PRELIMINARY STUDY ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIES OF OVERRIPE TEMPEH

By

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ABSTRACT

PRELIMINARY STUDY ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIES OF OVERRIPE TEMPEH

Esther Lim Shi Lin

Tempeh is a famous soy-based product originated from Indonesia with high nutritional content. The nutritional content of tempeh makes it a high-quality source of protein, which is an ideal choice for vegan as the meat-substitute product. There is also another variation of tempeh which is overripe tempeh. Fundamentally, it is just a tempeh with extended fermentation. With the presence of fresh tempeh and overripe tempeh, a comparison was done to understand which tempeh is better to be consumed in terms of its organoleptic properties and nutritional value. Results showed that overripe tempeh exhibited a darker yellowish colour followed by a slightly reddish hue and had a higher physicochemical properties and proximate composition as shown in Table 4.1, 4.2, and 4.3 respectively. For instance, titratable acidity (0.20%), moisture (58.05%), ash (1.61%), and carbohydrate (17.75%) content. In addition, overripe tempeh demonstrated a lower hardness (1.63 N), springiness (0.83), and cohesiveness (0.20) value, while having a higher fracturability (3.09N) as compared to fresh tempeh. Besides, most of the panellists from the sensory evaluation as shown in Figure 4.1 does not prefer the softer texture as well as the stronger taste of overripe tempeh due to its sourness, and bitterness when compared to fresh tempeh. Furthermore, fungal communities were the dominant

communities for both tempeh during the fermentation process. However, bacterial communities would take over fungal communities in overripe tempeh during extended fermentation. Throughout the 14 days of cold storage (4℃), the microbial population of overripe tempeh started to decline significantly on day 7 during shelf-life analysis as shown in Table 4.5. Future study would emphasise on a detail analysis of the changes in nutritional content and composition of microbial community.

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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.

ESTHER LIM SHI LIN

APPROVAL SHEET

This final year project report entitled "**PRELIMINARY STUDY ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIES OF OVERRIPE TEMPEH**" was prepared by ESTHER LIM SHI LIN and submitted as a 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 **ESTHER LIM SHI LIN** (ID No: **19ADB03699**) has completed this final year project entitled "PRELIMINARY STUDY ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIES OF OVERRIPE TEMPEH" under the supervision of Mr. SIM KHENG YUEN 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 thesis 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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(ESTHER LIM SHI LIN)

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a_w	Water activity
a*	Redness
CFU	Colony forming units
b*	Yellowness
L*	Lightness
PCA	Plate count agar
PDA	Potato dextrose agar
SSF	Solid-state fermentation
TFTC	Too few to count
TPC	Total plate count
TYPC	Total yeast mould count
wb	Wet basis

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Tempeh is a traditional food produced from soybeans through fermentation. It is originated from Indonesia and its versatility, flavour, and texture are distinctive. It is also a popular vegetarian cuisine as its chewy texture and unique flavour makes it suitable as a meat-substitute product (Guo, 2009). Tempeh is a nutritious food, which contains protein, fat, carbohydrates, fibre, various minerals, and vitamin B. Besides that, it is also act as prebiotic and probiotic due to its unique nutritional and bioavailability. There are a lot of benefits regarding consumption of tempeh. First, it can help to increase satiety, lower cholesterol levels, reduce oxidative stress, improve health of gut, and may decrease inflammation (Link, 2017). In comparison to its parent soybean, tempeh can improve digestion and nutritional absorption. Tempeh contains a monosaccharide which is called arabinose. It is used as a protective ingredient in children food to control the enterotoxigenic *Escherichia coli* that will cause diarrhoea (Puteri et al., 2018). The consumption data report showed that the market size for tempeh will grow over years with a compound annual growth rate of 15.50% during 2022-2029. This is because the high demand of consumer for meat substitutes product, and widely availability of tempeh on the market (Data Bridge Market Research, 2022).

Tempeh was discovered accidentally due to an unplanned fermentation of okara during the tofu making process (Karina, 2021). Nowadays, the production of tempeh is fully discovered, it is through the solid-state fermentation (SSF). The fermentation process relies heavily on *Rhizopus* spp. which includes *R. oligosporus, R. arrhizus, R. stolonifer*. During the fermentation process, *R. oligosporus* will secrete beta-glycosidase enzyme that hydrolysed the isoflavone glycosides to produce isoflavone aglycones. The digestive tract of an adult will take time to digest isoflavone glycosides and it is not guaranteed that it can be digested perfectly, whereas isoflavone aglycones can be easily absorbed by an adult. The enzymes produced by the mould will also eliminate antinutritional components and forms a preferable texture, flavour, and aroma of product (Xiao, 2011). However, there are microorganisms other than *Rhizopus* spp. inside tempeh as well, namely lactic acid bacteria, mould and yeasts. Besides that, there are also Firmicutes, which are carried over to the tempeh during the fermentation process. It is found as the dominant microorganism in the water that is used to soak the soybeans (Ahnan-Winarno et al., 2021).

Since the fermentation process of tempeh can be controlled, overripe tempeh is introduced. All the procedures of making overripe tempeh are identical to fresh tempeh, the only difference is that overripe tempeh has a longer solid-state fermentation period. Bacterial fermentation will take over in overripe tempeh, as the fungal microorganisms cannot survive due to the longer fermentation process compared to fresh tempeh (Hassanein, Prabawati and Gunawan-Puteri, 2015). It is normally used in flavouring, snack, and medication to improve appetite in

Indonesia (Puteri et al., 2018). Overripe tempeh has higher degrees of organoleptic properties compared to fresh tempeh (Gunawan-Puteri et al., 2015).

It is known that overripe tempeh has distinct difference in taste and quality compared to fresh tempeh. However, there are very limited studies in detail of the quality of overripe tempeh. With the existence of fresh tempeh and overripe tempeh, it is optimal to compare both tempeh. Hence, the objectives of this study were:

1. To compare the nutritional contents, physicochemical properties of the fresh and overripe tempeh.
2. To study the consumer preference level on the overripe tempeh.
3. To evaluate the microbial quality of the overripe tempeh using total plate and total yeast mould count method.

1.2 Problem Statements

There are a lot of research paper that revolves around the benefits of consuming tempeh. Recent studies claim that overripe tempeh can improve the appetite of consumers while also provide rich nutritional value (Puteri et al., 2018). However, there are no research paper that compares the differences between fresh tempeh and overripe tempeh regarding their nutritional value, physicochemical properties, sensory attributes, and the microbial stability. Overripe tempeh might undergo changes in texture, colour, flavour, and nutritional compositions during its extended fermentation. These changes are very important for both the consumers and producers to determine because the overall acceptability and nutritional value of the food will be impacted. In addition, extended fermentation time may increase microbial activity which might lead to food safety risks. Therefore, a thorough preliminary study that research on the physicochemical properties and microbial composition of overripe tempeh must be conducted.

1.3 Hypothesis

There are two hypotheses in this research:

1. Null hypothesis (Ho)
    a) There is no significant difference in proximate analysis.
    b) There is no significant difference in physicochemical properties.
    c) There is no significant difference in sensory evaluation.
2. Alternative hypothesis (Ha)
    a) There is significant difference in proximate analysis.
    b) There is significant difference in physicochemical properties.
    c) There is significant difference in sensory evaluation.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Soybean

Soybean (*Glycine max*), which is a part of the pea (legume) family of plants was first discovered in East Asia. The domestication of the soybean is believed to have originated in China (Li et al., 2010). Since centuries ago, soybean has been used as a source of food and drinks that are high in protein. Many other countries throughout the world, including China, Indonesia, Korea, Japan, and others also employ soybean in their popular cuisines. Aside from being a prominent component in local specialties, it is also commonly used in the form of soybean oil, or sauce which serves as a flavour enhancer in family cooking. Soybean production begins with the cultivation of the beans themselves as seeds (Naresh et al., 2019). Yellow soybean is harvested after it is ripened and harden on the plant. The soybean can also be harvested in the form of immature green soybean, which is known as edamame, it is often served in its pod after cooked (Garone, 2022).

Figure 2.1: Soybean (*Glycine max*).

2.1.1 Composition of Soybean

Soybean is a complete source of dietary protein; it includes all nine necessary amino acids. It is food that is rich in protein, carbohydrate, fat, fibre, vitamins, and minerals. It also includes a range of bioactive compounds, such as saponins, bioactive peptides, protease inhibitors, and isoflavones. Isoflavones are part of a group of substances known as phytoestrogens, which are substances found in plants with structures similar as oestrogen (Dukariya et al., 2020). In soy, genistein predominates; the remaining isoflavones are daidzein and glycitein. Isoflavones plays important role for the health benefits of soybeans (Arnarson, 2023).

2.1.2 Health Benefits of Soybean

The intake of soybeans has been linked to several possible health advantages as well as a decrease in a few chronic conditions like obesity, diabetes, immunological disorders, some forms of cancer, and cardiovascular disease. Furthermore, soybeans are also able to prevent osteoporosis, keeping the thyroid in good health and influences fertility (Dukariya et al., 2020).

2.2 Fermentation

Fermentation is the metabolic process of microorganisms which cause chemical changes to food. It is a method used to preserve food by producing antibacterial metabolites since ancient times (Dimidi et al., 2019). During this process, microorganisms will consume the carbohydrates and convert it into acid or alcohol. Fermentation in food can be categorised as two main types, which is lactic acid fermentation, and ethanol fermentation. (Faden, 2020).

2.2.1 *Rhizopus Oligosporus*

Rhizopus oligosporus is a fungus that is frequently used to ferment food in Asian country, particularly used in the production of tempeh (Nout and Aidoo, 2002). It will secrete enzymes that degrade protein, fat, and starch, acting as growth substrates and contributing to metabolic activities. The metabolism of the mould will cause rupture of the soybean cells, resulting in an increased ratio of mature mould, as seen by its darker hue (Handoyo and Morita, 2006).

2.2.2 Succession of Microbial Community During Fermentation

The concept of microbial succession provides us a structure for comprehending the temporal and geographical changes that occur during a microbial fermentation (Luo et al., 2020). An ideal environment for microbial succession can be established through fermentation process as it produces a wide range of nutrients that are required for the microbial development. As it continues to ferment, competition will arise among various kind of microorganisms for these nutrients. Those that can best utilise the available nutrients are going to survive, while the others will starve to death. This microbial succession mechanism will greatly influence the tempeh quality during the tempeh fermentation process. Different microbial strains that present in tempeh contribute significantly to its flavour, texture, and overall quality. Therefore, the tempeh would be less appealing without this microbial succession mechanism (Cooper, 2022).

2.2.3 Tempeh Fermentation

It is a polymicrobial fermentation as the process involves various kind of microorganisms such as, lactic acid bacteria, and few kinds of *Rhizopus* moulds (Puteri et al., 2018).

The first stage of fermentation starts by the soybean naturally undergoes fermentation which is lactic acid fermentation when it is soaked and left overnight. Soaking is an important step to increase the moisture level of the beans, making them appropriate for eating and boosting microbial activity during fermentation. This procedure also aids in the extraction of naturally occurring antibacterial compounds, such as saponins and the bitter components. Therefore, the soaking liquid should be removed and the beans are cooked in fresh water (Nout and Aidoo, 2002). The second stage of fermentation starts by mixing the soybeans with the starter culture and left to ferment at room temperature (BC Centre for Disease Control, 2016). At the beginning of the second stage fermentation, the temperature of soybean inoculant rises slowly. After that, the mould grows in rapid succession until it reaches the peak and subside slowly. The mould growth is closely related to the increase and decrease in temperature. The peak temperature can go up to 45^{o}C (Liu, 2008). At this temperature, the dense cottony mycelium is formed during the fermentation process which will bind the soybeans together and form a compact cake. The desirable fermentation temperature to produce tempeh is between 27–30^{o}C which is the room temperature. Good air circulation is required during the fermentation process (Dwiatmaka et al., 2022).

2.2.4 Health Benefits of Tempeh

Tempeh is a quality source for vitamin B, iron, calcium, fiber and so on. The notable nutritional content of tempeh served in 100g is as followed:

Table 2.1: Comparison on nutritional composition between tempeh and beef.

Attributes	Tempeh	Beef	Unit
Energy	192	243	kcal
Protein	20.3	17.5	grams
Fat	10.8	19.4	grams
Carbohydrates	7.64	0	grams
Calcium	111	7	mg
Iron	2.7	1.96	mg
Vitamin B-6	0.215	-	mg
Cholesterol	0	68	mg

Data obtained from (U.S. DEPARTMENT OF AGRICULTURE, 2018).

From the nutritional value of tempeh, it can be derived that it is relatively high protein, moderate fat, and low carbohydrate food. It also does provide some essential vitamins and minerals. As compared to grounded raw beef with 80% lean meat and 20% fat, it has lower calories, higher protein, lower fat, and better minerals. This makes tempeh one of the best choices of meat substitutes for vegans and patient with health issues as it has a lot of benefits.

The main benefit of consuming tempeh includes decrease blood pressure, lower risk of heart problems, better insulin resistance, decrease inflammation and promote bone health. This can be directly derived from the nutritional content. Low cholesterol resolved blood pressure and heart issues, while high calcium content is beneficial for bones. It is also a favourable alternative for people with digestive disorder to consume beans (Wiginton, 2021).

2.2.5 Factors Affecting Quality of Tempeh

During the tempeh fermentation process, there are a few factors that can affect the quality of tempeh. First, the rate of mycelial growth on the tempeh can be affected by few environmental factors, such as temperature, pH, a_w, and concentration of carbon dioxide (Sparringa et al., 2002). Furthermore, nutritional and chemical changes that happen during the tempeh fermentation will also affect organoleptic properties of the tempeh. For instance, the flavour of the tempeh can change as the metabolites change throughout the fermentation process. Sour flavour of tempeh is contribute by organic acids, while sweet, umami, and bitter taste of tempeh is contributed by certain type of amino acid, such as serine, glutamine, and methionine respectively (Prativi et al., 2023). Other than that, introducing other microbe along the fermentation process can affect the quality of the tempeh as well. Studies have shown that introducing S. *cerevisiae* to the fermentation process can enhance the nutritional content of tempeh. Besides, the duration of fermentation can also affect the quality of tempeh. The increment in fermentation time could potentially improve the tempeh quality, for example moisture, protein, and ash content. However, the fermentation needs to be controlled. If the tempeh is left fermented for too long, the tempeh will soften and release undesirable smell. In addition, the nutritional quality will also deteriorate (Rizal et al., 2022).

2.3 Physicochemical Analysis

2.3.1 pH Analysis

The pH is a gauge of the acidity of alkalinity in an aqueous solution. In food industry, the primary use of pH is to regulate the chemical and physical reactions required for food production and inhibit the proliferation of pathogens. It is especially important for the cases of fermented food, such as tempeh. The pH will change throughout the fermentation process. At the first step of fermentation, the pH value will be lower to below 4.6 as lactic acid is produced. This is important to keep Bacillus cereus and Staphylococcus aureus from developing when the beans are soaked overnight. While during the second stage of fermentation, it is an alkaline fermentation. The mould will release ammonia which will increase the pH value to approximately between 6.8-8.0 (BC Centre for Disease Control, 2016). Accurate pH measurement is important as it ensures good product quality and complies with the regulations of food safety (Aliouche, 2019). The pH value of a 48 hours fermented tempeh is in the range of 7.7-8.4 (Bhowmik, Balasubramanian and Yadav, 2013).

2.3.2 Colour Analysis

Food colour analysis is the process to quantify food colour using equipment. For instance, the utilization of spectrophotometer for colour measurement enables food producers and retailers to understand and meet the food quality standards. This is important because human eye is subjective, which means that every person reads colour differently (Phillips, 2023). This is done by measuring the L*, a*, and b* values. L* values indicate the degree of lightness from 0 (black) - 100 (white). Positive a* values indicate redness, while negative a* values is green colour. Positive b* values show yellow colour, while negative b* values show blue colour (Pathare, Opara and Al-Said, 2012). The mycelium is white colour but once it is sporulates, it will turn black as the spores is black. Therefore, overripe tempeh will have darker colour compared to fresh tempeh (BC Centre for Disease Control, 2016).

2.3.3 Water Activity

Water activity is a gauge of the amount of accessible water that is available for microorganisms to utilize for growth (Mermelstein, 2009). It increases with temperature. Majority of food contains water activity more than 0.95, which provides enough moisture for the bacteria, yeasts, and mould to grow. This amount can be reduced to the point where the growth of these organisms will be inhibited (Public and Service, 2020). Measuring water activity is important for food safety in terms of microbial growth by determining the lower limit of available water. The lowest water activity required for most food spoilage bacteria to grow is about 0.90 (Sungtong, 2023).

2.3.4 Titratable Acidity

Titratable acidity is the calculation of sum between total free protons and un-dissociated acids within a solution. It expressed the total acidity of a solution as an approximate value. The main difference of pH and titratable acidity is that pH does not have unit whereas titratable acidity uses grams per litre (Madhu, 2018). The purpose of titratable acidity is similar to pH measurements, which is to ensure food quality and complies with food safety regulations.

2.3.5 Texture Profile Analysis

In texture profile analysis, there are a few parameters that could be analysed. For instance, hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. Hardness is defined as the force that is required to change the shape of food sample in a certain way. For example, a cheese is easily dented when pressed, it means that the hardness is low. Next, fracturability is defined as the force where the first major changes happen is observed. For instance, if it takes a lot of pressure to snap a carrot in half, its fracturability is lower. Then, adhesiveness refers to the work needed to unstick the food sample from the tool. For example, a melted cheese on the spoon is very difficult to be removed, hence its adhesiveness is high. Besides, springiness refers to the speed of a squished food sample getting back to normal shape. For instance, if a marshmallow is squished and it expand back to its original form very quickly, its springiness is high. Other than that, cohesiveness means the strength of internal bonds inside a food sample. For example, if a food crumbles easily, it has low cohesiveness and if a food stays together after bitten or pulled, it has high cohesiveness. Furthermore, gumminess refers to the energy required to break down soft food to swallow. For instance, gummy candy needs to be put in the mouth for manipulation before swallowing. Higher gumminess means higher amount of manipulation needed. Moreover, chewiness is defined as the energy required to chew a hard food until it is swallowable. For example, comparing chewing a piece of steak and a piece of bread, the steak has higher chewiness as it takes longer to chew it until it is swallowable. Lastly, resilience measures the ability of the material to recover or bounce back after it has been compressed. For example, if a sponge cake is pressed down and it recover to original shape quickly, it is high resilience, if it stays squished, it is low resilience (Trinh and Glasgow, 2012).

2.4 Proximate Analysis

It is a method used to determine the macronutrient content in a food samples, which includes moisture content, ash content, crude fat content, crude protein content, carbohydrate content. To obtain reliable result, sampling and sample preparation must be done carefully so that homogenous and representative sample can be analyse (Nielsen, 2006).

2.4.1 Moisture Content

Moisture content is the quantity of water that is present in a material. The measurement of water content is a challenging task because of the complex intermolecular bonding within the material's structure. To accurately assess and maintain suitable water levels during processing, the term moisture content is used to define the measurable water content of a sample. Moisture content can impact the physical properties of a substance and is unavoidable in a processing environment. Hence, it is important to precisely identify and manage moisture content levels for scientific operations or production to be done successfully (Wesolowski, 2023). In a 45 hours fermented tempeh, 65.65% of moisture content is recorded (Rizal et al., 2022).

2.4.2 Ash Content

Ash content, in food terms, refers to the substances that are left once the food sample is exposed to intense heating which can eliminate moisture, volatile compounds, and organic matter. In food terms, ash content determines the overall mineral content that exist within a food. It is important because it is used to determine the nutritional content of the food (Mukherjee, 2019). In this study, the author cited Astawan et al. whom states that tempeh contains 2.01–2.47% of ash content (Rizal et al., 2022).

2.4.3 Crude Fat Content

Crude fat refers to the fat-soluble material crude mixture that is found in a food sample. It is used to define the overall quantity of fat that exist within the sample. It can be done by extracting the fat from food sample using solvent like petroleum ether to calculate the lipid content. The use of crude fat in a human body is to provide energy. It is important to define the crude fat content because consuming too much crude fat can cause health issues like obesity and heart disease (Sarmila, 2023). In a 45 hours fermented tempeh, 8.47 fat content is recorded (Rizal et al., 2022).

2.4.4 Crude Protein Content

Crude protein in food terms is basically how much protein is in the food. Determination of crude protein is by using the measurement of nitrogen content in a food to approximate the protein content within the food (Hobson, 2019). The accurate measurement of crude protein content is important, especially in cases like milk because it determines the business value of the food product and influences the business feasibility of emerging industries that focuses on alternative protein production (Hayes, 2020). In a 45 hours fermented tempeh, 17.14% of protein content is recorded (Rizal et al., 2022).

2.4.5 Carbohydrate Content

Carbohydrates, or saccharides is also a source of energy for the body. Body can break down carbohydrates into glucose, which serves as the main energy supply for the muscle and brain. Human are recommended to consume certain range of calories in form of carbohydrates every day depending on their activity level, body size and blood sugar control. After all, too much carbohydrate intake can lead to diabetes, which is why determination of carbohydrate content is important for human to calculate the calories intake (Brazier, 2023). In this study, the author cited Astawan et al. whom states that tempeh contains 6.12%–6.57% of ash content (Rizal et al., 2022).

2.5 Sensory Evaluation

2.5.1 Hedonic Test

Hedonism is a word derived from the Greek word ‘hedone’ that is defined as pleasure (Ray, 2017). Hedonic test can also be defined as the degree of consumer satisfaction on the food samples. Hedonic test is the most common method for sensory evaluation. There are various type of hedonic test depending on the hedonic scales. The most widely used scale is the nine-point scale but there are flaws because it mainly uses language like for a scale of 1 – 9, 1 means dislike extremely and 9 means like extremely. It does not work well in other languages. Besides that, the adjectives are very subjective to each respondents (Meullenet, 2004). Hence, there are different hedonic scales which are improvised and is better to be used, such as 5-point hedonic scales, where 1 – dislike extremely, 2- dislike slightly, 3- neither like or dislike, 4- like slightly, 5-like extremely (Graham, Agbenorhevi and Kpodo, 2017). Hedonic test is very important to determine the acceptability of the consumer on a food sample.

2.6 Microbial Analysis

2.6.1 Total Plate Count

Total plate count (TPC) is a technique used to approximate the total number of microorganisms like mould, yeast and bacteria present in a substance (Arifan et al., 2019). The purpose of TPC is to analyse whether the product is safe for consumption, and the health risk associated with consumption of the product (G

A Hanum, A Kurniawati and R Normaliska, 2018). From one of the studies conducted, there are 3 different manufacturers of fresh tempeh. The plate count of the tempeh from each manufacturers differs, ranging from 6.04–10.76 log CFU/g (Erdiansyah et al., 2022).

2.6.2 Total Yeast Mould Count

Total yeast and mould count (TYMC) is a technique used to calculate the number of colony forming units present in every gram of product (CFU/g). It is the scientific method to count and report the population of living bacteria, yeast, and Mould in a food sample. The purpose of TYMC is to indicate the general cleanliness of the product in the entire lifecycle, including aspects like growth environment, material handling, storage facilities and processing conditions. Having Mould is not necessarily bad, but a high Mould count can indicate that the food is spoiled, which is detrimental to the health of consumer (Yaghmaee, 2017). In this study, the microbial count using potato dextrose agar in soybean tempeh is more than or equal to 10^4 UFC/g, which is equivalent to CFU/g, where UFC/g is more commonly used in Italy (Bento et al., 2021).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials and Instruments

Yellow soybeans were used for tempeh production was purchased from the local supermarket, Econsave Cash & Carry Sdn Bhd in Kampar. While the tempeh starter culture was purchased from the same authorised online store, Unchang Tea in Shopee throughout the research to achieve consistent results. The powdered tempeh starter culture used was composed of a dried mixture of live culture with rice as the substrate.

The instruments used were, weighing scale, induction cooker, thermometer, drying oven, Mettler Toledo pH meter, Interscience BagMixer, Sorvall Legend Micro 21R centrifuge, Philips Series 5000 Blender Core, TA-XT Plus Texture Analyser, CM-600d Colorimeter, A&D Moisture analyser MX-50, Lab Swift Portable Water Activity Meter, Nabertherm Muffle Furnace, Gerhardt Fat Analyser, Desiccator, SpeedDigester K-436, Scrubber K-415, Distillation Unit K-350, Hirayama Autoclave Machine, ESCO Laminar Flow Cabinet.

3.2 Tempeh Preparation

The fresh tempeh and overripe tempeh were fermented in the Food Processing Lab in UTAR, D209. The main ingredients needed for tempeh preparation were 100 g soybean, 10 g tempeh starter culture, and 10 mL of distilled white vinegar.

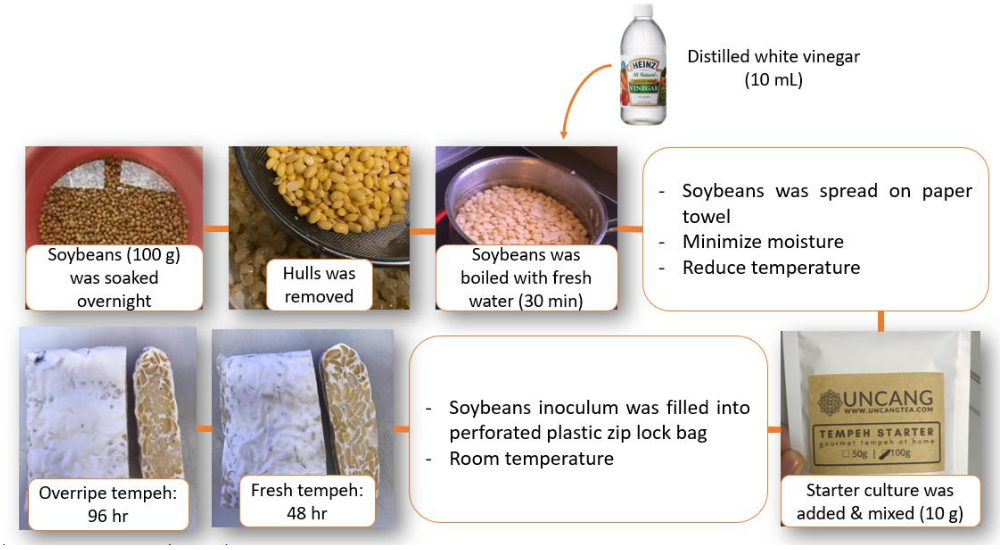

Figure 3.1: Fresh tempeh and overripe tempeh preparation flow.

Firstly, the soybeans were soaked in tap water overnight to ease the dehulling process, and the excessive water was drained off after that. The soaked soybeans were rinsed with tap water a few times and to remove the hulls. The utensils required for the step later were all brought to boil in a large pot as a sterilisation process. Next, the soybeans were boiled in a large pot filled with 700 mL tap water for 20 minutes to soften the soybeans. Distilled white vinegar was then added into the pot and continue cooked for 10 minutes. After that, the excess water was drained off from the large pot. The soybeans were spread on a paper towel to remove all the remaining liquid on the soybeans. The soybeans were then left to cool in the pot to at least lower than 35°C using a thermometer. This was an important precaution because the tempeh starter cannot survive in temperature higher than 35.6°C. The tempeh starter culture was added into the pot and mixed well with a sterilised utensil to allow fermentation process.

The soybeans inoculated with the starter culture were filled into a perforated 12 cm X 20 cm plastic zip lock bag. The holes on the plastic bag were perforated in a 4 X 4 manner to allow air circulation for the aerobic fermentation to occur. Then, the zip lock bag was closed and folded into a size that allow the inoculated soybeans to spread evenly and achieve a desirable thickness of tempeh. The perforated zip lock bag containing the inoculated soybean was placed into a ventilated plastic container to prevent uncontrolled factor such as insects. Finally, the zip lock bags were kept room temperature for a duration of 48 hours for fresh tempeh and 96 hours for overripe tempeh as shown in Figure 3.2 below.

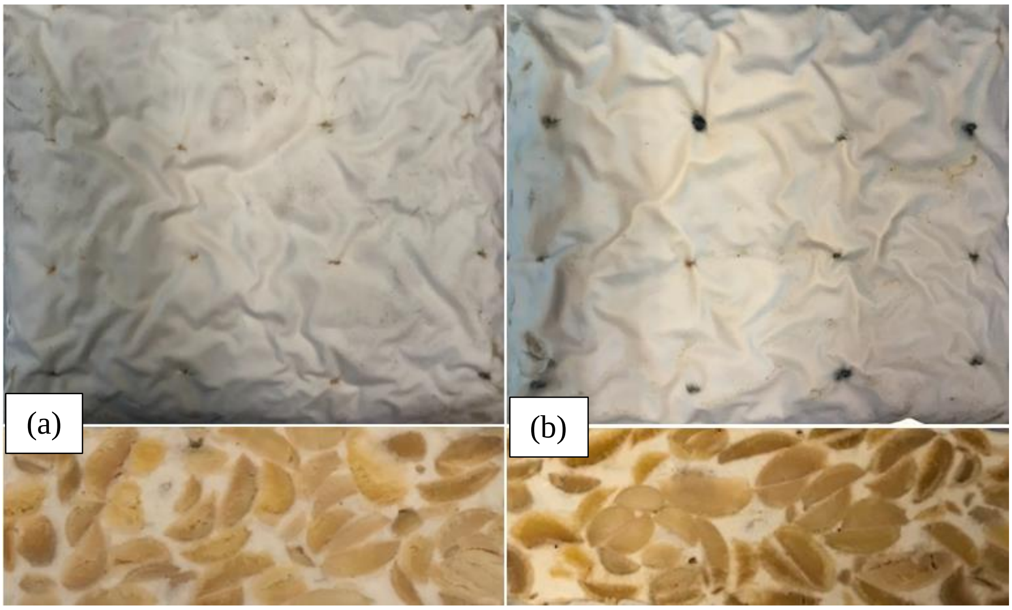

Figure 3.2: From left to right (a) Fresh tempeh covered with mycelium; (b) Overripe tempeh covered with sporulated mycelium.

3.3 Physicochemical Analysis

3.3.1 Determination of Water Activity

The water activity of the fresh tempeh and overripe tempeh was analysed using Lab Swift Portable Water Activity Meter. First, the samples were homogenised using Philips Series 5000 Blender Core. The water activity meter was preheated and calibrated with the salt standard provided. A layer of homogenised sample was spread evenly on the sample dish and placed into the water activity meter. All the sample were run in triplicate and the water activity was recorded.

3.3.2 Determination of pH

The pH value of the fresh tempeh and overripe tempeh was analysed using Mettler Toledo pH meter. About 1 g of sample was mixed with 5 mL of distilled water and the mixture was homogenised for 10 minutes (Handoyo and Morita, 2006). The probe of the pH meter was rinse with distilled water and dried with Kimwipes. The pH meter was first calibrated with buffer solution. The probe was then placed into the sample mixture to record the measurement. All the sample were run in triplicate and the pH value was recorded.

3.3.3 Titratable Acidity of Tempeh

The titratable acidity of fresh tempeh and overripe tempeh was determined using titration method. Approximately 20 g of sample was homogenised with 180 mL of distilled water using a Interscience BagMixer. The mixture was then centrifuged at 3100 rpm for 10 minutes at 4℃. About 10 mL of supernatant was aliquoted into a conical flask using pipette. Few drops of phenolphthalein were added into the conical flask as an indicator. The solution in the conical flask was titrated with 0.1 N NaOH until the solution turns to pink colour (Nurdini et al., 2015). The volume of NaOH titrated was recorded. All the sample were run in triplicate and the titratable acidity was calculated as shown in Equation 3.1 below.

Equation 3.1: Formula used to calculate titratable acidity.

$$\text{Titratable acidity (\%)} = \frac{(volume\ of\ NaOH\ used)\ \times 0.1 \times 100}{volume\ of\ sample}$$

3.3.4 Colour Measurement of Tempeh

The colour of the fresh tempeh and overripe tempeh was analysed using CM-600d Colorimeter. The colorimeter was calibrated with the white calibration cap provided. The measurements collected from the sample was categorised as mycelium-covered surface, and cross-sectional surface as shown on the Figure 3.3 below.

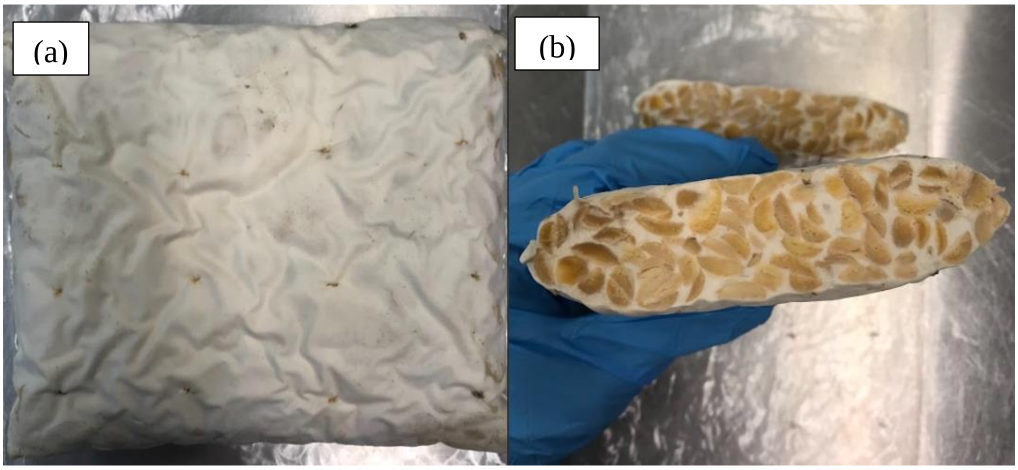

Figure 3.3: From left to right (a) mycelium-covered surface; (b) cross-sectional surface.

The measurements were collected according to the CIE-Lab parameters in three dimensions: L*, a*, and b*. All the sample were run in triplicate and the L*, a*, and b* was recorded (Zahidah and Lo, 2022).

3.3.5 Texture Profile Analysis of Tempeh

The texture analysis of the fresh tempeh and overripe tempeh was determined using TA-XT Plus Texture Analyser. First, the sample was prepared and sliced into an equal size of 2 cm^3 dimension. The load cell and probe height of the texture analyser was calibrated and fitted with the 2 mm diameter cylinder probe. The hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience were the eight parameters recorded using the Exponent Software (Zahidah and Lo, 2022). All the sample were run in triplicate.

3.4 Proximate Analysis

All the sample used for determination of ash content, crude fat content, and crude protein content was dried using a drying oven at 102℃ for 5 hours and homogenised using Philips Series 5000 Blender Core. The sample was then kept in Schott bottle for further used as shown on the Figure 3.4 below.

Figure 3.4: Homogenised dried sample for fresh tempeh and overripe tempeh.

3.4.1 Determination of Moisture Content

The moisture content of the fresh tempeh and overripe tempeh was analysed using A&D Moisture analyser MX-50. First, the samples were homogenised using Philips Series 5000 Blender Core. The moisture analyser was preheated, calibrated, and the temperature was set to 105℃. The sample pan cleaned before analysing each measurement. Approximately 1 g of homogenised sample was used and spread evenly on the sample pan to start the analysis. All the sample were run in triplicate and the percentage of moisture content were recorded in wet basis (wb).

3.4.2 Determination of Ash Content

The ash content of fresh tempeh and overripe tempeh was analysed using dry ashing method. The crucible was prepared by pre-ashing in the Nabertherm Muffle Furnace at 550℃ for 12 hours. The weight of each empty crucible was then recorded. About 2 g of homogenised dried sample was measured into the crucible. The sample was incinerated in the muffle furnace at 500℃ for 8 hours (Gaines, 2023). The crucible was cooled to room temperature in the desiccator and the weight of the ashed sample was recorded. All the sample were run in triplicate and the percentage of the ash content was calculated and converted to wb.

3.4.3 Determination of Crude Fat Content

The crude fat content of the fresh tempeh and overripe tempeh was analysed using Soxhlet method. The extraction beaker together with three boiling stone in it was preheated in a drying oven at 105℃ for 1 hours. It was then cool down in desiccator for 30 minutes. The weight of the extraction beaker with boiling stone was recorded. About 5 g of homogenised dried sample was wrapped with filter paper, then placed into a thimble and cotton wool was inserted to cover it. The thimble was inserted into the extraction beaker and about 90 mL of petroleum ether was added in a fume hood. The Gerhardt Fat Analyser was preheated and used to extract fat from the sample. The extraction beaker was dried in a drying oven at 105℃ for 1 hours followed by cooling down in desiccator for 30 minutes. The final weight of the extraction beaker was again recorded. All the sample were run in triplicate and the percentage of the crude fat content was calculated and converted to wb.

3.4.4 Determination of Crude Protein Content

The crude protein content of the fresh tempeh and overripe tempeh was analysed using Kjeldahl method which involve digestion, distillation, and titration.

The SpeedDigester K-436 was preheated to 8.5 level for at least 10–15 minutes. About 2 g of the homogenised dried sample, 7 g of potassium sulphate (K_2SO_4), and 0.8 g of copper sulphate ($CuSO_4$) was added into the digestion tube. Approximately 20 mL of concentrated sulphuric acid was added into the digestion tube in a fume hood. The digestion tube was placed into the rack on the digester to start the digestion process. The digester was turned off when all the sample turn to a clear blue colour. The digested sample in digestion tube was cooled down below 40°C and it was ready to proceed to the distillation step.

The Distillation unit K-350 was preheated. A digested sample in digestion tube was placed into the distillation unit. A conical flask containing 25 mL of 4% boric acid with few drops of indicator (Bromocresol green) was placed at the receiving vessel. Distilled water and 32% sodium hydroxide (NaOH) were added into the digestion tube until the sample solution turns brown in colour. The volume of distilled water used was 40 mL while volume of 32% NaOH used was 60 mL. The distillation was processed for 2 minutes for each sample until the solution in the conical flask at the receiving vessel turns from pink to blue in colour. It was ready to proceed to the titration step.

The distilled solution in the conical flask was titrated with 0.1 M standardise hydrochloric acid (HCl) (Gaines, 2023). The titration process was done as the colour of the distillate turns pink in colour. The volume of HCl titrated was recorded. All the sample were run in triplicate and the percentage of crude protein content was calculated and converted to wb.

3.4.5 Determination Carbohydrate Content

The carbohydrate content (wb) of the fresh tempeh and overripe tempeh was determined using difference method. It was calculated as the Equation 3.2 shown below.

Equation 3.2: Estimation of carbohydrate content using difference method.

$$\text{Carbohyrate (\%)} = 100\% - (Moisture + Ash + Crude\ Fat + \ Crude\ Protein)\%$$

3.5 Sensory Evaluation of Respective Samples

A 5-point hedonic scale was used to evaluate the preference level between fresh tempeh and overripe tempeh. This test was conducted in the Sensory Analysis Laboratory of UTAR with 50 untrained panellists from UTAR. The sensory attributes evaluated was appearance, aroma, taste, texture, and overall acceptability. The score sheet was prepared as shown in **Appendix A.**

All the samples were sliced into 0.5 cm width and 5.5 cm ± 0.5 cm length, then shallow fried on steel pan with hot oil for 2 minutes each side. Each sample was assigned with three random digits, and the sequence of the sample presentation was randomised according to the master sheet prepared as shown on **Appendix B**. The sample was placed into a sample cup and the random digits assigned was wrote on the sample cup (Drake, 2021). The samples were served to the panellists on a tray. The data collected was analysed, and the mean score for each attribute was compared using paired t-test.

3.6 Microbial Analysis of Respective Samples

Two method were used to analyse the microbial quality in this study, which is total plate count method, and total yeast mould count method. The agar media used for total plate count method was plate count agar (PCA) while for total yeast mould count was potato dextrose agar (PDA). Molten PCA was prepared by mixing 4.7 g of PCA powder with 200 mL of distilled water. While molten PDA was prepared by mixing 7.8 g of PDA powder with 200 mL of distilled water. The 200 mL of molten PCA, 200 mL of molten PDA, 90 mL of distilled water, five test tube containing 9 mL of distilled water, and micropipette tips was autoclaved at 121℃ for 15 minutes at 975 kPa.

3.6.1 Sample Preparation

The utensils used to prepare the sample was sterilised at first place. The sample was then sliced and kept into plastic zip lock bag for shelf-life study. Each plastic zip lock bag contained 10 g of sample which is the amount required for homogenisation in the serial dilution. This was done as shown on the Figure 3.5 below.

Figure 3.5: Sample prepared in zip lock bag to prevent cross contamination.

3.6.2 Serial Dilution

About 10 g of sample was homogenised with 90 mL of sterile distilled water using stomacher for 2 minutes to get 10^{-1} dilution. A micropipette was then used to aliquot 1 mL of the 10^{-1} dilution into a test tube containing 9 mL of sterile distilled water to prepare the 10^{-2} dilution. This step was repeated accordingly until 10^{-6} dilution.

3.6.3 Microbial Population Analysis

The microbial population in fresh tempeh and overripe tempeh was determined using PCA and PDA (Charles Vegdahl, 2022). The sterile molten PCA was cooled to around 40–45°C. Micropipette was used to aliquot 1 mL of 10^{-4} dilution inoculum on to a sterile petri dish. The cooled sterile molten PCA was poured into the sterile petri dish. The petri dish was rotated vertically, clockwise, horizontally, and anticlockwise to mix the media with the inoculum gently. The step was run in duplicate and repeated for 10^{-5} dilution, 10^{-6} dilution, and sterile molten PDA for all the sample. All the inoculated petri dish were incubated upside down in the incubator at 37℃ for 24 hours (Dahal, 2022). The surface and subsurface colonies was counted. All the petri dish that contained colonies within the range of 20 – 200 was enumerated. The petri dish that contained 10^{-5} dilution of inoculum was used in this study as the number of colonies was calculated in log CFU/g.

3.6.4 Shelf-Life Study on Overripe Tempeh

The shelf-life study on overripe tempeh was determined using PCA and PDA (Charles Vegdahl, 2022). The plastic zip lock bag containing 10 g of overripe tempeh was stored in refrigerator at 4℃ for 14 days. The analysis in this study was done on day 0, day 3, day 7, day 10, and day 14 of storage period. The sterile molten PCA was cooled to around 40–45℃. Micropipette was used to aliquot 1 mL of 10^{-4} dilution inoculum on to a sterile petri dish. The cooled sterile molten PCA was poured into the sterile petri dish. The petri dish was rotated vertically, clockwise, horizontally, and anticlockwise to mix the media with the inoculum gently. The step was run in duplicate and repeated for 10^{-5} dilution, 10^{-6} dilution, and sterile molten PDA. All the inoculated petri dish were incubated upside down in the incubator at 37℃ for 24 hours (Dahal, 2022). The surface and subsurface colonies was counted. All the petri dish that contained colonies within the range of 20–200 was enumerated. The petri dish that contained 10^{-5} dilution of inoculum was used in this study as the number of colonies was calculated in log CFU/g.

3.7 Statistical Analysis

IBM SPSS Statistical Software Version 29.0.1.0 was used to analyse the proximate composition, physicochemical properties, sensory evaluation, and microbial load using independent t-test. It was conducted to determine the significant difference between samples. All the measurement was then presented as mean ± standard deviations in the results.

CHAPTER 4

4.0 RESULTS

4.1 Physicochemical Analysis

Table 4.1 represented the physicochemical properties for fresh tempeh and overripe tempeh. The results showed that both the fresh tempeh and overripe tempeh exhibited similar water activity result (0.96, $p>0.05$). Likewise, there was also no significant difference ($p>0.05$) detected between the pH value of fresh tempeh (6.66) and overripe tempeh (6.43). Furthermore, the total titratable acidity of overripe tempeh (0.20%) was relatively higher than fresh tempeh (0.13%). These results indicated a significant difference ($p<0.05$) for the titratable acidity between these two tempeh.

Moreover, the colour measurement for both mycelium-covered surface and cross-sectional surface in these two tempeh showed similar results ($p>0.05$). The results of mycelium-covered surface for overripe tempeh appears diminished in L* value (79.27), while slightly higher in a* value (0.49), and b* value (13.82) when compared to fresh tempeh (L*=88.91, a*=0.43, b*=11.17). On the other hand, the results of cross-sectional surface for overripe tempeh also showed lower L* value (69.90), higher a* value (5.51), but lower in b* value (29.20) compared to fresh tempeh (L*=74.20, a*=4.86, b*=30.11).

Table 4.1: Physicochemical properties of fresh tempeh and overripe tempeh.

			Tempeh samples	
			Fresh Tempeh	**Overripe Tempeh**
Water activity (a_w)			0.96 ± 0.00^{a}	0.96 ± 0.00^{a}
pH			6.66 ± 0.05^{a}	6.43 ± 0.04^{a}
Titratable acidity (%)			0.13 ± 0.06^{a}	0.20 ± 0.00^{b}
Colour	Mycelium-covered surface	L*	88.91 ± 1.38^{a}	79.27 ± 4.57^{a}
		a*	0.43 ± 0.29^{a}	0.49 ± 1.06^{a}
		b*	11.17 ± 2.12^{a}	13.82 ± 3.43^{a}
	Cross-sectional surface	L*	74.20 ± 4.23^{a}	69.90 ± 3.29^{a}
		a*	4.86 ± 1.85^{a}	5.51 ± 0.99^{a}
		b*	30.11 ± 3.30^{a}	29.20 ± 3.38^{a}

*Different superscript letters within the same row indicates there is statistical difference between the mean value ($p<0.05$). While same superscript letters within the same row indicates no significant difference ($p>0.05$).

4.2 Texture Profile Analysis

The results of TPA for fresh tempeh and overripe tempeh was shown in Table 4.2. It was observed that overripe tempeh (1.63) exhibited lower hardness value ($p>0.05$) as compared to fresh tempeh (3.41). Similarly, overripe tempeh also showed a lower value ($p>0.05$) in springiness (0.83), and cohesiveness (0.20) as compared to fresh tempeh (0.91, and 0.42 respectively). Nonetheless, overripe tempeh showed a highest fracturability value which was 3.09 N among all the parameters. In fact, fresh and overripe tempeh showed significant difference ($p<0.05$) on adhesiveness, gumminess, and chewiness.

Table 4.2: Texture profile analysis of fresh tempeh and overripe tempeh.

Parameter (Unit)	**Tempeh samples**	
	Fresh Tempeh	**Overripe tempeh**
Hardness (N)	3.41 ± 1.31^{a}	1.63 ± 0.38^{a}
Fracturability (N)	2.39 ± 0.60^{a}	3.09 ± 0.28^{a}
Adhesiveness (N.s)	-0.71 ± 0.49^{a}	-0.84 ± 0.11^{b}
Springiness	0.91 ± 0.11^{a}	0.83 ± 0.12^{a}
Cohesiveness	0.42 ± 0.03^{a}	0.20 ± 0.05^{a}
Gumminess (N)	1.14 ± 0.54^{a}	0.78 ± 0.09^{b}
Chewiness (N)	1.46 ± 0.90^{a}	0.37 ± 0.10^{b}
Resilience	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}

*Different superscript letters within the same row indicates there is statistical difference between the mean value ($p<0.05$). While same superscript letters within the same row indicates no significant difference ($p>0.05$).

4.3 Partial Proximate Composition of Respective Samples

Table 4.3 showed the averages of each composition for fresh tempeh and overripe tempeh which includes moisture, ash, crude fat, crude protein, and carbohydrate content. Generally, overripe tempeh showed higher partial proximate level as compared to fresh tempeh. For instance, the moisture content of overripe tempeh recorded as 58.05%, which was relatively higher to those found in fresh tempeh (55.23%). Next, the ash content in overripe tempeh (1.61%) was slightly higher than fresh tempeh (1.58%). Furthermore, overripe tempeh (17.75%) showed a higher carbohydrate content in contrast to fresh tempeh (17.46%). These results demonstrated that there was no significant difference ($p>0.05$) in moisture, ash, and carbohydrate content between these two tempeh.

Nevertheless, overripe tempeh (5.67%) demonstrated a lower crude fat content as compared to fresh tempeh (7.35%). In fact, a lower crude protein content was also detected in overripe tempeh (16.92%) as compared to fresh tempeh (18.38%). These results indicated a significant difference ($p<0.05$) in the crude fat, and crude protein content between these two tempeh.

Table 4.3: Proximate composition of fresh tempeh and overripe tempeh.

Parameter (wb)	Tempeh samples	
	Fresh Tempeh (%)	**Overripe Tempeh (%)**
Moisture	55.23 ± 0.35 [a]	58.05 ± 0.83 [a]
Ash	1.58 ± 0.01 [a]	1.61 ± 0.04 [a]
Crude Fat	7.35 ± 0.23 [a]	5.67 ± 0.03 [b]
Crude Protein	18.38 ± 0.03 [a]	16.92 ± 0.40 [b]
Carbohydrate	17.46 ± 0.45 [a]	17.75 ± 0.42 [a]

*Different superscript letters within the same row indicates there is statistical difference between the mean value ($p<0.05$). While same superscript letters within the same row indicates no significant difference ($p>0.05$).

4.4 Sensory Evaluation

The significant difference and mean ± standard deviations from the total of 50 panellists in the 5-point hedonic scale sensory test was shown in **Appendix D**. The mean score for each sensory attribute was then tabulated in Figure 4.1 below. The preferences level of panellists on the aroma, and overall acceptability of the fresh tempeh and overripe tempeh demonstrated a significant difference ($p<0.05$). While the preference level of panellists on appearance, taste, and texture between both tempeh were not significant difference ($p>0.05$).

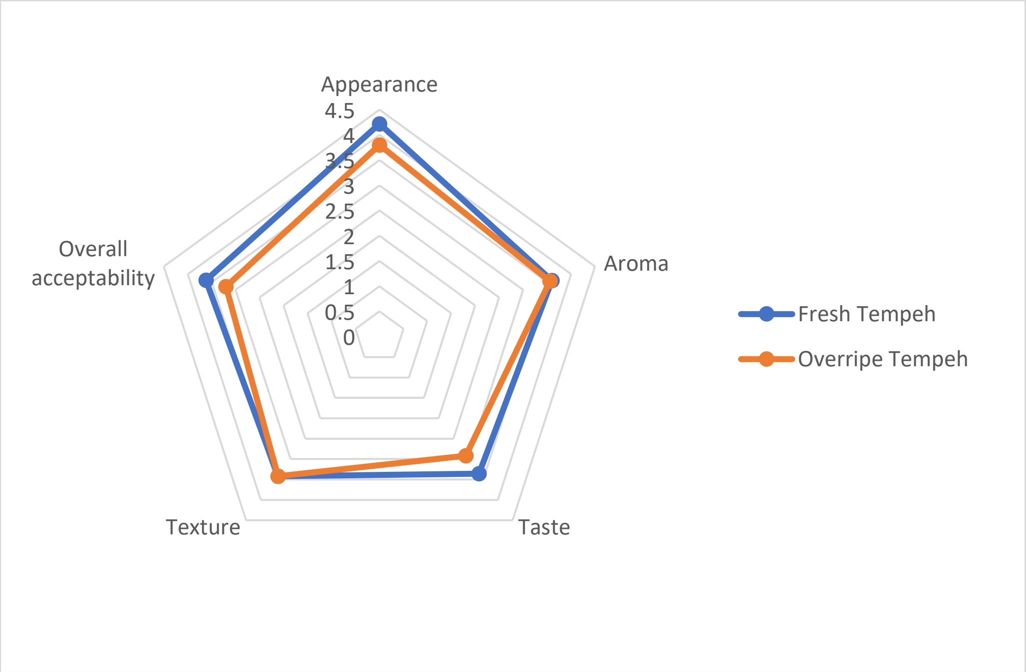

Figure 4.1: Sensory evaluation of the fresh tempeh and overripe tempeh.

4.5 Microbial Population Analysis

The microbial load of respective tempeh samples was evaluated in this study as the results was tabulated as Table 4.4. It was observed that the microbial load for both samples were not much difference ($p>0.05$), as the total plate count of the overripe tempeh was recorded as 5.64 log CFU/g, while for the fresh tempeh was 5.44 log CFU/g. In addition, the total yeast mould count of the samples exhibited similar population to the total plate count. For instance, the total yeast mould count of the overripe tempeh was 5.60 log CFU/g, while the fresh tempeh was recorded as 5.51 log CFU/g.

Table 4.4: Microbial load of the fresh tempeh and overripe tempeh samples.

Type of count	**Microbial load** (log CFU/g)	
	Fresh Tempeh	**Overripe Tempeh**
Total Plate Count	5.44 ± 0.11^{a}	5.64 ± 0.01^{a}
Total Mould Count	5.51 ± 0.02^{a}	5.60 ± 0.08^{a}

*Different superscript letters within the same row indicates there is statistical difference between the mean value ($p<0.05$). While same superscript letters within the same row indicates no significant difference ($p>0.05$).

4.6 Storage Test of Overripe Tempeh

The changes in microbial communities of overripe tempeh throughout the 14 days of cold storage (4°C) was tabulated in Table 4.5.

Initially, the total plate count (5.64 log CFU/g) and total yeast mould count (5.60 log CFU/g) for overripe tempeh exhibited relatively similar results ($p>0.05$). The total plate count for overripe sample shown steady increase until day 7 (5.80 log CFU/g) before it drops to 5.70 log CFU/g on day 10. However, the microbial load in total yeast mould count increased significantly ($p<0.05$) to 6.21 log CFU/g on day 3 before it started to drop to 5.89 log CFU/g, and 5.68 log CFU/g on day 7 and 10 respectively. In fact, the samples showed TFTC for both total plate and total yeast mould count after 14 days of storage time.

Table 4.5: Changes in microbial population of overripe tempeh throughout the 14 days storage period in fridge at 4°C.

Time (Days)	**Microbial load** (log CFU/g)	
	Total Plate Count	**Total Mould Count**
0	5.64 ± 0.01^{a}	5.60 ± 0.08^{a}
3	5.73 ± 0.03^{a}	6.21 ± 0.00^{b}
7	5.80 ± 0.00^{a}	5.89 ± 0.02^{a}
10	5.70 ± 0.12^{a}	5.68 ± 0.01^{a}
14	TFTC	TFTC

*Different superscript letters within the same row indicates there is statistical difference between the mean value ($p<0.05$). While same superscript letters within the same row indicates no significant difference ($p>0.05$).

CHAPTER 5

5.0 DISCUSSION

5.1 Effect of Extended Fermentation of Tempeh on Physicochemical Analysis

5.1.1 Water Activity

Water activity in tempeh indicated the availability of water in the product for microbial growth, enzymatic reactions, and other biochemical process. For tempeh, this was important because it was highly related to the fermentation, microbial stability, and shelf stability. It was understood that a water activity of at least 0.95 are required for bacteria, yeasts and mould growth (Public and Service, 2020). In this study, the water activity (a_w) for both fresh and overripe tempeh was recorded as 0.96 ($P>0.05$), indicated the major microorganisms especially the *Rhizopus Oligosporus* grew optimally throughout the fermentation process.

5.1.2 pH

The pH value assess the acidity and alkalinity of tempeh using a pH meter by measuring the negative log of the hydrogen ion concentration (Tyl and Sadler, 2017). The common pH value for a tempeh product falls between the range of 6.8−8.0 (BC Centre for Disease Control, 2016). Based on Table 4.1, the pH value of overripe tempeh (6.43) was lower than fresh tempeh (6.66). The reason for this is because yeast would change the substrate into alcohol and organic acids which result in the reduction of sugar content in the substrate. Besides, as the fermentation process was extended, the yeast growth would become dominant which indirectly contributed to the production and accumulation of organic acids (Rizal et al., 2023). Other than that, as tempeh fermentation involved conversion of protein into amino acids (Handoyo and Morita, 2006), negatively charged amino acid such as aspartic acid and glutamic acid would indirectly affect the pH value as well (Rawat, 2020). Furthermore, lactic acid bacteria presence in the tempeh would produce lactic acid which would also lower the pH (Hassanein, Prabawati and Gunawan-Puteri, 2015). Hence, prolonged fermentation process in overripe tempeh would exhibit a lower pH value.

5.1.3 Titratable Acidity

Intrinsic acids in the tempeh was titrated using standard base to assess the titratable acidity by measuring the amount of hydrogen ions that had neutralize (Boulton, 1980). This hydrogen ions could be found, as the organic acid dissociated. During the fermentation process, tempeh could contain various kind of organic acid. As an example, the predominant organic acid in soybean tempeh was acetic acid (Erkan et al., 2020). In addition, lactic acid would also produce by Lactic Acid Bacteria. These amounts of organic acid and lactic acid would increases as the fermentation prolonged (Prativi et al., 2023; Hassanein, Prabawati and Gunawan-Puteri, 2015), and thus increase the titratable acidity. This was in line with the result, where overripe tempeh had a higher titratable acidity as compared to fresh tempeh.

5.1.4 Colour Analysis

The colour analysis measures the visual appearance of tempeh in terms of hue, brightness, and saturation, denoted in the Lab* colour space. Based on the result in Table 4.1, for mycelium-covered surface, the L* values of overripe tempeh were lower than fresh tempeh, indicating that it was darker in colour. For the a* value, overripe tempeh was slightly higher than fresh tempeh. It indicates that the overripe tempeh had slightly more reddish hue than fresh tempeh. Both type of tempeh also had positive b* value, which means they were both yellowish in colour.

To sum it up, the mycelium-covered surface of overripe tempeh was darker, slightly higher reddish hue, and more yellowish than a fresh tempeh. This was because immature mould was white in colour while mature mould showed darker colour (Puteri et al., 2018). Another factor was due to the sporulation of *Rhizopus Oligosporus* on aged tempeh, where the spores are black in colour (Handoyo and Morita, 2006).

Whereas for the cross-sectional surface, it was similar in terms of L* values and a* values, as overripe tempeh had higher value than fresh tempeh. The reason for overripe tempeh to be darker on the cross-sectional surface was due to the increased number of fungi entering death phase. Besides, the amount of unsaturated fatty acid such as, linoleic, and linolenic were increased due to the prolonged fermentation process, and these fatty acids are susceptible to be oxidised, which causes darker colour. For the reddish hue, it was caused by the formation of vitamin B12, which was red in colour (Muzdalifah et al., 2017).

5.1.5 Texture Profile Analysis

The texture profile analysis was done to measure the mechanical characteristics of tempeh. The texture of the tempeh is one of the key quality features that is evaluated directly by consumers when consuming tempeh, a good tempeh were characterised as a compact and firm texture (Yuliani et al., 2022). From Table 4.2, overripe tempeh was observed to have a lower hardness, springiness, and cohesiveness, while had a higher fracturability.

Overripe tempeh was softer as compared to fresh tempeh due to the increment of mature mould would make the texture of the tempeh softer due to the weakening of mycelium network as a lot of the mycelia were not regenerated (Handoyo and Morita, 2006). In addition, the softer texture could also be due to the higher moisture content in overripe tempeh. Other than that, the texture of tempeh could also be affected by fat content, as well as the type and quantity of carbohydrates in the raw material (Wikandari et al., 2020).

Next, the springiness parameter uses the value of 0−1 to indicates a viscous sample, and elastic sample respectively. From the results, overripe tempeh demonstrated a less elastic characteristic as compared to fresh tempeh. When pressure was applied, the air in the cavities within the mycelium network was released. However, when the pressure was released, these cavities were not able to refill air due to the weak mycelium network of overripe tempeh. Therefore, overripe tempeh had a lower ability to retain its original shape (Wikandari et al., 2020).

Furthermore, the lower value of cohesiveness showed that overripe tempeh was less compact as compared to fresh tempeh. These values were affected by the capability of the fungal mycelia to spread through the soybeans. Whereas the weak mycelium network of overripe tempeh caused it to lose ability on binding the soybeans well to form compact cake.

Meanwhile, the fracturability was a measurement of the tempeh resistance to bend. The higher the fracturability of overripe tempeh, the shorter distance for the tempeh to break (Anis Jauharah, Wan Rosli and Daniel Robert, 2014), which means it was more brittle under applied force.

5.2 Effect of Extended Fermentation of Tempeh on Proximate Analysis

5.2.1 Moisture Content

The moisture content of this study indicated the amount of water present in the tempeh. It was understood that the moisture content could be used as an indicator of tempeh quality too. Based on Table 4.3, the moisture content of overripe tempeh (58.05%) was higher than fresh tempeh (55.23%). This situation might be attributed by the fact that the tempeh started to absorb moisture from its environment (Hassanein, Prabawati and Gunawan-Puteri, 2015), as it left to ferment for more days. Besides, another reason was due to the digestion of substrate during the microbial fermentation process, which results in producing water, carbon dioxide and energy (ATP). Hence, the longer the fermentation time, the higher the moisture content (Rizal et al., 2022).

5.2.2 Ash Content

The ash content was the inorganic minerals left after dry ashing in a muffle furnace. From Table 4.3, it could be observed that the ash content of overripe tempeh was slightly higher than fresh tempeh. This could be due to vitamin B12 that was formed across the tempeh fermentation period, which increases the ash content in tempeh due to existence of cobalt (Co) that exist in vitamin B complex (Sine and Soetarto, 2018). The higher ash content could also due to the decreased in moisture, carbohydrate, and fat content as the prolonged fermentation process (Obadina et al., 2013).

5.2.3 Crude Fat Content

The crude fat content of tempeh was measuring all fat-soluble component extracted from tempeh. Table 4.3 shows that overripe tempeh had lower crude fat content than fresh tempeh. This was also related to the fermentation period, where fat content decreases when fermentation period increases. The reason for that was because *Rhizopus Oligosporus* would produce lipase enzyme which functions to hydrolyse triacylglycerol into glycerol and fatty acid during the prolonged fermentation process. This fatty acid would then be used by *Rhizopus*

Oligosporus for growth by acting as an energy source during tempeh fermentation process. In the end, it results in lower fat content (Rizal et al., 2022).

5.2.4 Crude Protein Content

The crude protein content for tempeh measured the total nitrogen content in tempeh. Similarly, overripe tempeh had lower crude protein content than fresh tempeh. This was due to the cell structures of soybean were broken down by enzymes that was secreted by *Rhizopus Oligosporus* during fermentation process. In this cell disorganization process, protease would break down proteins into amino acid and peptide fragment (Handoyo and Morita, 2006). This also means that more nitrogen components were available upon degradation by *R. Oligosporus* throughout fermentation since the crude protein of tempeh mostly came from the soy protein.

On contrary, overripe tempeh exhibited relatively lower protein content as compared to the fresh one that found in this study. These phenomena could be attributed by the conversion of protein into soluble protein or amino acid during the extended fermentation (Puspitojati et al., 2019). Then, these degraded proteins were utilised in mould biomass production, which were oxidised afterwards. Hence, the crude protein content decreased as the fermentation period extended because they were utilised for self-growth of fungi (Handoyo and Morita, 2006).

5.2.5 Carbohydrate Content

The carbohydrate content was calculated with the formula provided in Equation 3.1. It could estimate the total carbohydrate present in tempeh. Based on Table 4.3, overripe tempeh had higher carbohydrate content than fresh tempeh. Soybeans consist of carbohydrate that is simple and complex. As the fermentation process extend, the activity of α-amylase enzyme would increase, and polysaccharides which are complex carbohydrate would be broken down into simple carbohydrates (Astawan, Cahyani and Wresdiyati, 2023).

5.3 Sensory Evaluation

From the statistical analysis done towards the 5-point hedonic test shown in Table 4.4, the average overall acceptability of fresh tempeh was higher which indicates that most of the panellists could accept fresh tempeh. In addition, most of the panellists could not accept overripe tempeh was due to its appearance, aroma, and taste. Generally, the process of enzymatic hydrolysis for proteins would yield a bitter taste, and the presence of peptides could also provide umami taste (Utami, Wijaya and Lioe, 2016). During the extended fermentation process, there would be more hydrolysis of protein, hence yielding a stronger and richer flavour, as well as more sourness and bitterness in overripe tempeh. Next, the texture attribute shows similar results for both fresh tempeh and overripe tempeh which indicates the panellists do not have a preferred texture between two tempeh. This could be due to the tempeh samples used for sensory evaluation was prepared using frying process, which would cause Millard reaction that could diminish the texture, hence causing little to no differences in the texture. Based on the comments written by the panellists as shown in **Appendix C**, a few numbers of panellists wrote that overripe tempeh was more bitter and sour, had a richer and heavier taste, one even mentioned that there was artificial taste. Overall, there were more panellists that prefer fresh tempeh.

5.4 Effect of Extended Fermentation of Tempeh on the Microbiological Quality

Tempeh is a fermented soybean product which consist of a diverse microbial population, which might consist of the bacterial, and fungal community. The aim of microbial analysis was to evaluate the microbiological qualities of overripe tempeh. Therefore, the fungal communities were estimated through total yeast and mould count method, while bacterial communities were estimated through total plate count method (Charles Vegdahl, 2022). Although, the microbe presence in the overripe tempeh were observed to be dominant in bacterial community as it had a slightly higher total plate count value (5.64 log CFU/g) as compared to total mould count (5.60 log CFU/g). This could be due to the bacterial community were taking over the fungal community during the extended period of fermentation process (Hassanein, Prabawati and Gunawan-Puteri, 2015). However, the microbial load observed for fresh and overripe tempeh found in this study were not much different ($p>0.05$), indicated that the fermentation was successfully carried out without any sign of contamination. Besides, the total yeast mould count for both conditions (fresh and overripe fermentation) were comparable to the total plate count found in this study (Table 4.5). This clearly signified that as the fermentation progressed, there were more microorganisms became engaged (Djunaidi et al., 2017). Furthermore, this situation was attributed by the fact that the fungal community was dominant throughout the fermentation.

5.5 Storage Test of Overripe Tempeh

As tempeh is a highly perishable food product, the storage analysis is of utmost importance to understand the shelf life of overripe tempeh. In Table 4.6, the 14 days shelf life of overripe tempeh was evaluated by estimating the microbial population within the tempeh. Initially, both the bacterial and fungal community showed relatively similar results ($p>0.05$). This was because both bacterial and fungal communities were still adapting to the cold environment ($4^{o}C$). Then, the microbial load in total yeast mould count multiplies rapidly signifying the fungal species becoming the dominant community among the microbial population on day 3. Whereas total plate count experiences a gradual growth from day 0 until day 7. This could be due to the fungus species in the overripe tempeh were well adapt to the cold storage more quickly, allowing them to develop themselves and proliferate in the environment.

However, the fungal community was observed to had significant decline on day 7. This could be due to the metabolic interaction between the microbial population. For instance, some bacteria species in the overripe tempeh might produce metabolites that would inhibit the fungal growth. After day 7, the microbial population of overripe tempeh experienced a gradual decline and finally showed TFTC on day 14. This indicated that the microbial growth was entering death phase. The depletion of essential nutrients was one of the main reasons for this, alongside with the suppression of the microbial development due to the waste product production and the physical space was becoming insufficient (Bruslind, 2022).

5.6 Limitation and Future Study

In this study, there were a few limitations that could be improved. As the tempeh was kept inside a ventilated container to be fermented, the environmental factor was uncontrolled. The most significant one was temperature, alongside with humidity of the surrounding environment, which could play a role to impact the fermentation process. Besides that, the origin of the soybeans was unknown. Even though the soybeans were bought from the same supplier, the origin of the soybeans might come from different places, which might slightly impact the proximate analysis result. Lastly, there should be another way for the preparation of tempeh in sensory evaluation. This was because when the tempeh was fried, the appearance, aroma and texture might be similar, and causing the panellists to face difficulties when evaluating. Using another method like steaming tempeh could highlight the differences in sensory properties between two tempeh, thus providing a better insight and result for sensory evaluation.

For future study, further analysis could be done on the changes in nutritional content and microbes. For instance, the type of protein that was present and undergo changes or to identify the identity of microbes in the tempeh and observe the changes of these microorganisms. A better understanding on the microbial growth of tempeh can be achieved if study is conducted in detail for these field.

CHAPTER 6

6.0 CONCLUSION

This project was done to compare the differences in proximate composition, physicochemical properties, consumer preferences and microbial quality between fresh and overripe tempeh through statistical analysis. The findings revealed that there were a few properties that exhibits significant differences.

First, the overripe and fresh tempeh showed significant difference in titratable acidity. The titratable acidity of overripe tempeh (0.20%) was slightly higher than fresh tempeh (0.13%). In the texture profile analysis, it was found that adhesiveness, gumminess, and chewiness of both tempeh were significant different. Overripe tempeh showed lower value than fresh tempeh in these three parameters.

Next, crude fat and crude protein content showed significant differences in proximate composition. Overripe tempeh has lower values for both properties, signifying that it has lower protein (16.92%) and fat (5.67%) content compared to fresh tempeh (18.38%; 7.35% respectively).

For sensory evaluation, the preference of panellists on aroma and overall acceptability showed significant different between these two tempeh. The overall acceptability score of overripe tempeh (3.20) was lower than fresh tempeh (3.62), indicating that more people prefer fresh tempeh instead of overripe tempeh.

In terms of the microbial quality, the microbial population analysis showed that there were no significant differences among the microbial population of these two tempeh. This showed that the microbial population for fungal community and bacterial community were similar. Whereas for the shelf-life study, a statistical difference could be seen in day 3. The total mould count (6.21 log CFU/g) was more than the total plate count (5.73) signifying that the fungal community was dominant in day 3.

In conclusion, the objectives of the study were achieved. There were significant differences in nutritional contents like crude fat and crude protein content. There were also significant differences in physicochemical properties like titratable acidity and adhesiveness, gumminess, chewiness in texture profile analysis. The consumer preference level on tempeh were also determined. Majority of the people preferred fresh tempeh over overripe tempeh. Lastly, the microbial quality in overripe tempeh were also evaluated. There were no significant differences in bacterial community and fungal community. On the other hand, the shelf-life analysis had proven that the microbial population started to decline on day 7. Hence, overripe tempeh had lower nutritional value, lower sensory performance when compared to fresh tempeh. It also had a bad shelf-life performance, meaning that it is highly perishable.

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APPENDICES

Appendix A

Score Sheet

Questionnaire for Hedonic Scaling Test

Panel No./Name : ____________________

Product : ____________________

Date : ____________________

Instruction : ____________________

Please taste these samples and indicate how much you like or dislike for both samples. Please rinse your mouth with water before tasting each sample.

Taste the sample on the left first. Then, rate your preference level based on this scale:

5 Like very much
4 Like moderately
3 Neutral
2 Dislike moderately
1 Dislike very much

Sample codes		
Appearance		
Aroma		
Flavour/taste		
Texture		
Overall acceptability		

Comments:

____

Thank you.

Appendix B

Table 1: Master sheet prepared for sensory evaluation.

Panel No.	Sample codes and Order of presentation		
	Permutation	**A (Fresh)**	**B (Overripe)**
1	**AB**	1 **132**	2 **168**
2	**BA**	2 **212**	1 **003**
3	**AB**	1 **990**	2 **478**
4	**BA**	2 **001**	1 **509**
5	**AB**	1 **605**	2 **064**
6	**BA**	2 **912**	1 **183**
7	**AB**	1 **974**	2 **142**
8	**BA**	2 **352**	1 **386**
9	**AB**	1 **389**	2 **466**
10	**BA**	2 **107**	1 **527**

Table 1 continued: Master sheet prepared for sensory evaluation.

11	**AB**	1 **362**	2 **278**
12	**BA**	2 **709**	1 **669**
13	**AB**	1 **996**	2 **947**
14	**BA**	2 **720**	1 **157**
15	**AB**	1 **240**	2 **655**
16	**BA**	2 **749**	1 **146**
17	**AB**	1 **355**	2 **716**
18	**BA**	2 **356**	1 **127**
19	**AB**	1 **748**	2 **675**
20	**BA**	2 **452**	1 **880**
21	**AB**	1 **765**	2 **470**
22	**BA**	2 **196**	1 **903**

Table 1 continued: Master sheet prepared for sensory evaluation.

23	**AB**	1 **427**	2 **353**
24	**BA**	2 **119**	1 **226**
25	**AB**	1 **965**	2 **486**
26	**BA**	2 **357**	1 **586**
27	**AB**	1 **397**	2 **427**
28	**BA**	2 **970**	1 **664**
29	**AB**	1 **628**	2 **080**
30	**BA**	2 **255**	1 **229**
31	**AB**	1 **687**	2 **695**
32	**BA**	2 **179**	1 **008**
33	**AB**	1 **719**	2 **602**
34	**BA**	2 **546**	1 **936**

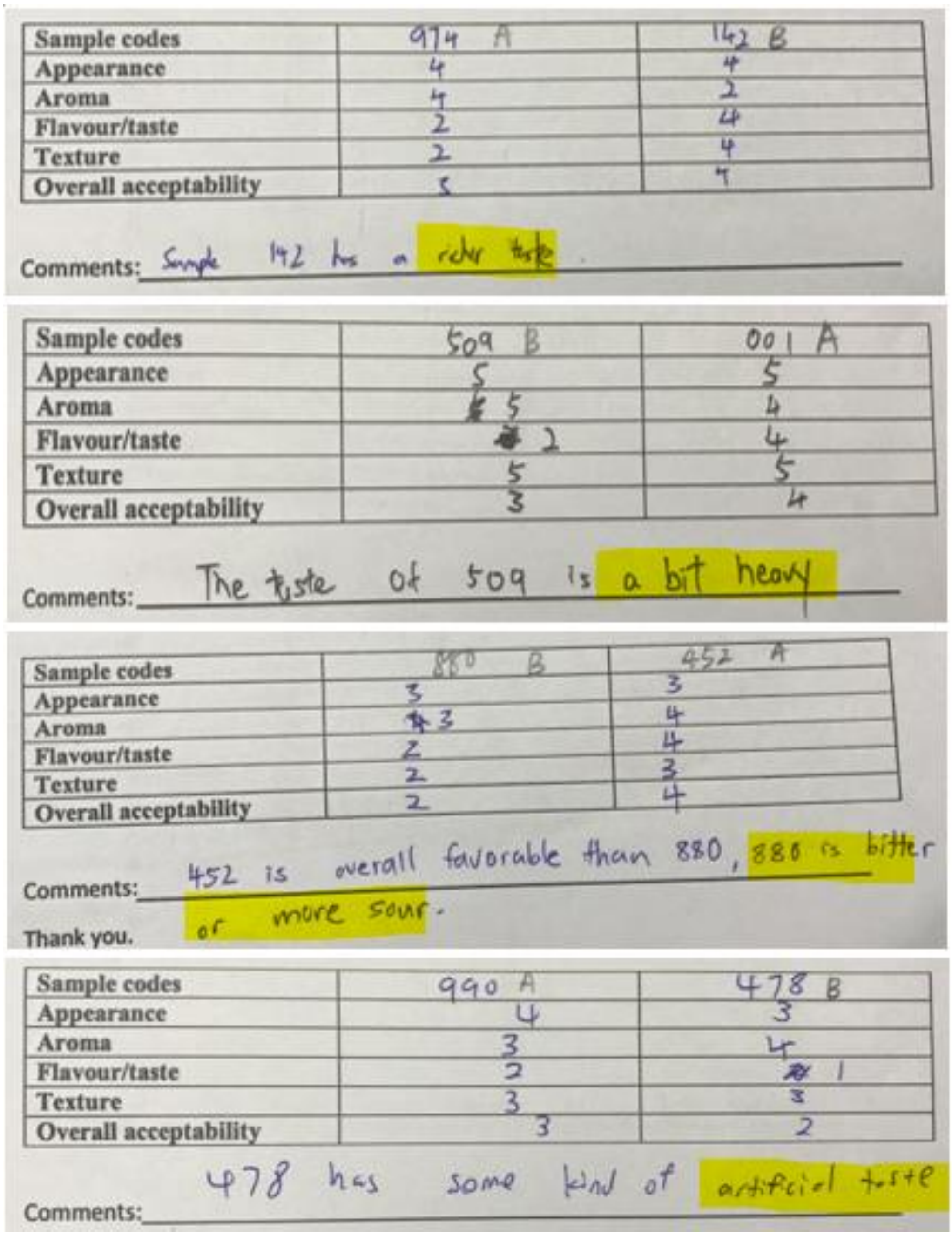
Table 1 continued: Master sheet prepared for sensory evaluation.

35	**AB**	1 **259**	2 **276**
36	**BA**	2 **013**	1 **393**
37	**AB**	1 **990**	2 **881**
38	**BA**	2 **520**	1 **454**
39	**AB**	1 **787**	2 **477**
40	**BA**	2 **252**	1 **691**
41	**AB**	1 **119**	2 **942**
42	**BA**	2 **116**	1 **137**
43	**AB**	1 **063**	2 **979**
44	**BA**	2 **762**	1 **757**
45	**AB**	1 **553**	2 **075**
46	**BA**	2 **780**	1 **909**

Table 1 continued: Master sheet prepared for sensory evaluation.

47	**AB**	1	2
		447	**433**
48	**BA**	2	1
		251	**193**
49	**AB**	1	2
		836	**466**
50	**BA**	2	1
		413	**816**

Appendix C



Sample codes	914 A	142 B
Appearance	4	4
Aroma	4	2
Flavour/taste	2	4
Texture	2	4
Overall acceptability	5	4

Comments: Sample 142 has a richer taste.

Sample codes	509 B	001 A
Appearance	5	5
Aroma	5	4
Flavour/taste	2	4
Texture	5	5
Overall acceptability	3	4

Comments: The taste of 509 is a bit heavy

Sample codes	880 B	452 A
Appearance	3	3
Aroma	3	4
Flavour/taste	2	4
Texture	2	3
Overall acceptability	2	4

Comments: 452 is overall favorable than 880, 880 is bitter or more sour.

Thank you.

Sample codes	990 A	478 B
Appearance	4	3
Aroma	3	4
Flavour/taste	2	1
Texture	3	3
Overall acceptability	3	2

Comments: 478 has some kind of artificial taste

Figure 1: Comments received from panellists in sensory evaluation for A: fresh tempeh; B: overripe tempeh.

Appendix D

Table 2: Mean score of each sensory attributes for fresh tempeh and overripe tempeh.

Sensory Attribute	**Tempeh Samples**	
	Fresh Tempeh	**Overripe Tempeh**
Appearance	4.22 ± 0.74^{a}	3.80 ± 0.83^{a}
Aroma	3.60 ± 0.76^{a}	3.56 ± 1.09^{b}
Taste	3.36 ± 0.85^{a}	2.92 ± 1.14^{a}
Texture	3.42 ± 0.78^{a}	3.42 ± 0.99^{a}
Overall acceptability	3.62 ± 0.78^{a}	3.20 ± 1.16^{b}

*Different superscript letters within the same row indicates there is statistical difference between the mean value ($p<0.05$). While same superscript letters within the same row indicates no significant difference ($p>0.05$)

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FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	ESTHER LIM SHI LIN
ID Number(s)	19ADB03699
Programme / Course	BACHELOR OF SCIENCE (HONOURS) FOOD SCIENCE
Title of Final Year Project	PRELIMINARY STUDY ON THE PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITIES OF OVERRIPE TEMPEH

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
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Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

____
Signature of Supervisor

Name: SIM KHENG YUEN

Date: 31/8/2023