by using primary method then be analyzed once again by using NIR. The random samples taken from Kuantan area also being analyzed by using NIR to determine the amount of nutrient content.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

The experiments being conducted are to determine the nutrient content of tempeh produced from small scale producer in Kuantan area and also to compare the results from primary method analysis and the results from the NIR instrument analysis. The primary method analysis was done to a few samples from Kuantan area. The analysis was then being used to secure a standard in the NIR instrument. These samples the being analyzed again by using NIR analysis. From here, the two results from primary method analysis and NIR analysis then are compared to find out the percentage of differences between the two methods. Comparison graph then being plotted.

Random samples from Kuantan area also taken to be analyzed by using NIR instrument. These random samples were analyzed to determine the average nutrient content in the tempeh produced in Kuantan area by these small scale producers. While analyzing the nutrient content of the tempeh samples, the growth rate of the nutrient content in different packaging and fermentation time also been analyzed by using NIR analysis.

4.2 Analysis of Tempeh samples for primary analysis

For primary method analysis, the nutrient content analyzed are protein (Kjeldahl method), Fiber (filter bag method), Fat (Soxhlet method), ash and moisture.

4.2.1 Results of Tempeh samples for primary analysis

Table 4.1: Results of tempeh samples for primary analysis

Samples	Analysis (%)			
	Protein	**Fat**	**Fiber**	**Ash**
Tempeh A1	46.75	38.69	5.08	2.41
Tempeh A2	45.69	37.35	7.15	2.41
Tempeh A3	29.43	36.62	9.93	2.39
Tempeh B1	48.64	17.13	5.98	1.52
Tempeh B2	18.09	17.92	5.34	1.46
Tempeh B3	43.43	17.30	9.73	1.50
Tempeh C1	47.30	22.14	6.72	1.86
Tempeh C2	44.30	20.89	14.98	1.88
Tempeh C3	42.55	20.94	4.93	1.89

4.2.2 Discussions of results of tempeh samples analysis using primary method

From the results in table 4.1, we can see that the most noticeable nutrient content in the tempeh samples are from its protein content. From the USDA nutritional tables, the protein content in 100 g of tempeh consist about 20 % of the tempeh (USDA Nutrient Database for standard referee). However, from the results, the protein content in the tempeh samples consists about 50 % of the tempeh samples.

From results also, the fat content of the samples were quite high. However, from the article on tempeh (Sharyn Passeretti, 2002), it is stated that the quality of the protein content in tempeh is due to the Rhizopus Oligosporus. Where the Rhizopus produce enzymes protease and lipase which will results in the breakdown of proteins to amino acids and fats are hydrolyzed into fatty acids. Therefore, because of this, tempeh has high quality and digestible.

The fiber content in the tempeh samples are also quite high. This is because, tempeh is made from whole soybeans, and therefore, it is not shocking as it has high fiber content. Because soybeans is a part of plant-based food, soybeans can be a good source of fiber which can help lower blood cholesterol levels and also reduce the risk of heart disease (Kerr et al, 2001) and fermentation process utilizes more of the proteins and fiber content.

For fiber analysis, there are a few errors during the experiments, therefore the results are less trustable. During the experiment, the equipment suddenly having overpressure which forcing the chemical solutions out. Therefore to continue the experiment, later on, for the cleansing process, it was done manually.

4.3 Near Infrared (NIR) analysis on tempeh samples

The results from the primary method analysis are then being used as the standard in the NIR analysis. The samples analyzed by using primary method are then analyzed again by using NIR analysis to determine the difference of both analysis.

4.3.1 Results of NIR analysis on tempeh samples

Table 4.2: Results of tempeh samples by using NIR analysis

Samples	Analysis (%)			
	Protein	Fat	Fiber	Ash
Tempeh A1	53.09	29.47	8.50	2.74
	52.49	29.86	8.48	2.75
Tempeh A2	50.85	29.61	8.51	2.74
	50.60	29.84	8.50	2.75
Tempah A3	52.91	29.67	8.51	2.74
	52.58	29.93	8.51	2.75
Tempeh B1	48.45	33.51	8.24	2.88
	48.03	33.83	8.24	2.90
Tempeh B2	47.93	32.29	8.37	2.84
	47.77	32.52	8.37	2.85
Tempeh B3	48.03	31.00	8.46	2.79
	47.82	31.31	8.45	2.81
Tempeh C1	47.42	31.29	8.52	2.83
	46.84	31.62	8.52	2.84
Tempeh C2	47.83	31.68	8.49	2.84

	47.02	31.88	8.48	2.85
Tempeh C3	46.82	32.71	8.40	2.87
	46.62	33.02	8.39	2.89

4.3.1.1 Discussions on the results of NIR analysis on tempeh samples

The result from Table 4.2 above is from the same tempeh samples that were analyzed by using primary method analysis. The samples analyzed by using NIR were duplicated to make sure that the analyses are as accurate as possible. The reason the samples were duplicated are because of the nature of NIR that is very sensitive to particles size. From a review article written by Melchor et al, it is stated that NIR reflectance is very sensitive to particle size, shape and distribution of powders or granular samples. In order to minimize the effect of particle sizes effect, the samples are supposedly grinded to a near-uniform size (Melchor et al, 2001).

However, for this study, the particle sizes for each sample can be varied. Therefore, instead of making sure that the particle sizes of the samples are near-uniform in size, the samples were analyzed twice each time. The samples were duplicated to minimize the particle sizes error while doing the analysis.

Tempeh known for its high protein content. However, it also has high moisture content. However in this study, the moisture content of the samples are not analyzed because for the samples to be analyzed by using NIR, the samples must be in dry matter state. Therefore, almost all of the moisture content in the samples are already being suck out before analysis.

4.3.2 Results of random tempeh samples analysis using NIR

Table 4.3: Results of random tempeh samples analysis using NIR

Samples	Analysis (%)			
	Protein	Fat	Fiber	Ash
Tempeh D1	55.78	32.13	8.49	2.82
	55.32	32.42	8.49	2.83
Tempeh D2	51.48	30.94	8.63	2.79
	51.39	31.11	8.63	2.79
Tempeh D3	54.49	31.44	8.59	2.80
	54.18	31.60	8.59	2.81
Tempeh E1	59.87	24.18	8.80	2.54
	59.69	24.49	8.79	2.55
Tempeh E2	57.08	27.07	8.58	2.63
	56.61	27.32	8.57	2.64
Tempeh E3	56.25	25.34	8.78	2.59
	55.91	25.53	8.78	2.59
Tempeh F1	59.88	23.22	8.86	2.51
	59.78	23.63	8.84	2.52
Tempeh F2	57.69	23.04	8.88	2.49
	57.50	23.35	8.87	2.50
Tempeh F3	57.75	24.77	8.73	2.55
	57.45	25.11	8.72	2.57
Tempeh G1	53.00	29.39	8.34	2.71
	52.93	29.78	8.32	2.73
Tempeh G2	53.19	30.96	8.22	2.76
	52.68	31.21	8.21	2.77
Tempeh G3	53.39	31.25	8.21	2.77
	53.20	31.40	8.21	2.78

4.3.2.1 Discussion for random tempeh samples analysis

Table 4.3 above is the results for random samples taken from Kuantan area. From the results, the average nutrient content in tempeh samples is about 55 %. For fiber analysis, the average amount in the tempeh samples is 27 %. The average fat amount is around 8 % and average ashing in the tempeh samples are around 2 %. The amounts of the nutrient content in these random samples were almost the same.

However, there is slightly difference in the samples of E1 until F3. From the results, the amount of protein content for these samples are slightly higher than the other samples (samples from A1 until D3 including G1 until G3). The fat content for these samples are also slightly lower than the others. The difference in the analysis is because these samples are actually from different type of packaging.

Up until now, the packaging for all the samples are from plastic packaging with small holes in the plastic (Refer Appendix B1). However, for samples E1 until F3, the packagings for the samples are from paper packaging. These samples were packaged traditionally rather than using plastics. The difference between plastic packaging and paper packaging will be discussed later in this chapter.

4.4 Comparison between Primary method analysis and NIR analysis

Table 4.1 is the result for primary method analysis while Table 4.2 is for the results of NIR instrument analysis. To determine the difference between primary method analysis and NIR instrument analysis, comparison graph between primary analysis and NIR analysis are made.

Figure 4.1: Graph of comparison between primary analysis and NIR analysis (Protein)

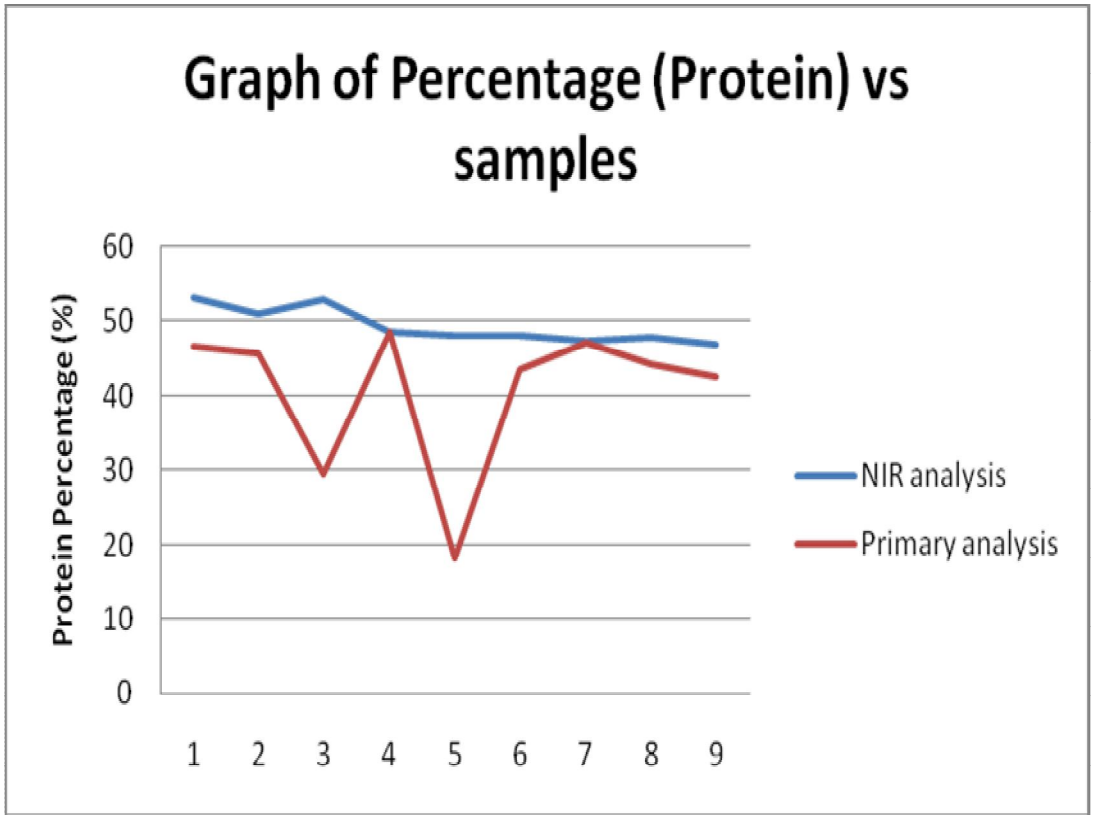


Figure 4.2: Graph of comparison between primary analysis and NIR analysis (Fat)

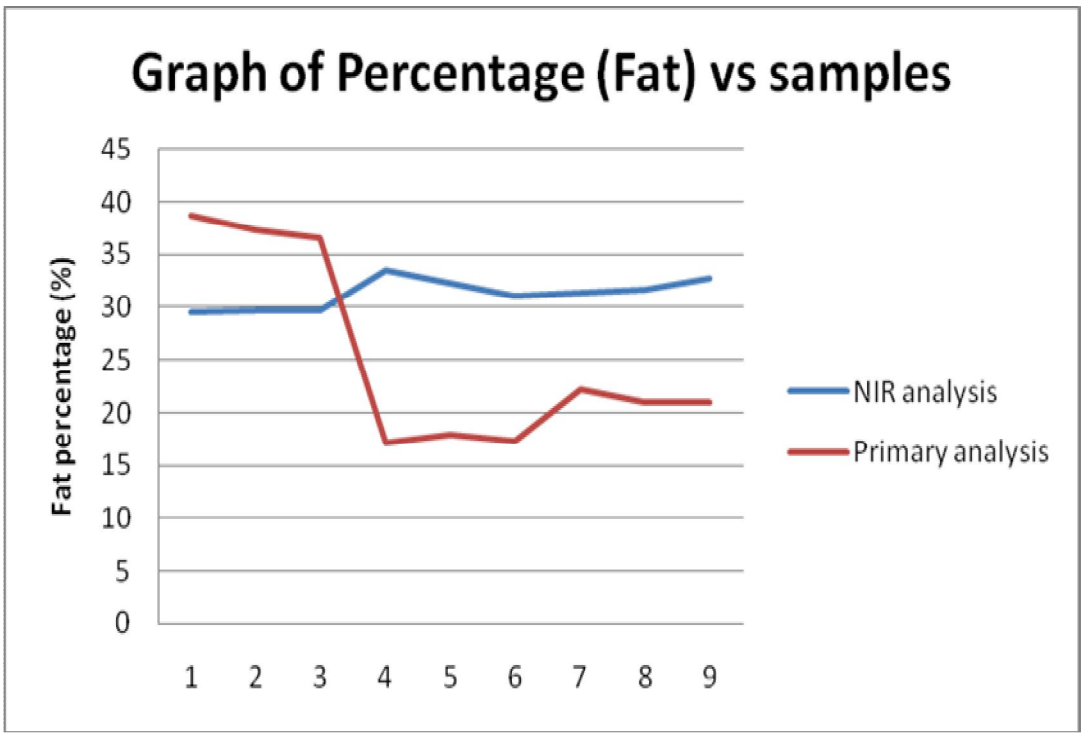


Figure 4.3: Graph of comparison between primary analysis and NIR analysis (Fiber)

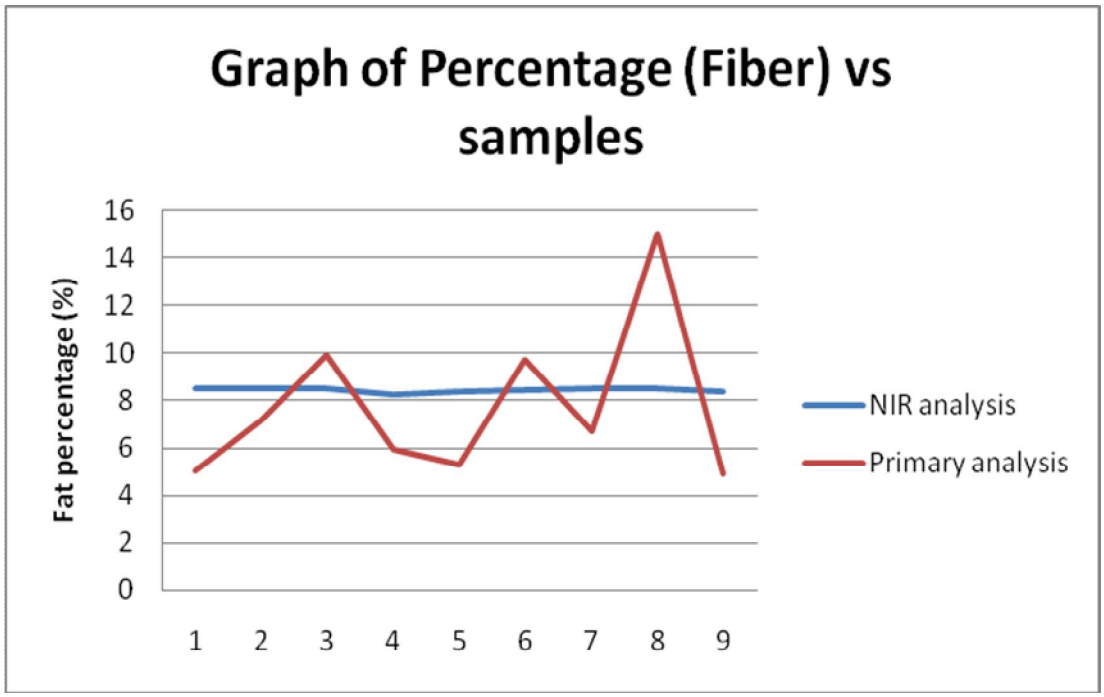
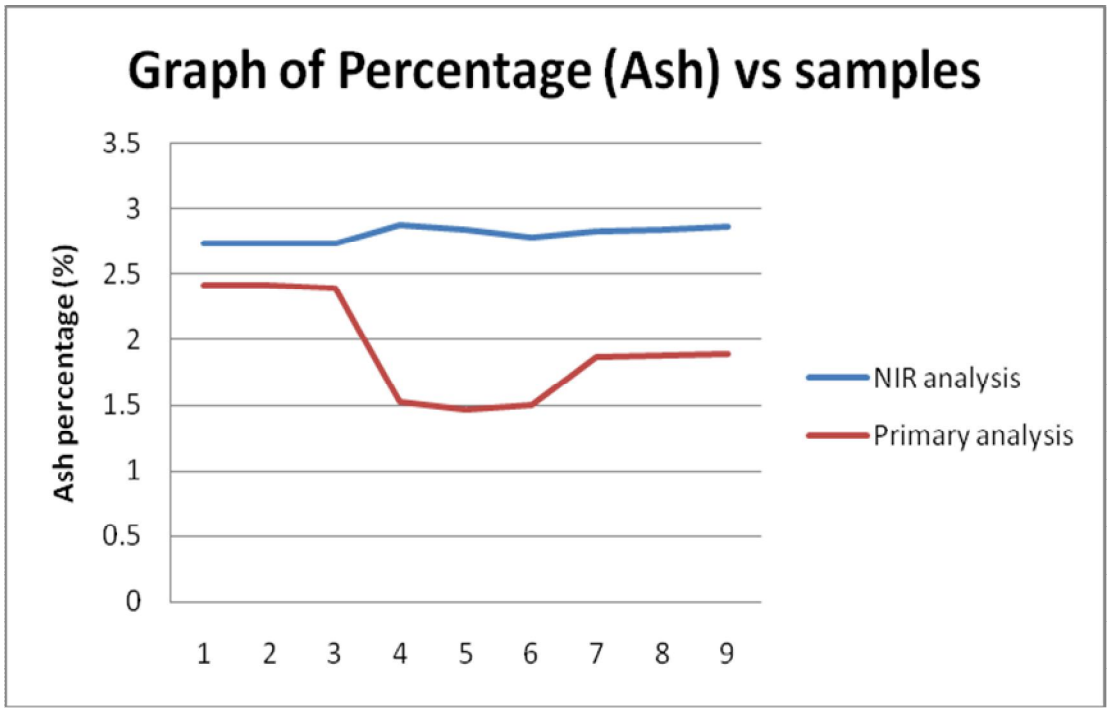


Figure 4.4: Graph of comparison between primary analysis and NIR analysis (Ash)



From the comparison between both primary and NIR analysis, we can see that there are differences between both analysis. By comparing both types of analysis, for overall differences, there are about 5 to 20 % differences between both analysis depending on the errors during analysis. For protein analysis, the percentage difference of both analysis is about 10 %. There are some similarities between both analysis with some of the samples having the same protein reading.

For Fat analysis, from Figure 4.2, we can see that there are also about 10 % differences between both analysis. For the analysis of fiber content (Figure 4.3), the differences are about 2 to 4 % differences also depending on the errors during analysis. For ash analysis (Figure 4.4), the percentage differences are about 2 to 3 %. Although there are differences between the analysis using primary method and NIR instrument analysis, there are similarities between both analysis.

This is because the NIR analysis is based from the primary analysis. Based from the results from the primary method analysis, the standard for NIR instrument analysis can be plotted. After plotting the standard, then the NIR analysis can be used to analyzed tempeh samples. Analyzing the tempeh samples by using primary method need to be done one by one and it consumes a lot of time and energy. However, by plotting the standard from the results on the primary method analysis into the NIR instrument, the analysis of tempeh can be done quicker.

Apart from itd rapidity, NIR spectroscopy offers a number of other important advantages over traditional chemical methods. It is physical, nondestructive method which required no sample preparation. The precision is also very high. In contrast with traditional chemical analysis, there are no reagents are required and there are also no wastes are produced. It is a multi-analytical technique with several determinations can be done simultaneously (D. I. Givens et al, 1997). However, there are disadvantages in using NIR analysis. The chief disadvantages are the need for high precision spectroscopic instruments, dependence on time consuming and laborious calibrations procedures, complexity in the choice of data treatment and lack of sensitivity for minor constituents (Norris, 1989).

From the comparison also, it is safe to assume that the NIR analysis reads the primary method analysis but by average readings. From the graph of comparison between both methods, we can see that the readings between both methods are almost the same with the analysis from NIR instrument is more stable. The similarities between the analysis are unavoidable as the NIR standard were based from the primary method analysis.

4.5 Analysis of nutrient content of tempeh with different packagings

There are two types of packaging for the tempeh samples analyzed. The two types of packaging are plastic packaging and paper packaging. After analyzing the nutrient content of both methods, there slightly difference in the nutrient content of these packaging. Therefore, for more understanding and clearer, analysis were done on both packaging for matured tempeh and also aged tempeh. The aged tempeh has been fermented for another one week (1 week) from the day of fresh tempeh analysis. Figure for mature tempeh (refer Appendix B2) and aged tempeh (refer Appendix B3) are in the list of Appendix.

4.5.1 Results of nutrient content of tempeh with different packaging and fermentation time

The tempeh's nutrients packed in two different packaging which is paper packaging and plastic packaging were analyzed to determine the difference of nutrient utilized during the fermentation process

4.5.1.1 Analysis for Paper packaging

4.5.1.1.1 Analysis for matured tempeh

Table 4.4: Mature samples analysis (Paper packaging)

SAMPLES	COMPONENT ANALYSIS			
	PROTEIN	FAT	FIBER	ASH
TEMPEH H1	60.23	40.52	7.38	3.01
	59.88	40.83	7.37	3.02
TEMPEH H2	60.74	40.42	7.39	3.01
	60.28	40.68	7.39	3.02
TEMPEH H3	58.73	42.18	7.28	3.07
	58.25	42.32	7.28	3.08

4.5.1.1.2 Analysis for Over fermented tempeh (aged tempeh)

Tables 4.5: Aged tempeh analysis (Paper packaging)

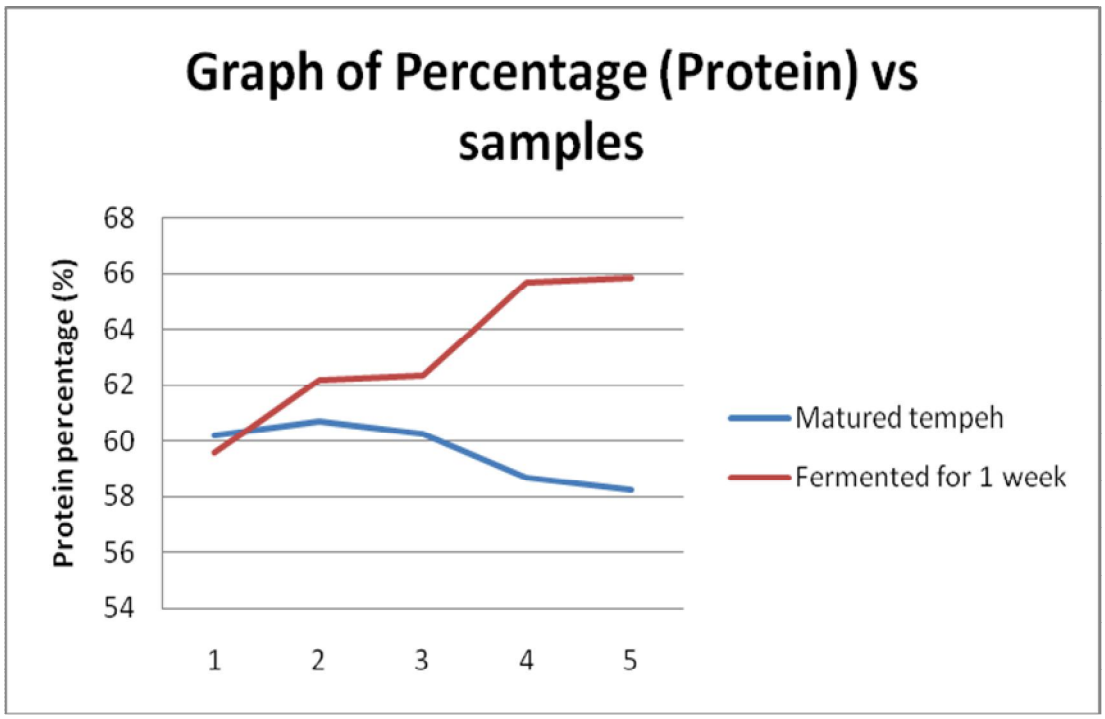
SAMPLES	COMPONENT ANALYSIS			
	PROTEIN	FAT	FIBER	ASH
TEMPEH H1	59.58	-4.90	11.14	1.69
TEMPEH H2	62.15	-5.50	11.20	1.67
	62.32	-5.10	11.18	1.68
TEMPEH H3	65.66	-4.54	11.13	1.69
	65.82	-4.22	11.11	1.70

4.5.1.2 Discussions for analysis of tempeh with Paper packaging nutrient content

In Table 4.3 which consists of NIR analysis on the random samples taken from Kuantan area, it has been mentioned that there are slight difference on the nutrient content between plastic packaging and paper packaging. Table 4.4 and Table 4.5 above are the results from the analysis using NIR instrument on the matured tempeh and aged tempeh that used paper as the packaging material.

From the result of protein consumption, the protein content in the samples is quite high with average protein content at about 60 %.

Figure 4.5: Graph of comparison of protein content between matured tempeh and aged tempeh with paper packaging



From figure above, the average protein content of matured tempeh is about 59 % while for the aged tempeh, the protein content is around 62 %. From the result, protein content is believed to have utilized during the one week (1 week) fermentation process. This can happened because in tempeh, the high protein content are utilized because of the fermentation process. The fermentation process or the inoculation process between the soybean and the *Rhizopus Oligosporus* mould produce many nutrient contents including protein.

Figure 4.6: Graph of comparison of Fat content between matured tempeh and aged tempeh with paper packaging

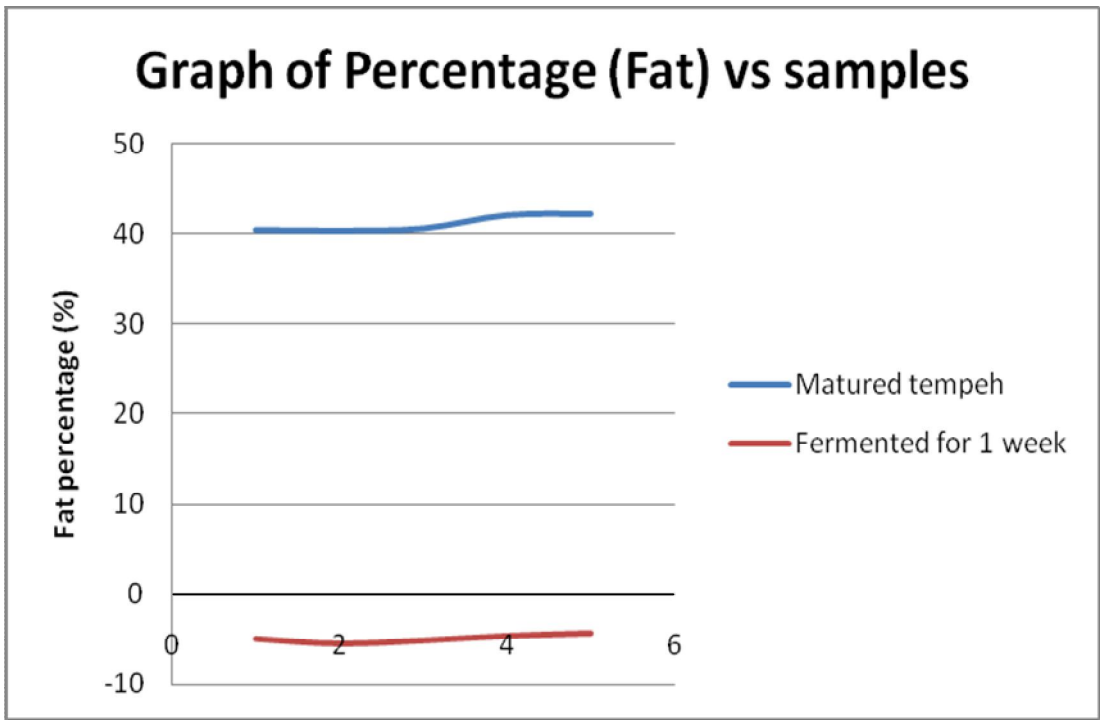
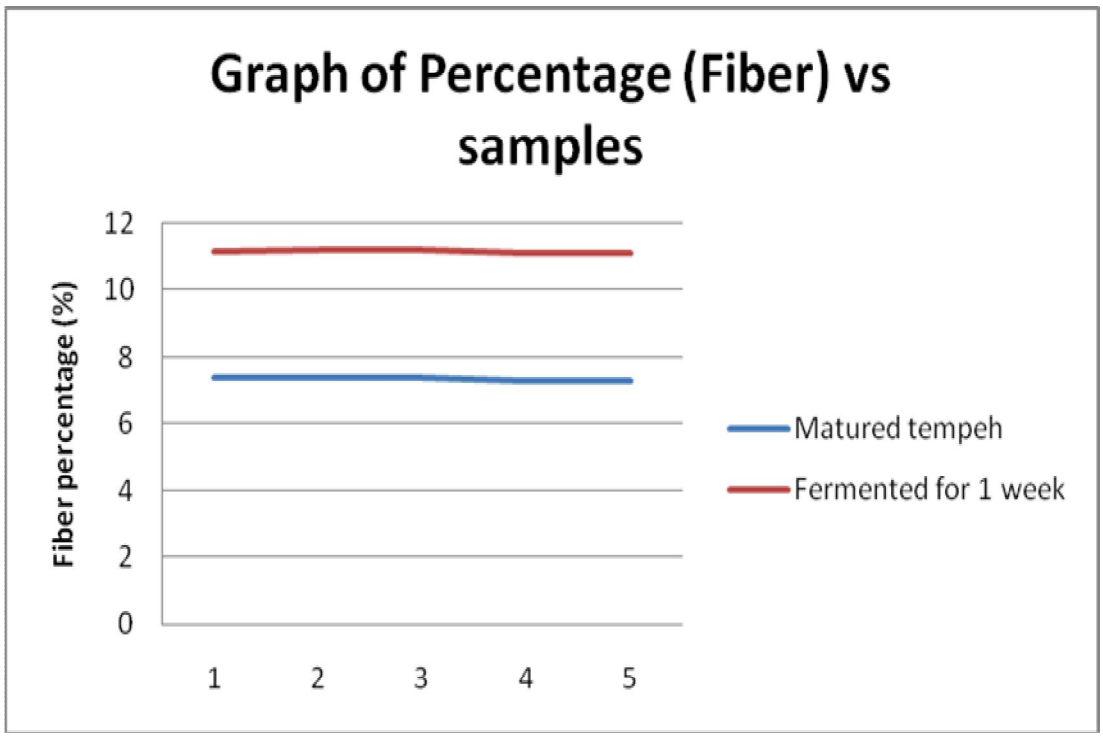


Figure 4.6 above is the comparison of fat content in matured tempeh and aged tempeh with paper as the packaging material. From the graph, the fat content for the matured tempeh is about 40 %. However, for the aged tempeh, the fat content cannot be determined using NIR instrument analysis. The negative mark in the reading of the fat content for the aged tempeh emphasize that the fat content in the aged tempeh cannot be determined.

Figure 4.7: Graph of comparison of Fiber content between matured tempeh and aged tempeh with paper packaging



For Fiber content analysis of the matured tempeh and aged tempeh with paper packaging as the packaging material, the fiber content of the samples can be seen slightly higher than the aged tempeh with percentage of 11 %. The fiber percentage difference between both matured tempeh and aged tempeh is about 4 % difference. The fiber content is higher in the aged tempeh can be because the fermentation process of the tempeh samples and also because the samples are already in the state of stale.

Figure 4.8: Graph of comparison of Ash content between matured tempeh and aged tempeh with paper packaging

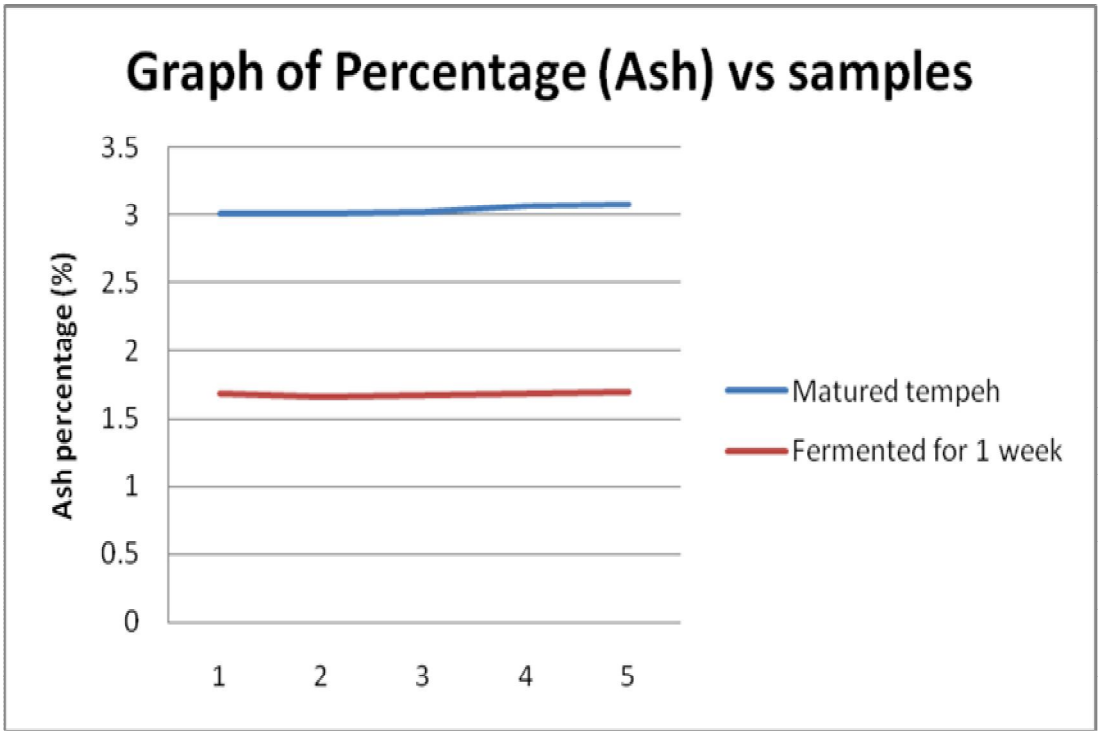


Figure 4.8 above shows the comparison of ash content comparison between matured tempeh and aged tempeh with paper as the packaging material. From the graph, the percentage difference of the ash content between both matures tempeh and aged tempeh is about 1.5 % difference. The ash content of the matured tempeh are slightly higher than the aged tempeh. This may because of the stale state that the samples are in after 1 week of fermentation.

4.5.1.3 Analysis for Plastic packaging

4.5.1.3.1 Analysis for matured tempeh

Table 4.6: Mature samples analysis (Plastic packaging)

SAMPLES	COMPONENT ANALYSIS			
	PROTEIN	FAT	FIBER	ASH
TEMPEH I1	47.08	36.69	7.78	2.94
	47.03	36.78	7.78	2.94
TEMPEH I2	47.40	38.30	7.64	2.99
	47.32	38.46	7.63	2.99
TEMPEH I3	48.23	37.17	7.73	2.95
	48.07	37.33	7.73	2.96

4.5.1.3.2 Analysis for Over fermented tempeh (aged tempeh)

Table 4.7: Aged tempeh analysis (Plastic packaging)

SAMPLES	COMPONENT ANALYSIS			
	PROTEIN	FAT	FIBER	ASH
TEMPEH I1	35.45	8.25	10.23	2.17
	35.65	8.62	10.21	2.18
TEMPEH I2	36.49	9.66	10.14	2.20
	36.14	9.89	10.12	2.21
TEMPEH I3	33.82	10.24	10.11	2.23
	33.66	10.44	10.10	2.24

4.5.1.4 Discussions for analysis of tempeh with Plastic packaging nutrient content

Table 4.6 and Table 4.7 above shows the results of nutrient content analysis between matured tempeh and aged tempeh with plastic as the packaging material. Compared to the nutrient content for the tempeh samples with paper as the packaging material, the nutrient content of tempeh with plastic packaging is slightly lower. For more understanding, graph of comparison between both matured tempeh and aged tempeh has been plotted.

Figure 4.9: Graph of comparison of Protein content between matured tempeh and aged tempeh with plastic packaging

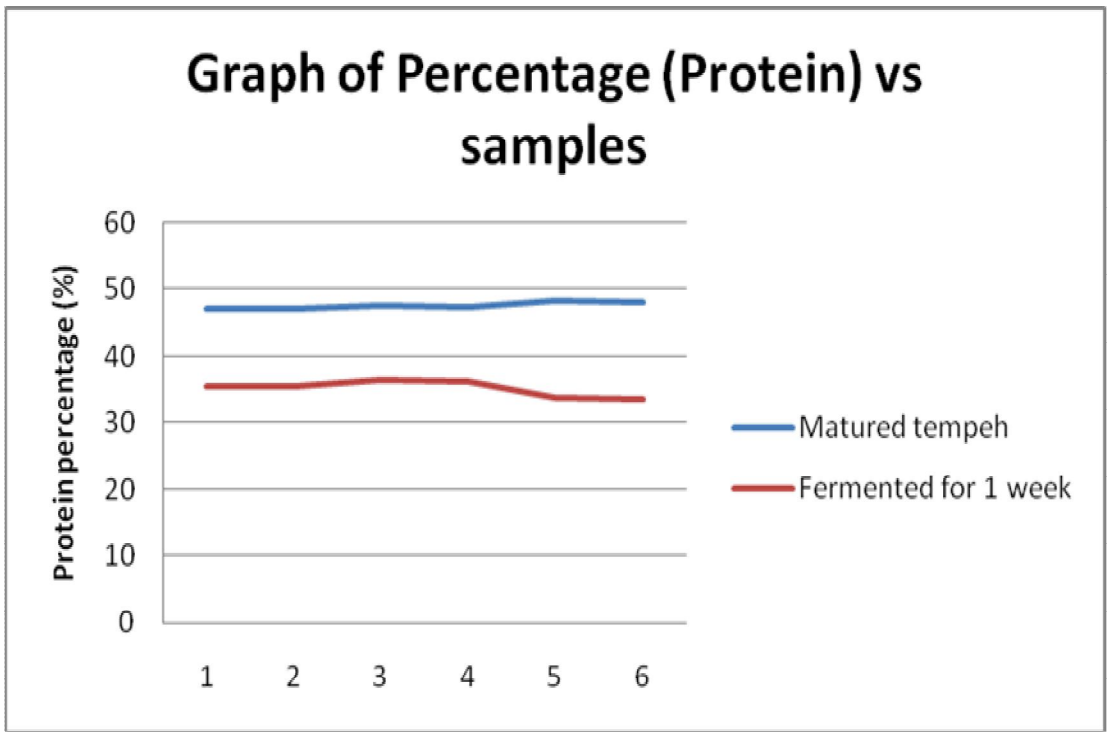
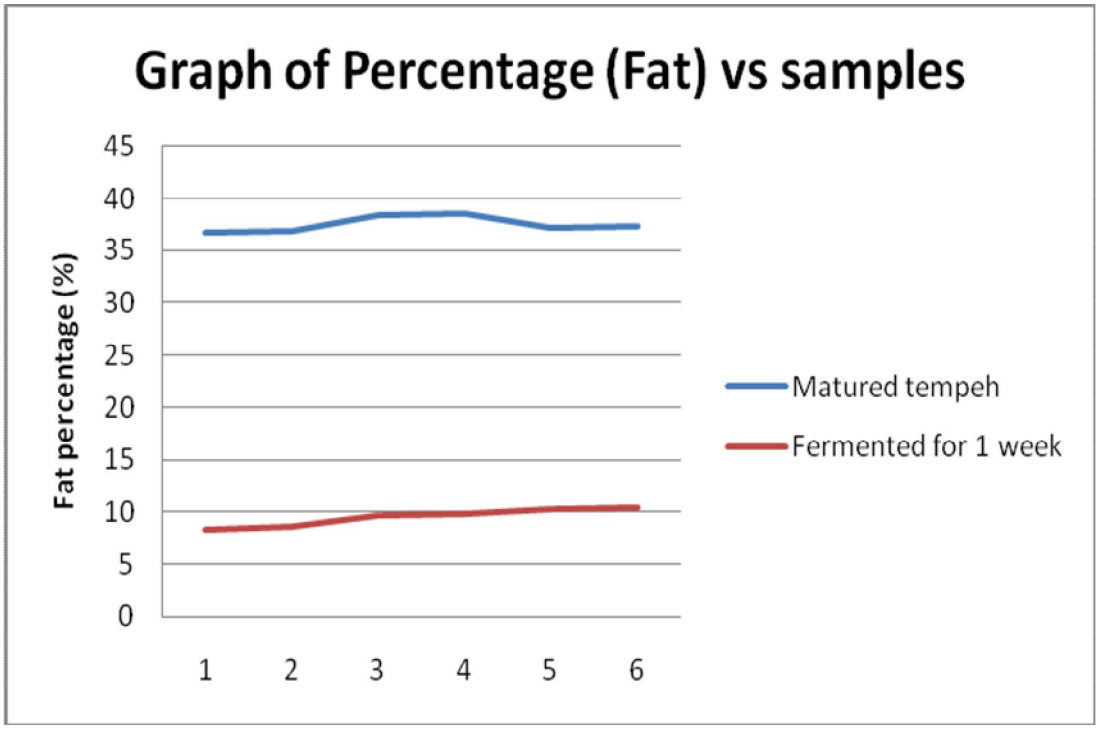


Figure 4.9 above shows the percentage of protein content in mature tempeh and aged tempeh with plastic as the packaging material. From the graph, it shows that the percentage of protein difference between both matured tempeh and aged tempeh is about 10 % difference. The protein content of the aged tempeh can be seen deteriorated from the matured tempeh.

Figure 4.10: Graph of comparison of Fat content between matured tempeh and aged tempeh with plastic packaging



From the graph in figure 4.10 above, the fat content of the matured tempeh can be seen deteriorated about 25 % after been fermented for another one week (1 week). The deterioration is slower than the fat content in tempeh samples that use paper as the packaging material.

Figure 4.11: Graph of comparison of Fiber content between matured tempeh and aged tempeh with plastic packaging

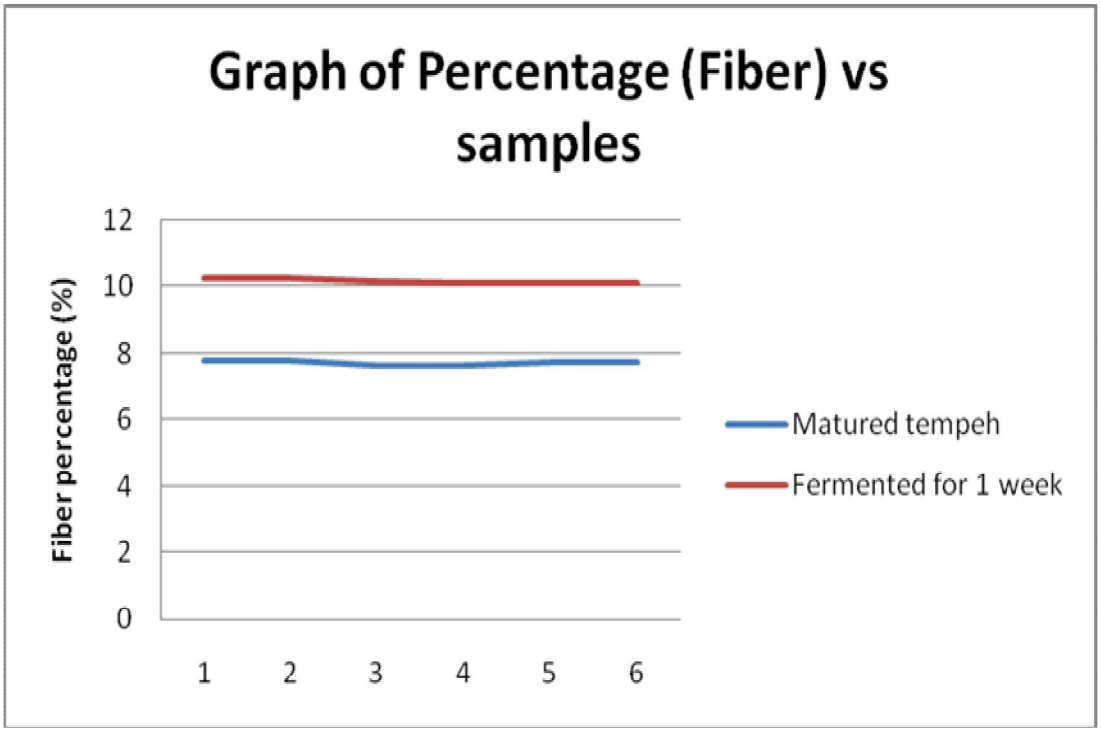


Figure 4.11 above shows the comparison of fiber content between matured tempeh and aged tempeh with plastic as the packaging material. From the graph above the percentage difference between the matured tempeh and aged tempeh is about 2 % difference. The aged tempeh has slightly higher fiber content than matured tempeh.

Figure 4.12: Graph of comparison of Ash content between matured tempeh and aged tempeh with plastic packaging

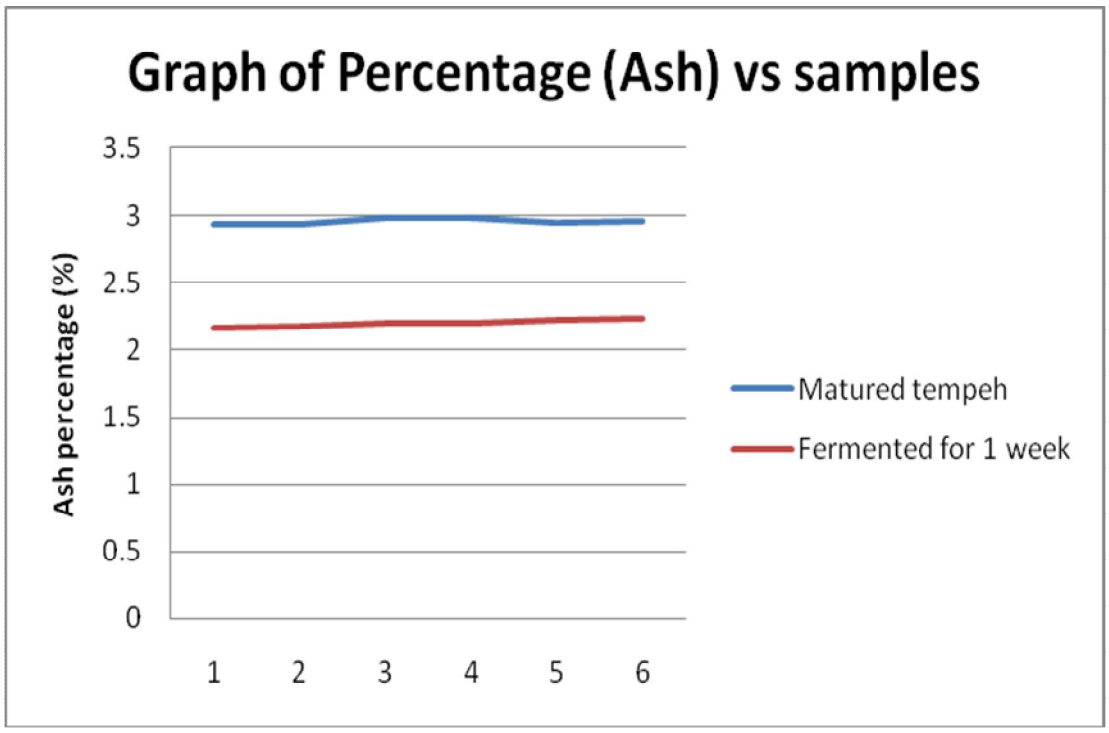


Figure 4.12 above shows the graph of comparison of ash content between matured tempeh and aged tempeh with plastic as the packaging material. From the graph above the percentage difference of ash in matured tempeh and aged tempeh is about 1.5 % difference. The ash content in the matured tempeh is slightly higher than in the aged tempeh.

4.5.2 Summary of the nutrient content results on different packaging

From the nutrient content results of both types of packaging, the main nutrient content utilization such as protein is higher in the paper packaging while in plastic packaging, the utilization is lower. However, even though the nutrient utilization is higher in paper packaging, as the fermentation is let happened for

another one week (1 week), the nutrient contents in plastic packaging deteriorated slower than in paper packaging.

Tempeh depends on its fermentation process to utilize its nutrient content. The process will continue to happen until the tempeh are in the state of stale and cannot be eaten anymore. The fermentation process however produces heat. This heat needed to be release to make sure that the tempeh can stay fresh. In plastic packaging, the plastic have little holes so that the heat produce can be release. However, for the paper packaging, it is a tight packaging with no room for the heat produced to be release outside the package. Therefore, it is easier for the tempeh to sweat in paper package. Tempeh needs to stay in dry state to make sure its freshness. With the sweating problem and no place for the heat to be release, the tempeh in paper packaging is easier to be spoiled.

4.6 Precausion

During the experiment, there are a few details that need to be reviewed so that the experiment can be done with minimum errors. While doing the experiment on the fat content by using primary method analysis, to make sure that the petroleum ether boil in the flask did not over boil and causing accident, anti bumping is needed. Before filing the amount of petroleum ether needed in the flask, fill in a bit of the anti bumping in the flask. Make sure also to weight the flask with the anti bumping so that there will be no errors while weighing the flask with the fat extracted.

For protein analysis on the primary method, make sure that the samples must be weighted in the weighing boat provided for the equipment. The weighing boat will not produce reaction while running the experiment with 98% hydrochloric acid. In the method of analyzing the protein content, 10 mL of 98% hydrochloric acid is needed during the experiment. The molarity of the solution is very high. Therefore,

proper equipments are needed to prevent accident. While weighing the samples in the weighing boat, make sure that the samples weight did not exceed 1 g. This is because flooding can happen if there are too much samples

During the fiber analysis by using primary method analysis, there are a few precautions that needed to be paid attention to. While grinding the samples, make sure that the samples particle size is not too small. This is because if the samples particles sizes are too small there is possibility that the samples will leak from the seal bag. The ideal particle size for the sample is around 2 mm in size. For the seal bag, to prevent the samples from leaking and causing errors in the results make sure that the seal bag is sealed properly. While doing the experiment also, need to be careful with the equipment so that overpressure will not happens

As been stated above, NIR is a very sensitive instrument. Therefore, before starting the analysis, make sure that the instrument has gone through a warm up for 1 hour. To minimize the error during the analysis also, make sure that the samples analyzed is grinded to a near-uniform size to minimize the particle size error. By duplicating each samples also can reduce the error.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the results and discussions discussed above, both the analysis using NIR instrument and also primary method analysis have their own unique advantages and also disadvantages. However, from the results discussed, there are some similarities between primary method analysis and NIR analysis. The NIR analysis' standard is based from the primary method results. The food industrialist now prefer using NIR as the test instrument because it is quicker, however, for more accurate results, the primary method is still a better choice.

The two different types of packaging of tempeh also have their own advantages and disadvantages. The nutrient can be utilized higher in paper packaging however the analysis results, the nutrient can be preserved longer in the plastic packaging.

5.2 Recommendations

For further study, the research study can be extended by studying on the effect of the processing process to the nutrient content. In this study, the nutrient content of the finished tempeh is being studied. However, there are no specific facts on what kind of nutrient gain and loss during the processing process of tempeh. Therefore, it will be a good study to know which part of the process actually losing the nutrient content so that proper prevention can be done.

In the analysis between the paper packaging and plastic packaging, there are analysis of the nutrient content on the aged tempeh. By studying more the the aging effect on the nutrient content in tempeh can uncover why the some of the nutrient content deteriorated and some of the are actually gaining more.

The samples area studied also needed to be widen so that the research study is more reliable and universal.

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